Introduction...

1. Discovery of nitric oxide:

Nitric oxide (NO) is a versatile gaseous molecule that relays some major roles in cell signaling, stress response as well as in immunity. This novel molecule was first reported by a Belgian scientist J. B. van Helmont, which was prepared in his lab in 1648. Later in 1671, one of the greatest physicists, Robert Boyle also reported nitric oxide as 'volatile nitre' in the air, which supports combustion. After a century, Sir Joseph Priestley first time characterized the chemical properties of nitric oxide in 1772. He generated the gas by the action of 'spirit of nitre' (now known as nitric acid) on copper and named as 'nitrous air' and reported its spontaneous reactivity with 'common air' to generate a soluble brown gas (now known as nitrogen dioxide).

$$3Cu + 8H^{+} + 2NO_3^{-} \rightarrow 3Cu^{2+} + 2NO + 4H_2O$$

2. Chemical and physical properties of nitric oxide:

Nitric oxide (nitrogen monoxide, NO) is a molecule of interest for physicists, chemists and biologists for over 200 years; thus, a huge database of information is already present. NO is an uncharged, small lipophilic molecule contains total odd number (8 bonding and 3 antibonding electrons, i.e. in total 11) of electrons, thus it contains an unpaired electron [1]. The oxidation state of nitrogen (N) atom in NO is +2 and it is second of the oxides in which the oxidation states of nitrogen ranges from +1 to +5 (N_2O^{+1}; NO^{+2}; $N_2O_3^{+3}$; NO_2 and $N_2O_4^{+4}$; $N_2O_5^{+5}$). The bond order of NO is around 2.5 and the bond length of N-O is 1.15 Å. Though it is one of the smallest stable molecule, but the presence of the unpaired electron helps it to react only with those molecules which contain unpaired d electron/s [2]. Thus, it can easily react with oxygen (O_2) and reactive oxygen species like O_2^{-}. It can also react with transition metals containing *d* orbital like iron (Fe) present in different proteins e.g. NO can rapidly react with oxyferrohemoglobin and ferriheme can be formed [1].

3. Biosynthesis of nitric oxide:

Biosynthesis of NO is mainly dependent on the activity of nitric oxide synthases (NOSs) [3]. But NOS-independent NO synthesis also takes place *in vivo* [4]. Hence, biosynthesis of NO is mainly classified into two groups: NOS-dependent and NOS-independent NO synthesis.

3.1. Nitric oxide synthase-dependent nitric oxide synthesis:

Nitric oxide synthases [NOSs] (EC1.14.13.39) are member of cytochrome P450 enzyme family [5]. NOSs utilize L-arginine as the substrate [3-5]. NO is formed as the byproduct, during the conversion of L-arginine to L-citrulline. It is a two-step reaction. The first step is the formation of the intermediate Nω-hydroxy-L-arginine (NHA) from L-arginine and in the next step, NHA is converted to NO and citrulline. The reaction has a 1:1 L-citrulline/NO product stoichiometry [6].

3.1.1. Structure and mechanism of action of nitric oxide synthases:

Nitric oxide synthase is a homodimeric enzyme. Each of the monomers contain two domains: reductase and oxygenase domain [3, 7]. NOSs need cofactors like flavin adenine dinucleotide (FAD), flavin mono-nucleotide (FMN), tetrahydrobiopterin (BH_4), calmodulin and haem for its activity [8, 9]. The carboxyl terminal reductase domain provides the binding sites for nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) (providing reducing energy), FAD, FMN and calmodulin whereas amino terminal oxygenase domain provides binding site for BH_4 and cysteinyl thiolate-ligated haem group [3, 7]. This haem group contributes to the functional dimer formation [10]. The activity of NOS is dependent on the dimer formation. Zinc (Zn) is another factor that involves in the formation of functional dimeric NOSs [11]. The binding of calmodulin is regulated by the intracellular calcium (Ca^{2+}) ion concentration [12, 13]. Each of the monomers of NOSs, are capable of transferring electrons from reduced NADPH to FAD and FMN but has a very limited capability to reduce molecular O_2 to superoxide [14]. Electrons transfer from reductase domain to oxygenase domain only becomes possible after the formation of the dimer. Monomers of NOSs are unable to bind BH_4 and L-arginine. Hence, NO production can't be catalyzed by monomers [15]. In the functional form of NOSs, electrons are transferred from the reductase domain of the one monomer to oxygenase domain of the another monomer and consecutively two oxidation reactions take place to form NO and L-citrulline from the L-arginine via the generation of NHA as the intermediate [16,17] **[Fig. 1]**.

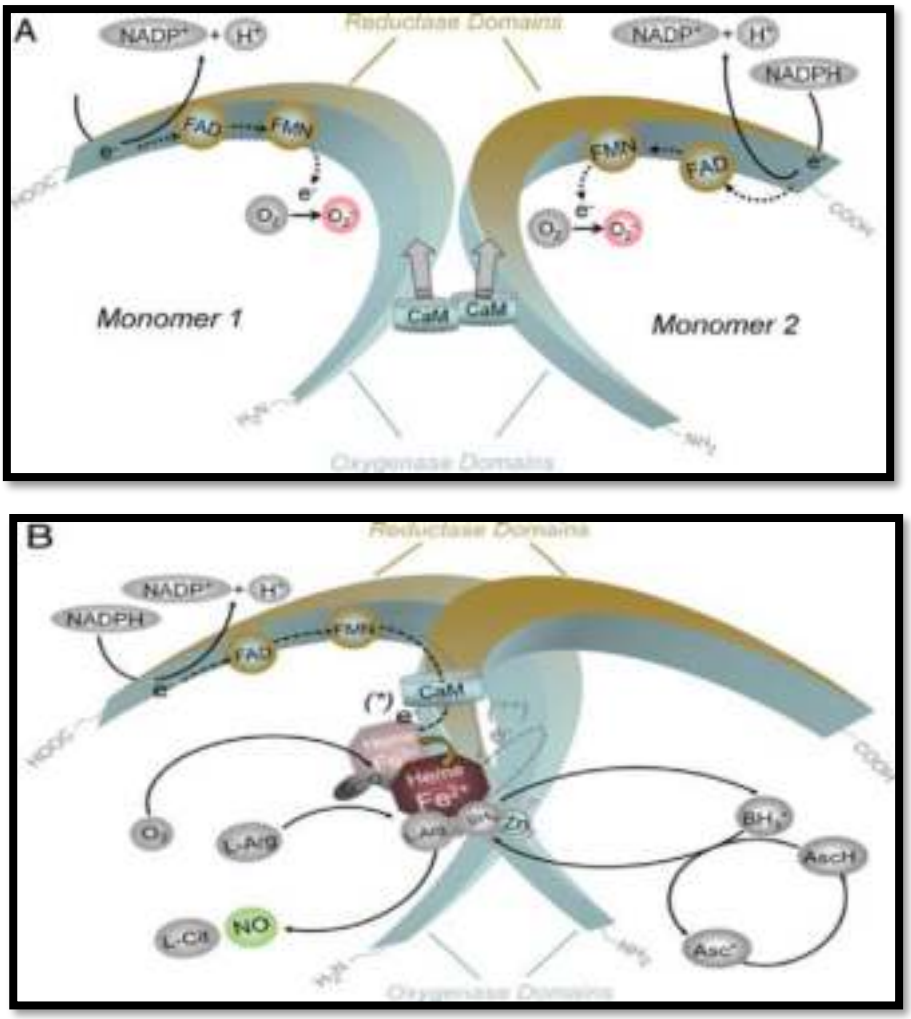

Fig. 1 Schematic representation of mechanism of action of nitric oxide synthases. (Adapted from Förstermann *et al.* 2012)

3.1.2. Mammalian nitric oxide synthases (NOSs):

Three isoforms of NOSs are present in mammals, referred to as neuronal 'n'NOS (NOS1), inducible 'i'NOS (NOS2) and endothelial 'e'NOS (NOS3). These NOSs play important roles in different pathophysiological functions. On the basis of the gene expression, isoforms of NOSs can be classified into two groups: constitutive (NOS1, NOS3) and inducible (NOS2) [7]. Localization of the isoforms is varied and isoforms have diverse roles in the regulation of different cellular processes [18-20].

3.1.2.1. Nitric oxide synthase 1 (NOS1):

nNOS is constitutively expressed in both the immature and mature neurons of the brain tissue and its activity is tightly regulated by the concentration of Ca^{2+} and calmodulin [21-26]. Immunohistochemistry analysis has revealed that nNOS is mostly present in

the spinal cord, adrenal cells, ganglia cells and vascular smooth muscle [18]. The subcellular localization of nNOS may be associated with its diverse functions. Presence of post-synaptic density protein, discs-large, zona occludens -1 (PDZ) domain is one of the significant characteristics of nNOS [27]. The PDZ domain may interact directly with the PDZ domain of another protein which results in the formation of dimer [27, 28]. Proteins containing PDZ, are believed to participate in different signal transduction pathways. The interaction of nNOS with the membrane is facilitated by the PDZ domain that may result in the alteration of NO signaling. nNOS also plays an important role in the synaptic signaling events [29, 30], like participation in the regulation of the long-term synaptic transmission [31]. NO produced by the activity of nNOS in nitrergic nerves can act as an unorthodox neurotransmitter that may stimulate NO-sensitive guanylyl cyclase in its effector cells, causing reduction in the tone of the different smooth muscles like blood vessels [18]. Inhibition in the activity of nNOS in the hypothalamus and medulla, causes systemic hypertension [32].

3.1.2.2. Nitric oxide synthase 2 (NOS2):

The expression of NOS2 is induced by the cytokines, antigens like bacterial lipopolysaccharide and other agents. Although this enzyme was first identified in macrophages, but reports suggest that iNOS has been expressed in all cell types in the presence of the appropriate agent [18, 33]. Interestingly, the expression of NOS2 is not dependent on the concentration of the intracellular Ca^{2+} [7]. The activity of this enzyme persists for a long time (many hours) after the immunological stimulation [34]. The expression of iNOS is regulated by the mitogen-activated protein kinase (MAPK) family of protein kinases [extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK), p38] and protein kinase B [35, 36]. NO, produced by the activity of iNOS in the cells, shows cytotoxic effect on the pathogens by interfering with the structure and function of the DNA and protein of the target cells [7, 37]. iNOS stimulated hepatocytes can use NO to eliminate malaria pathogens [37]. iNOS derived NO can also induce neuronal death due to inhibition of cytochrome oxidase, causing excitotoxicity [37, 38]. Large amount of NO produced by the iNOS play a significant role in the development of septic shock [39]. The fall in blood pressure is also indirectly mediated by excess production of NO by the iNOS in the vascular wall [40].

3.1.2.3. Nitric oxide Synthase 3 (NOS3):

NOS3 is mainly localized in the plasma membrane caveolae of the endothelial cells [41]. But this enzyme is also found in neurons of the brain, cardiac myocytes and platelets [18, 33]. Intracellular concentration of Ca^{2+} is very important for the activity of the eNOS. Ca^{2+} induces the binding affinity of the calmodulin (CaM) to the enzyme [42]. The bradykinin (BK) B2 receptor, a G-protein coupled receptor (GPCR), regulates the complex formation of Ca^{2+}/CaM [43]. The regulation of eNOS is also dependent on the protein-protein interactions. Heat shock protein 90 (hsp90) has a positive allosteric modulation effect on the activity of eNOS [44]. Translocation of eNOS from caveolae to other intracellular compartment like golgi bodies, is influenced by the activity of 'nitric oxide synthase interacting protein' (NOSIP) and 'nitric oxide synthase trafficking inducer' (NOSTRIN) [45]. Phosphorylation is another important factor for the stimulation of the eNOS. Specific tyrosine (Tyr), serine (Ser), threonine (Thr) residues of eNOS can be phosphorylated, activating the enzyme without depending on the concentration of Ca^{2+} [46, 47]. The best model to study such stimulation is fluid shear stress [47]. The inhibition of the activity of eNOS is mediated by the interaction of scaffolding domain of Cav-1(Caveolin-1) with the caveolin binding motif on eNOS [48]. eNOS stimulates soluble guanylyl cyclase and cyclic guanosine monophosphate (cGMP) in smooth muscle cells [49]. eNOS-derived NO decreases the expression of the genes involved in atherogenesis by inhibiting leucocyte adhesion to the vessel wall via interfering with the adhesion molecule CD11/CD18 (cluster of differentiation) [50]. NO, generated from eNOS, prevents cellular apoptosis and plays an important role in post-natal angiogenesis. Dysfunction of eNOS results in the development of cardiovascular disease [51].

3.1.3. Invertebrate nitric oxide synthase:

Like mammalians, NOS-derived NO plays an important role in bio-signaling in invertebrates [52-54]. In 1991, Radomsky *et al.* gave a clear idea about the presence of NO-mediated signaling as well as NOS activity in invertebrates like horseshoe crab (*Limulus polyphemus*) [52]. After this initial work, several studies reported the presence of NOS in invertebrates. In 1993, Eloffson *et al.* reported NADPH-diaphorase activity in osfradia, buccal ganglia, central nervous system (CNS) neurons, and in some peripheral organs [53]. In 1995, Jacklet *et al.* reported the cotransmitter activity of NO

in histaminergic synapses of *Aplysia* sp. [54]. In 1996, Moroz *et al.* reported the presence of Ca^{2+}-independent but calmodulin dependent NOS activity in mollusk species [55]. Domenech and Muñoz-Chápuli hypothesized that all the three isoforms of mammalian NOS may be derived from a single invertebrate ancestral gene through double whole genomic duplication that happened at the origin of vertebrates [56]. NOS-derived NO is an important component in defending invertebrates against pathogens. NOS from *Anopheles stephensi* (AsNOS) can be expressed inducibly in *Plasmodium*-infected mosquitoes. Inducible NOS activity is also found in *Rhodnius prolixus* when infected by *T. rangeli*, a South American Stercoraria trypanosome, pathogenic for vectors [57, 58]. NOS activity in invertebrates is also upregulated against the bacterial infection in invertebrates. It has been found that the expression of NOS is upregulated in response to systemic infection with *Escherichia coli* and *Micrococcus luteus* in *Anopheles gambiae*. Inhibition of the activity of NOS or lower NO production results in higher mortality rate of mosquito when infected by pathogenic bacteria, suggesting defensive role of NO in invertebrates [59].

3.1.4. Bacterial nitric oxide synthase (bNOS):

NOS activity is also present in prokaryotes. NOS activity in bacteria was first reported in *Nocardia* species, designated as NOS_{noc} [60] but till now none of the *Nocardia* sp. genome showed similar NOS sequence to that of the animal NOSs. Genome sequencing analysis first brought the clear evidence for the presence of NOS-like protein in bacteria which revealed that the bacterial ORFs (Open reading frames) coding for that protein with maximum sequence similarity to mammalian NOS_{ox} [61]. Key catalytic residues of NOS, are highly conserved from prokaryotes to eukaryotes. drNOS (NOS from *Deinococcus radiodurans*, a radiation-resistant bacteria) is the first NOS-like protein which was cloned, expressed and purified using *E. coli* as the host. The existence of NOS_{OX}-like proteins are mainly found in gram positive bacteria (*Bacillus* sp., *Deinococcus* sp., *Rhodococcus* sp. etc.) but gram negative bacteria like cyanobacterium also contains NOS like sequence [62]. A ~100 kD protein, found in *Rhodococcus* sp., was recognized by a human anti-iNOS antibody. In addition to it, the activity of the protein was reduced by mammalian NOS inhibitor and increased by BH_4, indicating the presence of NOS-like protein [63]. Unlike mammalian NOS, most of the bacterial NOSs only have an oxygenase domain but they can form dimer, indicating presence of different mechanism of dimerization [64, 65]. However, both the oxygenase domain

and reductase domain are present in *Sorangium cellulosum*, indicating both eukaryotes and prokaryotes may have evolved from a common ancestor [66]. NADPH-utilizing proteins like flavodoxin reductase/flavodoxin, transfers electrons to the bacterial NOS and support the production of NO [67, 68]. Bacterial NOS has essential role in cell physiology. It was found that bNOS can regulate the electron transfer to maintain membrane bioenergetics in *Staphylococcus aureus*, a human pathogen. This process is very important for the nasal colonization and resistance from membrane-targeting antibiotics like daptomycin in *Staphylococcus aureus* [69]. Thus, bNOS becomes one of the important drug target to inhibit the growth of methicillin-resistant *Staphylococcus aureus*(MRSA). It was reported that potent bNOS inhibitors like NG-nitro-L-arginine (L-NNA), can enhance MRSA killing [70]. Aminoquinolines, another compound, was found to inhibit the activity of bNOS by binding with the unique hydrophobic patch of bNOS [71].

3.1.5. Fungal nitric oxide synthase:

Orthologue of mammalian NOS is not found in fungus or yeast till today. However, some reports suggest that NOS-like proteins are present in different yeasts and fungi. In 1998, Kanadia *et al.* reported the presence of a NOS-like activity protein of 60 kD in the crude extract of yeast extract, peptone dextrose (YPD) grown *Saccharomyces cerevisiae* cells. The protein was detected by western blot using mouse monoclonal anti-neuronal NOS. That protein was activated by arginine and calmodulin whereas inhibited in the presence of NG-Nitro- L-Arginine Methyl Ester (L-NAME), a mammalian NOS inhibitor [72]. Another report suggests that the nitrite dependent-NO production can take place in mitochondria by the activity of cytochrome *c* under hypoxic condition in *S. cerevisiae* [73]. Calmodulin-dependent NOS-like activity was also found in *S. pombe*, a fission yeast [74]. In 2013, Nishimura *et al.* reported the Tah18-dependent NO production in *S. cerevisiae*. This Tah18-dependent NO production is positively regulated with generation of arginine via proline-arginine metabolic pathway, indicating NOS-like activity of Tah18. It is believed that Tah18 does not contain oxygenase domain. Tah18 may act as a reductase domain and transfer electrons from NADPH to an unknown oxygenase protein, which oxidizes arginine to citrulline and NO [75,76]. NOS-like activity has also been detected in *Blastocladiella emersonii*, an aquatic fungus. Vieira *et al.* reported that concentration of NO-derived products was increased at the sporulation stage and Ca^{2+}-NO-cGMP signaling pathway

was required for biogenesis of zoospores in *Blastocladiella emersonii* [77]. *Aspergillus oryzae* also carries a sequence which shows similarity with the arginine binding site of mammalian NOS oxygenase domain but till now there is no definite evidence of fungal NOS has been reported [78].

3.2. Nitric oxide synthase-independent nitric oxide synthesis:

Although NOS is the main catalytic enzyme for NO synthesis, but several reports suggest that NOS-independent NO synthesis also takes place *in vivo*. The main source of the NOS-independent NO generation is nitrite. In the process of denitrification, NO is produced as an intermediate product by the activity of nitrate reductase and nitrite reductase among the other enzymes. After NOS, the most important enzyme in terms of NO synthesis is nitrite reductase which catalyzes the reaction from nitrite to NO. On the basis of the requirement of co-factors, nitrite reductase is classified into two distinct unrelated groups i.e. heme containing enzyme i.e. cytochrome cd_1 and a copper containing enzyme. Soluble, dimeric Cytochrome cd_1 is widely present in the periplasmic space of the gram negative bacteria like *Pseudomonas aeruginosa*, *Thiobaccilus denitrificans* etc. While the copper containing nitrite reductases are found in both the gram positive (*Bacillus* sp.) and gram negative bacteria (*Alcaligenes* sp.). The copper containing nitrite reductase is bound tightly to the cell membrane of the gram positive bacteria [79]. Miyamoto *et al.* showed that TRPV3, a heat-activated transient receptor potential (TRP) ion channel can induce the NOS-independent NO generation from nitrite in keratinocytes [80]. Deoxyhemoglobin in erythrocytes and deoxymyoglobin in myocardial cells can convert nitrite to NO and form methemoglobin and metmyoglobin during hypoxic condition [81, 82]. Different globin proteins show nitrite reductase-like activities in neuroglobin, cytoglobin, and plant hemoglobins [83-85]. Xanthine oxidoreductase (XOR) can also act as the nitrite reductase during hypoxic conditions. XOR uses xanthine as the electron donor. The nitrite reductase-like activity of XOR is dependent on the concentration of nitrite [86, 87]. It was reported that complex III and complex IV along with cytochrome *c* of mitochondria can reduce the nitrite to NO under hypoxic condition during electron transport chain in liposome [88].

Non-enzymatic NO synthesis has also been reported in mammalian systems. The non-enzymatic NO production has been observed in the stomach under the acidic

condition. In the acidic condition nitrite is converted to nitrous acid (HNO_2). The unstable nitrous acid oxidizes to dinitrogen trioxide (N_2O_3) that subsequently decomposes to NO and nitrogen dioxide (NO_2). The conversion from HNO_2 to N_2O_3 is a second order reaction that indicates the process is comparatively slow [89, 90]. It has been also reported that ascorbate (AsA), an essential antioxidant, can induce non-enzymatic NO production by the reduction of nitrite to NO through the formation of monodehydroascorbate (MDA) radical [91, 92].

4. Beneficial role of nitric oxide:

The beneficial role of NO is associated with the regulatory activities in different biochemical processes in various organisms like mammals, plants, yeasts, bacteria [75]. In 1987, Ignarro *et al.* reported the role of NO as endothelium-derived relaxing factor (EDRF). After that researchers started to explore the NO-mediated signaling pathway and different roles of NO in complex biological processes like smooth muscle relaxation, platelet inhibition, anti-apoptosis etc. [93-96].

4.1. Nitric oxide and vascular tone:

Endothelium-derived NO stimulates soluble guanylate cyclase (sGC) that induces the formation of cGMP. cGMP is a secondary messenger and it can activate protein kinase G which inhibits the voltage-dependent calcium channel (VDCC) mediated calcium influx. Protein kinase G can also act on the SERCA (Sarco/endoplasmic reticulum calcium ATPase) to initiate reuptake of cytosolic calcium (Ca^{2+}) into the sarcoplasmic reticulum (SR), resulting in the reduction of intracellular calcium concentration. Intracellular calcium concentration is very important for the activation of calmodulin. With the reduction in the intracellular calcium concentration, the activity of calmodulin was found to have decreased. Calcium depletion inactivates myosin light chain kinase (MLCK) and upregulates the activity of myosin light chain phosphatase (MLCP). Due to the inactivation of MLCK, myosin phosphorylation is inhibited and actin-myosin cross-bridge breaks down, resulting in the relaxation of smooth muscle cells [97-99]. GPCR (G protein coupled receptors) activity is also associated with the relaxation of smooth muscle cells. Reports suggest that NO inhibits the activity of GRK2 (GPCR kinase 2), the negative regulator of GPCR signaling **[Fig. 2]**. Thus, phosphorylation of β-adrenoceptors is inhibited, that results in the inactivation of the desensitization of the

signal. This mechanism is very important for the relaxation of smooth muscle cells [100,101]. Under hypoxic condition, NO can efficiently regulate the signaling pathway for smooth muscle relaxation without activating the classical NO-sGC-cGMP pathway. The concentration of cyclic inosine monophosphate (cIMP) increases in this signaling pathway [102,103].

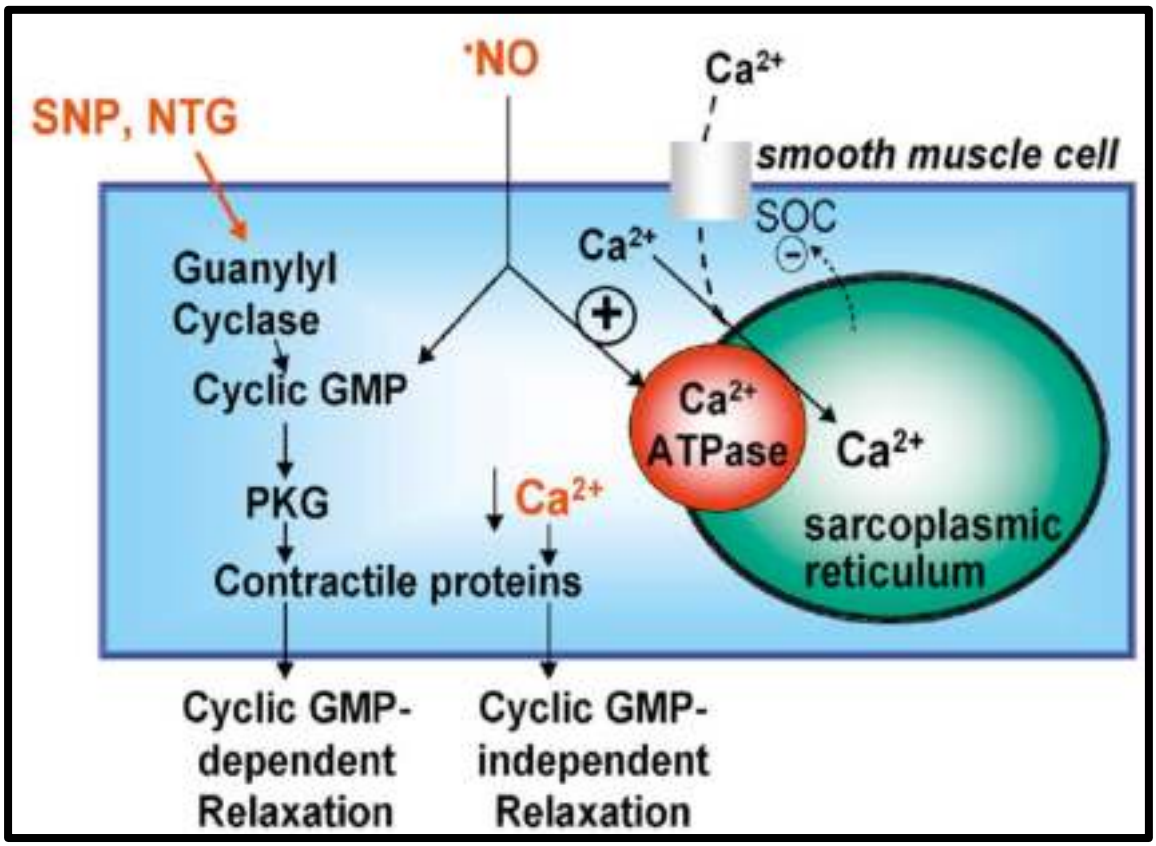

Fig. 2 Role of nitric oxide on relaxation of smooth muscle cell. (Adapted from Tong *et al.* 2010)

4.2. Nitric oxide and platelet inhibition:

Platelet inhibition is also mediated by NO-sGC-cGMP pathway. The key protein which is required for platelet inhibition is vasodilator sensitive phosphoprotein (VASP). This protein has been found in the platelets, endothelial and smooth muscle cells. Platelet activation is dependent on the interaction of the VASP with cytoskeleton proteins. The interaction leads to the polymerization of actin and the shape of the platelet changes, activating the aggregation. The interactive property of the VASP is negatively regulated by phosphorylation. Protein kinase G, the effector molecule of NO-sGC-cGMP pathway, can phosphorylate the Ser^{157} residue of the VASP that leads to the

conformational change of the protein. Due to conformational changes in VASP, the interaction of VASP with cytoskeleton proteins gets completely prevented, that result in the inhibition of platelet activation [104-106]. Report also suggest that interaction of Inositol-1,4,5-triphosphate receptor associated cGMP kinase substrate (IRAG) with inositol-1,4,5-trisphosphate receptor type I (InsP3RI) is also required for NO/cGMP-dependent inhibition of platelet aggregation [107]. Platelets have a little amount of eNOS. It has also been reported that endogenous NO may reduce the platelets response, without hampering the activation occurring at the site of blood vessel injury [104]. Dangel *et al.* showed that NO-sGC mediated cascade signaling is the only mechanism of platelet inhibition, no other mechanism is present to provoke platelet inhibition [108].

4.3. Nitric oxide as an anti-apoptotic factor:

Several reports suggest that NO can interact with the caspase and other proteins to act as an anti-apoptotic factor. NO involved anti-apoptotic event is also mediated through cGMP or cyclic adenosine monophosphate (cAMP) signaling pathway in different cells like eosinophils, PC12 cells, ovarian follicles, embryonic motor neurons and B lymphocytes etc. [109]. In general, apoptosis is initiated by the death receptor and mitochondrial signaling pathway [110]. NO can negatively regulate the activation of the both death ligand-dependent and independent apoptosis. Interaction of NO with the caspase proteins, including caspase-8, an important pro-apoptotic factor, leads to its structural alteration. Caspase-8 can upregulate the activity of Bax and cytochrome *c* in the death signaling pathway. Structural alteration of caspase-8 leads to the inhibition, preventing the death signal pathway of apoptosis [111-113]. TNF-receptor associated death domain protein (TRADD) can be inhibited in the presence of NO, preventing the stimulation of the apoptotic pathway by blocking the ceramide generation [114]. NO can also interfere with the mitochondrial function to inhibit apoptosis. Report suggests that NO can inhibit the PTP (permeability transition pore) reopening by membrane depolarization and accumulation of Ca^{2+}, that can reduce the release of cytochrome *c*, a pro-apoptotic factor [115]. Regulation of the anti-apoptotic activity of NO is concentration-dependent. In lower concentration, NO can act as an anti-apoptotic factor but in higher concentration NO can modulate the ratio of bcl-2 and Bax protein that leads to the activation of the apoptotic pathway [109].

4.4. Cytoprotective role of nitric oxide in yeast:

The cytoprotective role of NO has been reported under environmental stress conditions like high temperature, hydrostatic pressure, heat shock, redox stress etc. in yeast [75]. Nishimura *et al.* showed that the production of NO increases via Pro-Arg metabolic pathway. This elevated NO may help to overcome the stress induced by high temperature [4]. Another report suggests that NO can induce the activity of Mac1 protein by post translational modification under high temperature in *S. cerevisiae*. The higher activity of Mac1 stimulates the expression of ctr1 gene encoding high-affinity copper transporter, which in turn increases the intracellular copper concentration. This copper concentration stimulates the activity of Cu/Zn-SOD, an essential stress response enzyme [116]. NO can also activate the adaptive response by stimulating the peroxide scavenging activity and limiting the availability of iron in *S. pombe*, a fission yeast [117]. It has also been revealed that NO can act as an anti-aging agent. In yeast model, it has been observed that reduced NO production is one of the major cause of Batten disease [118]. Depletion of glucose concentration is one of the major nutrient stress for heterotrophs like yeast. Different cellular responses can be generated during glucose depletion condition by the activation of Rst2 protein in yeast. NO has been reported to induce the expression of the rst2 gene. The activity of Rst2 protein upregulates the expression of the fbp^{1+} gene, encoding a fructose-1,6-bis-phosphatase, via the STREP (stress-starvation response element of *Schizosaccharomyces pombe*) motif. This event leads to the activation of the stress response pathway to combat the hostile condition [119]. NO is also responsible for the metabolic shift in calorie restricted *S. cerevisiae* [120]. Overall, in presence of low concentration of NO, cytoprotective activities like stress resistance, fermentation, metabolism can be stimulated. In higher concentration, NO harms the cell directly or via the formation of reactive nitrogen species (RNS) [75].

5. Reactive nitrogen species (RNS) formation:

Reactive nitrogen species (RNS) is a family of reactive molecules which are derived from NO. RNS is the product of the reaction between NO with molecular oxygen (O_2) or reactive oxygen species like superoxide (O_2^-) [121]. The most common reactive nitrogen species are peroxynitrite ($ONOO^-$) and dinitrogen trioxide (N_2O_3). The reactive nitrogen species formation is dependent on the concentration of the NO. The steady-state concentration of NO is expressed by the ratio between the rate of reaction

(consumption and synthesis) and rate of diffusion [122]. Thus, the imbalance in the steady-state concentration of NO may result in the generation of reactive nitrogen species via several mechanisms like autooxidation of NO, reaction with superoxide etc. [121, 122].

5.1. Auto-oxidation of nitric oxide:

Auto-oxidation of NO is the reaction between NO with molecular oxygen (O_2) to form dinitrogen trioxide (N_2O_3). Different nitrosating agents like nitrosonium ion (NO^+), nitrous acidium ion (H_2ONO^+), can influence the reaction. The reaction is also favored under acidic condition [123]. It is a non-enzymatic, two-steps reaction.

$$2NO + O_2 \longrightarrow 2NO_2$$
$$NO + NO_2 \longrightarrow N_2O_3$$

As it is a third order reaction (second order in NO concentration and first order in O_2 concentration) [124], thus the rate equation of the reaction is expressed as

$$\text{Rate} = k[NO]^2[O_2]$$

[Where, k (rate constant) = 8.4×10^6 M^{-2} s^{-1} at 37°C]

N_2O_3 is also a reactive molecule and can react with the thiols like glutathione reduced, resulting in *S*-nitrosation ($k=1.6 \times 10^{-3}$ s^{-1}) [125]. This is an important reaction for the generation of nitrite anion.

$$RSH + N_2O_3 \longrightarrow RSNO + H^+ + NO_2^-$$

Some of the anions like bicarbonate, phosphate, and chloride have inhibitory effect on *S*-nitrosation under physiological pH. In acidic and physiological pH, nitrosyl halide is formed during the reaction between anions and N_2O_3 [126].

$$X^- + N_2O_3 \longrightarrow XNO + NO_2^-$$

[X represents bicarbonate, phosphate, and chloride]

5.2. Reaction of nitric oxide with superoxide:

At the high concentration, NO can react with the superoxide (O_2^-) to form peroxynitrite ($ONOO^-$). The rate constant of the reaction is varied from 6.6 x 10^9 to 19 x 10^9 $M^{-1}s^{-1}$, with the concentration of NO [127-129]. The rate of formation of peroxynitrite is 3-8 times greater than decomposition of superoxide by SOD [130]. Hughes and Nicklin proposed that hemolysis of peroxo bond was not a thermodynamically favorable reaction. Thus, the decaying of peroxynitrite mainly occurrs by two mechanisms: via the ion pairs or via intramolecular rearrangement, forming nitrate (NO_3^-) or nitrite (NO_2^-) respectively [131]. Proton catalyzed mediated decomposition of peroxynitrite is very rapid (k=1.3 s^{-1}) [132]. In addition to it, carbondioxide (CO_2) mediated peroxynitrite decomposition is also very rapid and end product of the reaction is NO_3^- with a half-life of ~50 ms [133-135]. Peroxynitrite is highly cytotoxic and several studies have been done with peroxynitrite [136, 137].

5.3. Formation of *s*-nitrosothiols:

S-nitrosothiols (RSNO) are the product of the reaction between NO and thiol group containing organic compounds. They are also known as thionitrites. RSNO can act as a signaling molecule in living systems. It plays a major role in platelet inhibition [138]. *S*-nitrosothiols can be formed via autooxidation of NO to N_2O_3, radical recombination between NO and a thiyl radical ($RS^{\bullet}$), and transition metal mediated pathways. It can't be formed without the presence of an electron sink. Hydrophobicity of the thiol-containing organic compound can positively influence the *s*-nitrosothiol formation [139, 140]. Activation of NOS can also contribute to the *s*-nitrosothiols generation via the formation of peroxynitrite. The most common intracellular *s*-nitrosothiol is *s*-nitrosoglutathione (GSNO), formed via the reaction of GSH (reduced glutathione) and NO [141]. The presence of oxygen is also a key factor for the formation of GSNO. The reaction of O_2, NO, and GSH is depicted below.

$$NO^{\cdot} + O_2 \longrightarrow ONOO^{\cdot} \text{ ……(i)}$$

$$ONOO^{\cdot} + NO^{\cdot} \longrightarrow 2\ {\cdot}NO_2 \text{……(ii)}$$

$$NO^{\cdot} + {\cdot}NO_2 \longrightarrow N_2O_3 \text{ ……(iii)}$$

$$GSH + N_2O \longrightarrow GSNO \text{…….....(iv)}$$

GSNO is not a stable molecule in the solution. It can be broken down to NO and GSSG. In the presence of acid, GSNO can release nitrosonium ion (NO^+). Thus, it is also referred to as a "NO donor" [141, 142]. Enzyme-dependent (GSNO reductase, Carbonyl reductase 1, Thioredoxin system) GSNO metabolism has also been found in different organisms [143].

6. Nitrosative stress:

When the ratio of nitrosants (NO and reactive nitrogen species) to antioxidants exceeds 1.0 inside the cell, in that situation, NO or reactive nitrogen species (RNS) interact with the biomolecules like DNA, lipid, proteins [109, 144]. Sometimes, this interaction is deleterious and may lead to the damage of DNA and DNA repair system, functional loss of proteins or enzymes, structural modification of lipids and also alter the redox homeostasis *in vivo* [109, 144-146]. This hostile condition is referred to as ***"nitrosative stress"***– a term first coined by Prof. Jonnathan Stamler and his co-workers in analogy with the "oxidative stress" [147]. Thus, in short, nitrosative stress can be defined as the belligerent condition, provoked by the imbalance in the concentration of NO [148].

6.1. Effect of nitrosative stress on cellular components:

Several reports suggest that NO or RNS can interact with the cellular macromolecules like DNA, lipid, protein etc. that may lead to its inactivation and structural modifications. These interactions influence the cellular homeostasis as well as its survival [109, 149] **[Fig. 3]**.

6.1.1. Nitrosative stress and DNA:

Nitrosative stress mediated DNA damage cannot be directly associated with NO but with RNS like HNO_2/acidified NO_2^-, dinitrogen trioxide (N_2O_3), and peroxynitrite ($ONOO^-$**)** [150]. The RNS mediated DNA damage can be processed through three chemical mechanisms: 1. direct interaction with DNA structure, 2. inhibition of DNA repair system and 3. via the production of genotoxic compounds like alkylating agents, and hydrogen peroxide (H_2O_2) [151]. N_2O_3 is a strong deaminating agent. It interacts with heterocyclic amines of DNA bases via the formation of diazonium ion and complete hydrolysis of diazonium ion leads to deamination. Hence, cytosine, adenine, and 5-methylcytosine are converted to uracil, inosine, and thymine respectively by the

action of N_2O_3 whereas guanine is converted to xanthine and oxanine. Deamination is the main reason for structural and characteristic alteration of the bases [152-154]. This deamination influences spontaneous depurination that leads to the break-down of DNA.

Peroxynitrite can interact with DNA during the replication and transcription process when DNA exists in the single-strand form [155]. It can damage both the sugar and bases of DNA. Guanine can be converted to 8-nitro-2′-deoxyguanosine (8-nitro-dG) and 8-Oxo-7,8-dihydro-2′-de-2 oxyguanosine by the treatment of peroxynitrite [156, 157]. These compounds are referred to as the marker of peroxynitrite mediated DNA damage [158]. Xanthine and hypoxanthine can also be formed by the treatment of peroxynitrite [159]. These changes decrease the integrity of DNA, promoting breakage of the strands. Peroxynitrite can also induce dG–dG and DNA–protein crosslinks that leads to DNA mutation [160, 150]. It can also induce fragmentation of the sugar moiety, generating strand breakage. It is believed that damage in sugar moiety involves hydrogen abstraction, generating highly reactive sugar radicals that participate in the radical mediated DNA strand breakage [158].

HNO_2/acidified NO_2^- can also induce DNA damage *in vivo* and *in vitro* [150]. It induces the DNA cross linking at G and CpG islands [161]. The indirect mechanism of DNA damage is associated with the inhibition of DNA repair system [162]. HNO_2/acidified NO_2^- can also interact with the components of DNA repair system. The Zn-finger motif of DNA repair enzymes can be affected by the NO released from acidified NO_2^-, resulting in the inhibition via losing the integrity [163]. DNA synthesis process can also be blocked by the action of NO via inhibiting ribonucleotide reductase [164, 165]. Furthermore, *in vivo* exposure of NO leads to the generation of genotoxic compounds, promoting apoptosis [109].

6.1.2. Nitrosative stress and lipid:

Nitrosative modification of lipids is associated with oxidation and nitration [166]. Lipid oxidation is a characteristic feature of inflammatory vascular diseases like atherosclerosis [167]. NO and its derivatives are usually involved in the modification of lipid and its biosynthesis [150]. But it has also been reported that at lower concentration, NO can inhibit the lipid oxidation by reacting with the lipid based radicals (L•, LO•' LOO•). LOO• mediated propagation can be blocked by the NO [168].

It was apparent that copper induced oxidation of low density lipoprotein (LDL) can be inhibited by lower concentration of NO in activated macrophages and endothelial cells [169-171]. Oxidation of liposomal cholesterol and phosphatidylcholine can be reduced in the presence of lower concentration of NO [172, 173].

In the presence of O_2 and singlet oxygen (O_2•), NO derived reactive species rigorously oxidize lipids [174]. NO_2^- mediated oxidation and nitration have been shown in unsaturated fatty acids, LDL, cholesterol etc. [175-177]. The protonated form of NO_2^- i.e. HNO_2 can react with ethyl linoleate and hydroperoxy-octadecadienoic acid and different nitrated species like nitroalcohol, nitroalkanes etc. are formed, leading to the lipid bilayer damage [178]. Peroxynitrite and its protonated form i.e. ONOOH are also involved in lipid damage [179]. Peroxynitrite mediated lipid oxidation leads to the generation of different nitrated species along with secondary oxygen species like singlet oxygen (O_2•) due to the rearrangement of unstable reactive peroxynitrite intermediates (LOONO) [180]. LOONO can form comparatively stable $LONO_2$ or breaks down to LO• and NO_2^- via hemolytic cleavage [181]. In addition to it, peroxynitrite mediated lipid oxidation also leads to the formation of lipid-protein adduct in LDL, indicating excessive breakdown of polyunsaturated acid [182].

6.1.3. Nitrosative stress and protein:

Proteins are one of the most vulnerable macromolecule in the presence of RNS including NO. Hence, the protein modifications, like, *S*-nitrosylation, protein tyrosine nitration (PTN), are referred to as the biomarkers of nitrosative stress. Both these forms are specific, inhibitory/toxic post translational modifications and can also participate in regulation of different cellular processes [183].

S-nitrosylation is a very important regulatory mechanism *in vivo* [184-186]. *S*-nitrosylation is a reversible post translational modification in which NO moiety covalently binds with specific cysteine residue(s) of a protein, yielding *S*-nitrosothiol [187, 188]. This reaction can be mediated by different chemical species like NO, metal-NO complex, nitrosonium ion (NO^+), *S*-nitrosoglutathione (GSNO) etc. *S*-nitrosylation induces the conformational changes of protein that may lead to acetylation, ubiquitylation, palmitoylation of different cellular components [189]. Proteins like hemoglobin, caspase-3, glyceraldehyde-3-phosphate dehydrogenase are involved in

transnitrosylation i.e. catalyzing the transfer of NO group to the adjacent protein [190-192]. Denitrosylation i.e. reversible reaction of *S*-nitrosylation, is also associated with the activity of proteins, protein-protein interaction, cellular signaling etc. It has been reported that caspase-3 can be activated via denitrosylation [193]. Compared to denitrosylated forms of protein, *S*-nitrosylated forms have a lower pK_a that leads to its stabilization. The presence of the bulky amino acids [e.g., phenylalanine (Phe), tyrosine (Tyr), arginine (Arg), and leucine (Leu)] near the cysteine (Cys) residue creates steric hindrance that leads to the blocking of *S*-nitrosylation [194]. However, at the higher concentration of NO or GSNO, enzymes can be inhibited. Mitochondrial proteins are the primary targets of *S*-nitrosylation mediated protein inhibition. Proteins like NADH dehydrogenase, aldehyde dehydrogenase, 2-oxoglutarate dehydrogenase can be inactivated via *S*-nitrosylation [195-197]. The activity of glyceraldehyde-3-phosphate dehydrogenase can also be inhibited via *S*-nitrosylation. The inhibition of the different proteins via *S*-nitrosylation is mainly associated with the conformational change of the active site [198, 199]. Researchers believe that *S*-nitrosylation is associated with a good number of cellular events ranging from bacteria to mammals [194]. A database (dbSNO 2.0 http://dbSNO.mbc.nctu.edu.tw) is designed to collect the *S*-nitrosylated protein from literature reviews [200]. Hence, the research is going on to uncover the *S*-nitrosothiol mediated response *in vivo*.

Protein tyrosine nitration (PTN) is another covalent post translational modification where nitro ($-NO_2$) group is added at the *meta* position of the phenolic ring of specific tyrosine residue/s of a protein [201]. Peroxynitrite, sodium nitroprusside, acidified sodium nitrite ($NaNO_2$) can form PTN via generation of $\bullet NO_2$ [202-204]. Professor Rafael Radi (Biochemist, Universidad de la República, Uruguay) and his co-workers have reported that PTN is a free radical process where not only $\bullet NO_2$ but also tyrosyl radical (•Tyr) is required [205]. Though it is not an enzymatic process but the process is still very selective and the formation of PTN is not dependent on the abundance of the tyrosine residue. PTN formation is mainly dependent on the local environment of the tyrosine residue and the secondary structure of the protein [201, 204]. Hence, PTN is considered as one of the most important biomarkers of nitrosative stress [206]. Addition of $\bullet NO_2$ radical at the tyrosine molecule, reduces the pKa value from 10.1 to 7.2 of the hydroxyl group present in the phenolic ring of the nitrotyrosine residue [207]. Nitrotyrosine residues are hydrophobic whereas tyrosine residues are mildly hydrophilic [201]. Tyrosine nitration generally contributes to the

generation of additional negative charge and are also able to add comparatively bulky substituent (due to the hydrophobicity) to the protein. This may lead to the alteration of local charge distribution as well as the configuration that results in inhibition [206]. Cellular mitochondrial matrix is the primary locus for PTN formation [208]. Reports suggest that Fe-S cluster proteins like aconitase can be inactivated by PTN [209]. Published reports suggest that peroxynitrite can form PTN in HDAC2 (Histone Deacetylase 2) at Tyr253, ascorbate peroxidase at Tyr5 and Tyr235, MnSOD at Tyr34, that results in inactivation [210-212]. The effect of protein tyrosine nitration on cellular signaling is not clear. It was hypothesized that tyrosine nitration might affect the cellular signaling due to alteration of the local environment. It has also been reported that PTN interferes with the protein phosphorylation which cannot be possible when the target Tyr residue is nitrated [213, 214]. However, previous report suggests that peroxynitrite induces both the nitration and phosphorylation of Tyr residue of protein (e.g. T-lymphocyte) [215]. Hence, the biochemistry of PTN and its functioning is not fully understood till now.

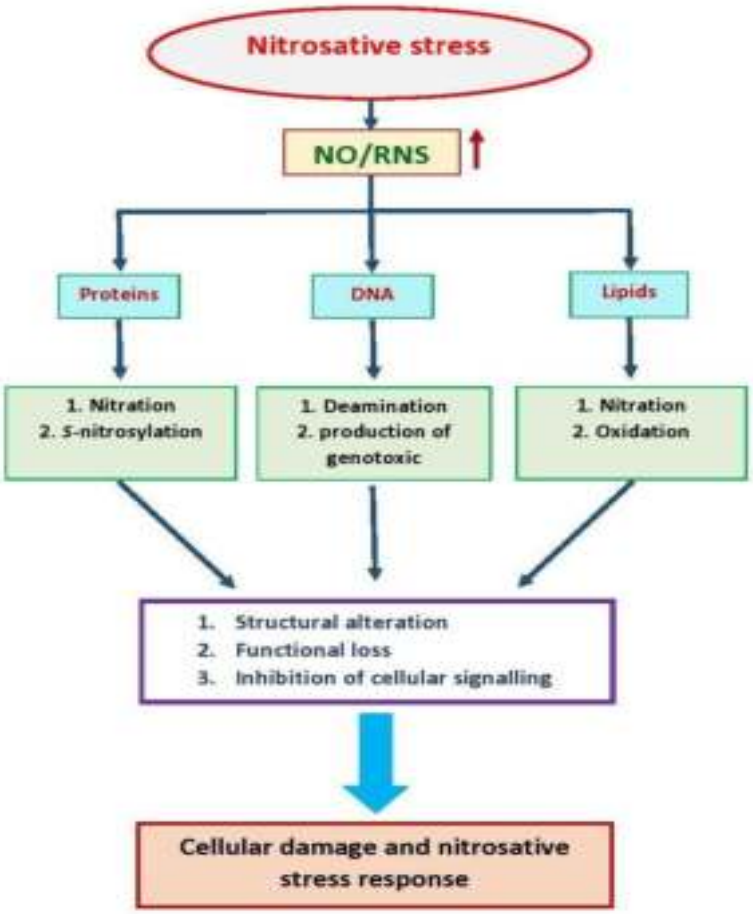

Fig. 3 Effect of nitrosative stress on protein, lipid and DNA.

6.2. Nitrosative stress and apoptosis:

At higher concentration, NO can act as a pro-apoptotic factor [109]. Intracellular higher concentration of NO can induce apoptosis in different cells like macrophage, neuroblastoma, smooth muscle cells etc. [216-219]. It has been reported that NO signaling is strongly associated with the apoptotic pathway in yeast, mammals etc. [109, 220]. It has also been mentioned that mitochondrial matrix proteins are vulnerable to nitrosative stress [201]. Higher concentration of NO can form more reactive peroxynitrite that can disrupt the mitochondrial transmembrane potential, resulting in release of cytochrome *c* due to transition in mitochondrial permeability [221-223]. The release of cytochrome *c* is the key event for the induction of apoptotic signaling pathway. This event can stimulate the downstream proteins (e.g. apaf-1, caspase-3 etc.) of the apoptotic signaling pathway [109, 224]. Caspases, a family of cysteine proteases, can induce DNase. The breakage of poly ADP-ribose polymerase (PARP), substrate of caspase-3, has been reported to increase in NO-mediated apoptosis, suggesting upregulation of caspase-3 during nitrosative stress [226]. Another important factor for apoptosis is the activity of p53, a tumor suppressor protein [224]. RNS induced DNA damage can stimulate the p53. Under nitrosative stress, induced p53 can stimulate the production of p21.

This event may lead to the blocking of cell cycle progression via inhibiting cyclin dependent kinases, an important factor for cell growth [226, 227]. In addition to it, report also suggests that activation of iNOS can induce the accumulation of p53, suggesting the role of NO as a pro-apoptotic factor [225]. NO induced p53 can also increase the expression of Bax, a pro-apoptotic protein and reduce the expression of Bcl-2, an anti-apoptotic protein [228]. The ratio of Bax to Bcl-2 is a very important factor for the induction of apoptotic pathway [109]. A recent publication by Almeida *et al.* (2020) has described the role of *S*-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in NO-mediated apoptotic signaling in yeast [220] suggest that the apoptosis rate in H_2O_2-treated yeast cells is induced with the level of *S*-nitrosylation of GAPDH. It has been reported that inhibition of GAPDH via *S*-nitrosylation leads to induction of DNase and apoptotic pathway. In addition to it, NO can stimulate cGMP pathway via binding with the heme-containing protein guanylyl cyclase, resulting in the production of cGMP, a secondary messenger that leads to the apoptosis [229, 230]. The reduced activity of protein kinase C (PKC), decrease in

extracellular signal-regulated kinases (ERK) phosphorylation, are also associated with NO-induced apoptosis [231, 232]. A schematic diagram of the role of NO on apoptosis is proposed in **Fig. 4**.

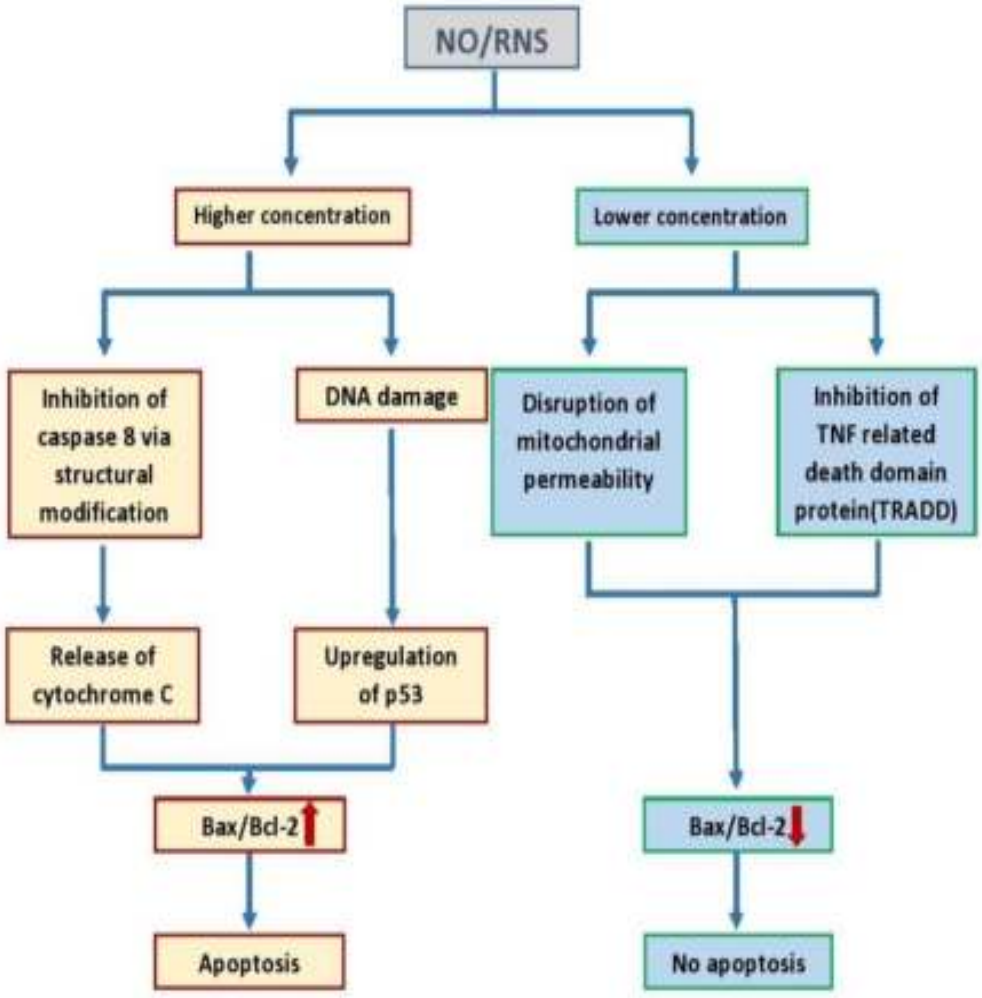

Fig. 4 Role of nitric oxide on apoptosis.

6.3. Nitrosative stress and aging:

"Aging" is a broad spectrum term, associated with chronological, replicative parameters [233, 234]. The study of aging is paradoxical, very complicated and associated with different factors like dietary restriction (DR), stress, genetic organization, environmental influences etc. [235]. To study the genetics and biochemistry of aging, *S. cerevisiae* has been the choice of organism since more than 50 years [234]. The aging of yeast, can be expressed by the two ways: replicative life span (RLS) and chronological life span (CLS). Replicative life span is defined as the number of daughter cells, generated from the mother cell before senescence, an

irreversible arrest of cell cycle. Whereas chronological life span is the length of survival time of a yeast cell in a non-dividing state [236]. These features give the opportunity to study the mechanism of aging of both the proliferating and non-proliferating cells using comparatively simple unicellular organism yeast [237]. Hence, it has become the choice of organism to characterize the underlying mechanism of aging [238].

It is well established that mitochondrial dysfunction and generation of ROS and RNS, are associated with aging [234, 239]. Though *S. cerevisiae* is petite positive (ability to survive without the mitochondrial DNA [240]) but still damaging of mtDNA, leads to aging [241]. Redox stress can lead to the accumulation of reactive species *in vivo* [236]. These reactive species can interfere with the macromolecules of the cell, resulting in the alteration of the cellular functions like metabolism, biogenesis etc. [242, 243]. The alteration in cellular metabolism (energy production, amino acid synthesis) as well as CLS is tightly associated with the TOR/Sch9 signaling and it is well established in dietary restriction mediated aging [244]. TOR/Sch9 signaling is an important factor for cell cycle progression. It also stimulates the translational process, but it represses the general stress response by restricting the localization of transcription factors Msn2 and Msn4 (important factor for redox stress response) in the cytoplasm [234, 245, 246]. Under the stress condition, the activity of TOR is reduced and cellular stress response is stimulated. It has been reported that TOR/Sch9 signaling is inhibited during redox stress due to the alteration in the localization of Sch9, increasing the CLS in yeast [247]. In addition to it, mitochondrial dysfunction also triggers retrograde signaling (a communication between mitochondria and the nucleus along with other cellular compartments [248]) which is also controlled by the TOR activity via regulating the expression of retrograde gene [249, 250]. Due to inhibition of the activity of TOR, the retrograde genes are expressed that may contribute to the extension of the chronological life span. Autophagy (recycling of the cellular macromolecules during stress condition [251]) also contributes to the extension of the CLS. It has been reported that TOR activity negatively regulates autophagy. Hence, the reduced activity of TOR increases autophagy which in turn leads to the expansion of CLS [252, 253]. The reduction in the activity of protein kinase A (PKA) is also associated with TOR/Sch9 signaling that contributes to the extension of CLS [245]. A schematic diagram of the role of NO on aging is proposed in **Fig. 5**.

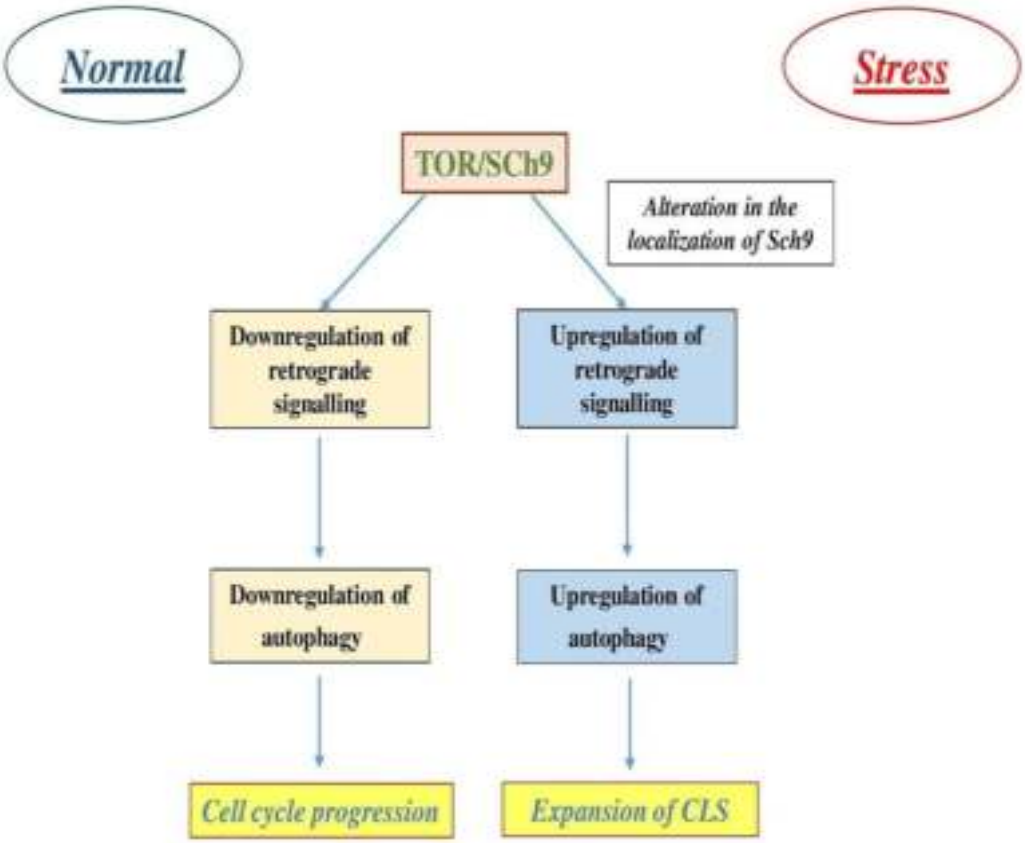

Fig. 5 Role of nitric oxide on aging.

Different studies have revealed the connection between the dysregulation of the biogenesis of ribosomes and aging. Enhanced ribosome biogenesis is one of the major characteristics feature of aging [254]. Under the stress condition, high rate of the ribosome biogenesis can promote aging through the excess translation. This event leads to the disruption of the global proteostasis, the process to maintain and regulate the proteins and their quality [255]. Loss of proteostasis is a hallmark of aging [256]. In addition to it, the accumulation of ribosomal DNA (rDNA) can also cause aging. The high rate of rDNA synthesis may lead to DNA damage that results in genetic instability, another vital cause of aging [257]. In addition to it, the inactivation of Fe-S cluster protein or the biogenesis of Fe-S may also contribute to the genetic instability [236, 258]. NO-signaling in aging is also a very complicated as well as self-contradictory subject. NO-signaling is very important for apoptosis but it can also act as an anti-aging component. Lewinska *et al.* showed that the level of NO was reduced rather than increased in dietary restriction mediated stress response. It indicates that NO-mediated pathways are involved in aging [259]. The elevated level of SOD is also associated with the extension of CLS, suggesting the hypothesis [260]. In mammalians, different

neurodegenerative diseases like alzheimer's disease (AD), parkinson's disease (PD), huntington's disease (HD), amyotrophic lateral sclerosis (ALS) etc. are related with aging [261-263].

6.3.1. Alzheimer's disease:

Alzheimer's disease (AD) is one of the most common neurodegenerative disease that is associated with nitrosative stress [264-266]. It has been observed that protein tyrosine nitration of tau protein is tightly associated with AD [267]. Tau is an axon-enriched microtubule associated brain protein, encoded by chromosome 17 in human [268, 269]. Protein tyrosine nitration in tau protein is very specific. It has been found that tyrosine residues are at the position of 18, 29, 197, 310, and 394, (using longest isoform of tau as the reference) prone to be nitrated in the presence of RNS like peroxynitrite. The sensitivity of amino terminal tyrosine resiudes (Y18, Y29) of tau proteins are more for the PTN as compared to other residues. PTN formation in Y310 is the rarest due to its localization at the hydrophobic microtubules binding repeats [270]. Nitration of tyrosine residues of tau, induces intracellular neurofibrillary tangle formation (due to polymerization) that may lead to the dysfunction of the protein as well as damage and destruction of synapses [268,271]. Another important biomarker of nitrosative stress mediated AD is the formation of extracellular senile amyloid plaques [264, 268, 272]. This event may lead to the accumulation of iron and copper inside the brain, resulting in the loss of metal homeostasis [273, 274]. In addition to it, loss of mitochondrial metabolism or dysfunction of mitochondria is also a characteristic feature of AD. Damaging of DNA and RNA, proteolysis are also associated with the nitrosative stress mediated AD [264, 275]. Overall, redox imbalance, permanent alteration cell signaling, gene expressions are also associated with AD [276, 277].

6.3.2. Parkinson's disease:

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder after than AD. One of the characteristic feature of PD is the movement impairment due to the selective degeneration of dopaminergic neurons [278]. Though it is considered as a sporadic disease but evidences reveal that it can be family-linked [279]. This aging-related disease is tightly associated with nitrosative and oxidative stress [280]. Reports suggest that degeneration of dopaminergic neuron is primarily caused by the reactive oxygen species (ROS) and RNS mediated lipid peroxidation, protein modification etc

in the brain tissue [281]. Astrocytes, star-shaped glial cells in CNS, play major role in metabolism and detoxification of reactive species. One of the most important function of astrocytes is maintaining the redox homeostasis in brain [282]. Astrocytic generation of RNS involves in the dysfunction of neuronal mitochondria [283]. Characteristic features of PD includes presence of eosinophilic cytoplasmic inclusions of fibrillar, misfolded proteins known as the lewy bodies containing ubiquitinated α-synuclein, parkin, syuphilin, neurofilaments and synaptic vesicle proteins [284].

α-synuclein is very prone to tyrosine nitration and considered as one of the most important cause of protein aggregation. It has been revealed that four tyrosine residues (Y39, Y125, Y133, Y136) are susceptible for nitration [285]. α-synuclein is very sensitive to the nitrating agents like peroxynitrite. Even a very low concentration of peroxynitrite is sufficient to cause PTN in α-synuclein [286]. Nitrated α-synuclein becomes resistance to proteolysis but prone for the aggregation that leads to the reduction in both lipid binding tendency and solubility in cell, suggesting the generation of toxicity due to misfolding of this protein [287]. The nitrated α-synuclein shows more immunogenicity that may leads to the stimulation of neuroinflammation by cytokines, NF-κB and others intracellular components [278, 288, 289]. Another important protein of PD pathogenicity is parkin, an ubiquitin E3 ligase encoded by the *PARK2* gene. This protein is involved in the maintaining of mitochondrial integrity and regulates mitophagic degradation [290]. Report suggests that overproduction of NO may interfere with the structural modification as well as activity of the protein via *S*-nitrosylation. The cysteine residues of the catalytic RING domains of parkin are prone to *S*-nitrosylation. *S*-nitrosylation induces the inactivation of parkin protein that leads to the neurotoxicity [291, 292]. In addition, loss of activity of DJ-1 and PINK, have been also identified in the pathogenesis of PD. Loss of activity of these proteins under nitrosative stress which in turn causes apoptosis and neurotoxicity [278, 293].

6.3.3. Huntington's disease:

Huntington's disease (HD) is a fatal genetic neurodegenerative disorder, associated with the progressive loss of memory, mood, behavior and cognition [294]. This autosomal neurological disorder is caused by the unusual expansion of CAG repeats (>36 repeats) within IT15 gene located at chromosome 4, that code polyglutamine (polyQ) tract in N-terminal site of the huntingtin protein, a ubiquitous protein, [295-

297]. It has been established that imbalance of NO contributes to the development of HD. There are two major pathways that can link the imbalance of NO and development of HD i.e. htt/HAP-1/calmodulin/NOS link and the CREB binding protein/htt/NOS link [295]. Dysfunction of NO may contribute to the Progressive striatal damage and abnormal cerebral blood flow (CBF), important markers of HD [298, 299]. The dysfunction of nNOS may lead to the alteration in NO production, resulting in neurodegeneration [299]. RNS like peroxynitrite mediated damage has been characterized in HD. Peroxynitrite may lead to DNA damage, lipid peroxidation, induction of iNOS, depletion of NAD^+ flux, that results in neurotoxicity [300]. This neurotoxicity inversely leads to the alteration of mitochondrial membrane permeability via pore formation, resulting in the release of cytochrome *c*, a proapoptotic factor [301]. Loss of activity of respiratory chain via inhibiting complex II, III, IV, has been well characterized in HD patients [302-304]. Report suggests that reduction in NO content in platelets, interferes with the eNOS phosphorylation at ser^{1177} during the advanced stages of HD [305].

6.3.4. Amyotrophic lateral sclerosis (ALS):

One of the most dangerous neurological disorder is Amyotrophic lateral sclerosis (ALS), most patient die less than 5 years after appearing the symptoms [306]. This neurodegenerative disease (also known as Charcot's disease or Lou Gehrig's disease) is marked by the selective loss of upper motor neurons of the motor cortex, and lower motor neurons of motor neurons of spinal cord and brain stem [307]. One of the major cause of ALS is mitochondrial dysfunction that leads to the death of the neurons [308, 309]. Mitochondrial dysfunction is associated with the lipid peroxidation, DNA damage and misfolding of important proteins [307]. Loss of activity of Cu, Zn superoxide dismutase (SOD1) via reactive species mediated misfolding was reported in the patient of ALS [310, 311]. SOD1 participates in the regulation of energy metabolism, cellular respiration, stress control etc. [312-314], thus the loss of activity of SOD1 leads to the suppression of the above processes along with the generation of superoxide or ROS, causative agents of mitochondrial dysfunction [315-318]. Elevated level of ROS has been reported in CNS of the patient with ALS [319]. Peroxynitrite, an important RNS, is also generated due to higher ROS production. Peroxynitrite mediated protein modification via formation of 3-nitrotyrosine in CNS is considered as the one of the important biomarker of ALS [320-324].

RNS-mediated lipid modification has also been found in the CNS of the patient with ALS [325]. One of the most abundant product of fatty acid peroxidation by RNS is 4-hydroxy-2-nonenal (HNE), act as 'second cytotoxic messenger', has been well characterized in the ALS patients [326]. It is a very toxic product that may stimulate cellular damage and apoptosis [327, 328]. It can move across the membrane and interacts with the different cellular components, leading to the cytotoxicity, one of the major reason for loss of activity of the motor neurons [306, 327]. Overall, due to the activity of reactive species, the structure of the membrane is affected that leads to the alteration in fluidity, permeability, transport and metabolic processes [306]. These alterations severy affect the neuronal function that causes ALS.

6.4. Nitrosative stress and metabolism:

It has been reported that RNS or NO can affect the cellular metabolic processes like tricarboxylic acid (TCA) cycle, electron transport chain, fatty acid biosynthesis, β-oxidation of fatty acid etc. via interfering with the enzymes of the different metabolic pathways [329, 330]. Activities of aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase have been found to be decreased under nitrosative stress due to the *S*-nitrosylation, resulting in the inhibition of TCA cycle [331-336]. RNS or NO mediated inactivation of aconitase has been characterized in chronological aging [337]. The nitration of the tyrosine residues of aconitase under nitrosative stress has also been reported [203]. Activity of glutamate dehydrogenase, catalyzes the conversion from glutamate to α-ketoglutarate has also been found to be nitrated in mice liver under nitrosative stress, that leads to the less generation of α-ketoglutarate for TCA cycle [338, 339]. Report also suggests that pyruvate dehydrogenase can be inactivated due to *S*-nitrosylation. Inactivation of pyruvate dehydrogenase directly affects the TCA cycle. Reduction in the TCA cycle via post translational modification under nitrosative stress contributes to the development of neurodegenerative disorders [301, 340-343]. In addition to it, enzymes of glycolytic pathway e.g. hexokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, enolase etc. are also very prone to *S*-nitrosylation [301, 329, 330]. Rate limiting enzyme of fatty acid biosynthesis i.e. very long-chain acyl-CoA dehydrogenase (VLCAD) has also been found to be *S*-nitrosylated in the presence of NO that leads to the generation of more acetyl-CoA, showing beneficial effect of *S*-nitrosylation [329]. In addition to it, metabolic reprogramming in *Pseudomonas fluorescens* under nitrosative stress has also been reported by Auger *et*

al. They hypothesized that metabolic reprogramming can act as bacterial defense strategy by coupling with antioxidant system to overcome the stress [344].

7. Cellular defense strategies against nitrosative stress:

Cells always try to overcome the hostile situation mediated by the different stress agents. These defense strategies include different enzymatic and non-enzymatic pathways. Though the defense strategies are not well characterized but still some of the components are found that play a major role to overcome the nefarious activity of RNS or NO inside the cell.

7.1. Non-enzymatic defense:

To combat the RNS or NO mediated stress, a good number of non-enzymatic components have been identified, among them γ-L-glutamyl-L-cysteinyl-glycine (GSH) or reduced glutathione has been found to be a potent candidate to protect the cell from RNS-mediated damage [345]. GSH is a very important component for maintaining the redox homeostasis *in vivo* that contributes to the cellular proliferation, differentiation and apoptosis. It can directly bind with NO to form GSNO, suggesting the role of GSH as NO scavenger [347]. It was reported that NO can stimulate DNA damage and protein modifications when the intracellular GSH level declines, indicating the protective role of GSH under nitrosative stress [348]. The alteration of mitochondrial GSH level is also associated with Ca^{2+} ion distribution, pyridine nucleotide oxidation status, mtDNA damage, and induction of membrane permeability transition [349-355]. *S*-glutathionylation (reversible binding of GSH with protein) helps to mask the proteins from irreversible oxidative damage. *S*-glutathionylation of proteins also contributes to the alteration of signal transduction that is required to respond against the reactive species mediated stress [356, 345]. Reports suggest that disruption of glutathione biosynthesis also contributes to the glutathione auxotrophy in *S. cerevisiae* and *S. pombe* [357, 358]. In addition to it, NADPH and nicotinamide adenine dinucleotide hydrogen (NADH) are also two important factors that provide the non-enzymatic defense to the cell under nitrosative and oxidative stress. NADH is an important cofactor for the antioxidant [359, 360]. Vitamin C and vitamin E also provide protection against nitrosative stress. These two vitamins together contribute to increasing the intracellular flux of GSH [361]. Vitamin E also breaks the chain of lipid

peroxidation and vitamin C also gives protection against the ROS like hydrogen peroxides [362, 363]. Lipoic acid (LA), another important non-enzymatic compound which gets digested, absorbed and converted to dihydro lipoic acid (DHLA). DHLA can neutralize free radicals and contribute to the generation of vitamin C. LA also helps to stabilize lysosome under nitrosative and oxidative stress via termination of free radicals and chelating of transition metals [364, 365]. In addition to it, melatonin, β-carotenes, and flavonoids are also found to show protective roles against the free radical mediated damage [366-368]. Melatonin also induces different antioxidant enzymes like superoxide dismutase, glutathione reductase, catalase etc. [366]. It also inhibits pro-oxidant enzymes like NOS, xanthine oxidase and lipoxygenase etc. It has also been reported that melatonin helps to stabilize or protect the cellular membrane from oxidative damage and increase the rate of electron transport chain (ETC) without increasing generation of reactive species [366]. β-carotenes and flavoniods play an important role to inhibit lipid peroxidation via scavenging peroxyl radicals [367, 368].

7.2. Enzymatic defense:

To overcome the reactive species mediated stress, activities of some enzymes have been found to be upregulated and detoxify the effect of NO or RNS, known as 'stress response enzyme' e.g. flavohemoglobin, Cu/Zn superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase (GR), catalase, GSNO reductase etc. [361, 369].

Flavohemoglobin (Yhb1) or nitric oxide dioxygenase (NOD) can detoxify the effect of NO by converting NO to NO_3^-, using NADPH as the reducing power [370]. Flavohemoglobin or NOD like activity is conserved from prokaryotes to higher eukaryotes [75]. In higher eukaryotes, hemoglobin shows NOD-like activity [371]. The amino acid sequence of flavohemoprotein (Hmp1) in *E. coli* shows high homological similarities with NOD of yeast [373]. In presence of NO, the overexpression of *YHB1* gene has been reported in *S. cerevisiae* and *C. albicans* [117, 372, 373]. Reduction or inhibition in the activity of Yhb1 increases the NO-mediated growth inhibition in the cells of *S. cerevisiae* and *C. albicans* [372, 374, 375]. It has also been observed that the loss of activity of Yhb1increases the intracellular RSNO production that may result in the generation of nitrosative stress condition [376]. Under the stress condition, cytochrome *c* oxidase (C*c*O) activity in mitochondrial respiratory chain complex

(MRC) can be downregulated that leads to the generation of superoxides and then it is subsequently converted to peroxynitrite, a potent nitrating agent [377, 73]. Yhb1 also helps the pathogenic yeast to resist from the macrophage-induced NO, showing its role as a virulence factor [373, 378]. The anti-nitrosative or anti-oxidative role of Yhb1 has been determined by its cytosolic and mitochondrial localization. In absence of superoxide Yhb1 is mainly present in cytosol but in the hypoxic condition, Yhb1 is mainly localized in the mitochondrial matrix, suggesting the protective role of Yhb1 under nitrosative and oxidative stress [379].

Another important anti-nitrosative enzyme is GSNO reductase (GSNOR) that breaks down toxic GSNO into oxidized glutathione (GSSG) and ammonia. GSNOR is very similar to GSH-dependent formaldehyde dehydrogenase (GS-FDH) [380], member of the formyldehyde dehydrogenase III that contains NADH binding site like alcohol dehdrogenase [381, 382]. The GSNOR activity has been reported in eukaryotes like *S. pombe*, *S. cerevisiae*, plants, and mammalians and most of bacteria [380, 381, 384, 385]. GSNOR or Fdh3 is considered as the marker of redox switch i.e. sensitive to the alteration of redox state of cell [386]. Earlier reports suggest that reduction in the activity of GSNOR can enhance nitrosative stress mediated damage [382, 387, 388]. It has also been reported that the loss of activity of GSNOR in *C. albicans* hampers the nitrosative stress response and virulent property. This suggests the role of GSNOR as the virulence factor that provides the protection from host immune response mediated by nitrosants like NO or its derivatives [382].

Catalase was the first characterized antioxidant enzyme that actively participates to combat the oxidative and nitrosative stress. Generally, the main function of catalase is the conversion of hydrogen peroxide to water and oxygen [389]. But the activity of catalase has been found to be upregulated to inhibit peroxynitrite mediated oxidation and nitration and also peroxynitrite catabolism in *S. cerevisiae* [390]. Report also suggests that catalase activity can be upregulated in *S. cerevisiae* in GSNO mediated nitrosative stress [391].

As mentioned earlier, GSH can act as the stress response component. Increase in the intracellular concentration of GSH is dependent on the activity of glutathione reductase (GR) that catalyzes the conversion from oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH as the reducing equivalent [392]. Activity of

GR is required for mitochondrial activity. It has been reported that GR can reduce the oxidation and nitration of Fe-S cluster proteins of mitochondria [393]. In addition to it, thioredoxin system also acts as a disulfide reductase. It has been reported that thioredoxin system helps to maintain the intracellular GSH concentration in GR mutant *E. coli* [394, 395].

Superoxide dismutase also indirectly helps to overcome the nitrosative stress. SOD reduces superoxide to hydrogen peroxide and oxygen atom. In the presence of excessive NO, SOD can reduce the concentration of intracellular superoxide which in turn inhibits or reduces the production of toxic peroxynitrite [396]. Peroxynitrite mediated lipid oxidation/peroxidation can also be reduced by the activity of glutathione peroxidase (GPx). Gpx uses GSH as the reducing power [397]. It is also considered as a H_2O_2-stress response enzyme [361].

8. Yeast as a model organism to study nitrosative stress:

Budding yeast (mainly *S. cerevisiae*) is a robust model system for basic biological research [398]. This eukaryotic organism carries 6692 genes in 12 megabase pairs of DNA that is distributed in 16 linear chromosomes present in nucleus. The genomic stability of the organism makes it one of the best system to study gene manipulation. Its full genome was sequenced in 1996 as the first eukaryote [399]. The integrity of the genetic and molecular toolbox of the yeast, has placed it as the primary system to study and develop the different high-throughput technologies involved in transcriptomics, proteomics, metabolomics and so on [400-405]. Like the budding yeast, fission yeast (*S. pombe*) has also become a powerful system to study the cell growth and division [406-409]. The whole genome sequencing of *S. pombe* was also reported in 2002 [410]. *CDC2* gene, an important factor for cell division, was first discovered in *S. pombe* [409]. Different cell cycle check points have also been characterized by using *S. pombe* and *S. cerevisiae*. Spindle check point genes, DNA damage checkpoint genes *etc.* have first been characterized in *S. cerevisiae* [411, 412]. In addition to it, the growth rate of yeast is very fast (~90 min/generation) as compared to animal model and it is comparatively easy to handle.

Another important feature to use yeast as the model organism to study the genetic and biochemical characterization, is the similarity with the metabolic and

cellular pathways that occur in higher eukaryotes like humans. Thus, yeast system has become a good model to study cancer and neurodegenerative diseases like Parkinson's disease, Alzheimer's disease and Huntington's disease [413-415]. Different gene mutations related to human cancer have been characterized by using yeast as the model system [416]. Another important factor, that is associated with different physiological disorders, is nitrosative stress. Reports suggest that formation of 3-nitrotyrosine and *S*-nitrosylation are two major markers of cardiovascular disease cardiovascular disease, obesity, diabetes mellitus and so on [417, 418]. To characterize the physiochemical properties and biochemistry of 3-nitrotyrosine, *S*-nitrosylation under nitrosative stress, one of the best system is yeast.

In the last decade a good number of studies have been done to characterize the nitrosative stress response using yeast (*e.g. S. cerevisiae*, *S. pombe*, *C. albicans etc.*) as the model organism. Several critical issues have been addressed in this last decade regarding nitrosative stress response that encourages researchers to explore more mechanistic pathways of nitrosative stress response. In response to nitrosative stress transcriptional regulations mediated by different transcriptional factors (TFs) in yeast have been well characterized. Yap1, an important transcriptional factor has been reported to be upregulated under nitrosative stress. It is one of the most important factor for the higher activity of SOD and catalase. Deletion of the *YAP1* gene fails to activate SOD and catalase. In addition to it, the subcellular localization of the Yap1 protein has been found in the nucleus of *S. cerevisiae* under nitrosative stress, suggesting its role under nitrosative stress [419]. On the other hand, Yap7 plays the exact opposite role regarding nitrosative stress response. Reports suggest that Yap7 represses Yhb1, an important anti-nitrosative enzyme. Hence, the deletion of Yap7 contributes to the enhancement of NO resistance in yeast. Binding of Yap7 to the *YHB1* promoter leads to the recruitment of Tup1 repressor, resulting in the downregulation of *YHB1* [420, 421]. Cwt1p, another negative regulator of Yhb1 has been found in *C. albicans* [422]. Nitrosative stress tolerance is based on different strategies possessed by different organisms. Anam *et al.* identified a nitrosative stress tolerance gene *RIB1* which encodes GTP cyclohydrolase II that catalyzes the first step in riboflavin biosynthesis. The byproduct of the reaction of GTP cyclohydrolase II, can scavenge RNS. Thus, the author claimed that riboflavin indirectly helps to overcome nitrosative stress [423]. Kar *et al.* discovered that transcription factor Atf1 was localized in the nucleus under

nitrosative stress in *S. pombe*. Though the mechanism is not clear but authors hypothesized that Sty1-Atf1 mitogen-activated protein kinase (MAPK) pathway may be required for the nitrosative stress response [424]. Till now different proteins have been identified that are sensitive under nitrosative stress. Redox homeostasis along with the antioxidant system, has also been characterized in the presence of different RNS. Using critical molecular biology tools, different transcription factors have been recognized as the nitrosative stress response element but there is a lacony regarding metabolic strategy under nitrosative stress.

References:

1. Lancaster JR Jr. (2015) Nitric oxide: a brief overview of chemical and physical properties relevant to therapeutic applications. Future Sci. OA1:FSO59.
2. Lancaster JR Jr. (1992) Nitric Oxide in Cells. Am. Sci. 80:248–259.
3. Stuehr, D. J., &Haque, M. M. (2019). Nitric oxide synthase enzymology in the 20 years after the Nobel Prize. British journal of pharmacology, 176:177–188.
4. Nishimura A, Kawahara N, Takagi H. (2013) The flavoprotein Tah18-dependent NO synthesis confers high-temperature stress tolerance on yeast cells. BiochemBiophys Res Commun. 430:137-43.
5. Li H, Poulos TL. (2005) Structure-function studies on nitric oxide synthases. J InorgBiochem. 99:293-305.
6. Bush PA, Gonzalez NE, Griscavage JM, Ignarro LJ. (1992) Nitric oxide synthase from cerebellum catalyzes the formation of equimolar quantities of nitric oxide and citrulline from L-arginine. BiochemBiophys Res Commun. 185:960-6.
7. Förstermann U, Sessa WC. (2012) Nitric oxide synthases: regulation and function. Eur Heart J. 33:829-37.
8. Crane BR, Arvai AS, Ghosh DK, Wu C, Getzoff ED, Stuehr DJ, Tainer JA. (1998) Structure of nitric oxide synthase oxygenase dimer with pterin and substrate. Science. 279:2121-6.
9. Alderton WK, Cooper CE, Knowles RG. (2001) Nitric oxide synthases: structure, function and inhibition. Biochem J. 357:593-615.
10. Klatt P, Pfeiffer S, List BM, Lehner D, Glatter O, Bächinger HP, Werner ER, Schmidt K, Mayer B. (1996) Characterization of heme-deficient neuronal nitric-oxide synthase reveals a role for heme in subunit dimerization and binding of the amino acid substrate and tetrahydrobiopterin. J Biol Chem. 271:7336-42.
11. Hemmens B, Goessler W, Schmidt K, Mayer B. (2000) Role of bound zinc in dimer stabilization but not enzyme activity of neuronal nitric-oxide synthase. J Biol Chem. 275:35786-91.

12. Cho HJ, Xie QW, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Nathan C. (1992) Calmodulin is a subunit of nitric oxide synthase from macrophages. J Exp Med. 176:599-604.

13. Hemmens B, Mayer B. (1998) Enzymology of nitric oxide synthases. Methods Mol Biol.100:1-32.

14. Stuehr D, Pou S, Rosen GM. (2001) Oxygen reduction by nitric-oxide synthases. J Biol Chem. 276:14533-6.

15. List BM, Klösch B, Völker C, Gorren AC, Sessa WC, Werner ER, Kukovetz WR, Schmidt K, Mayer B. (1997) Characterization of bovine endothelial nitric oxide synthase as a homodimer with down-regulated uncoupled NADPH oxidase activity: tetrahydrobiopterin binding kinetics and role of haem in dimerization. Biochem J. 323:159-65.

16. Nishimura JS, Martasek P, McMillan K, Salerno J, Liu Q, Gross SS, Masters BS. (1995) Modular structure of neuronal nitric oxide synthase: localization of the arginine binding site and modulation by pterin. BiochemBiophys Res Commun. 210:288-94.

17. Noble MA, Munro AW, Rivers SL, Robledo L, Daff SN, Yellowlees LJ, Shimizu T, Sagami I, Guillemette JG, Chapman SK. (1999) Potentiometric analysis of the flavin cofactors of neuronal nitric oxide synthase. Biochemistry. 38:16413-8.

18. Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H. (1994) Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension. 23:1121-31.

19. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. (1995) Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 377:239-42.

20. Kanwar JR, Kanwar RK, Burrow H, Baratchi S. (2009) Recent advances on the roles of NO in cancer and chronic inflammatory disorders. Curr Med Chem.16:2373-94.

21. Jiang X, Mu D, Manabat C, Koshy AA, Christen S, Täuber MG, Vexler ZS, Ferriero DM. (2004) Differential vulnerability of immature murine neurons to oxygen-glucose deprivation. Exp Neurol. 190:224-32.

22. Chen J, Tu Y, Moon C, Matarazzo V, Palmer AM, Ronnett GV. (2004) The localization of neuronal nitric oxide synthase may influence its role in neuronal precursor proliferation and synaptic maintenance. Dev Biol. 269:165-82.

23. Matsuda H, Iyanagi T. (1999) Calmodulin activates intramolecular electron transfer between the two flavins of neuronal nitric oxide synthase flavin domain. BiochimBiophysActa. 1473:345-55.

24. Roman LJ, Masters BS. (2006) Electron transfer by neuronal nitric-oxide synthase is regulated by concerted interaction of calmodulin and two intrinsic regulatory elements. J Biol Chem. 281:23111-8.

25. Guan ZW, Iyanagi T. (2003) Electron transfer is activated by calmodulin in the flavin domain of human neuronal nitric oxide synthase. Arch BiochemBiophys. 412:65-76.

26. Nathan C, Xie QW. (1994) Nitric oxide synthases: roles, tolls, and controls. Cell. 78:915-8.

27. Cui H, Hayashi A, Sun HS, Belmares MP, Cobey C, Phan T, Schweizer J, Salter MW, Wang YT, Tasker RA, Garman D, Rabinowitz J, Lu PS, Tymianski M. (2007) PDZ protein interactions underlying NMDA receptor-mediated excitotoxicity and neuroprotection by PSD-95 inhibitors. J Neurosci. 27:9901-15.

28. Zhou L, Zhu DY. (2009) Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. Nitric Oxide. 20:223-30.

29. Chanrion B, Mannoury la Cour C, Bertaso F, Lerner-Natoli M, Freissmuth M, Millan MJ, Bockaert J, Marin P. (2007) Physical interaction between the serotonin transporter and neuronal nitric oxide synthase underlies reciprocal modulation of their activity. Proc Natl AcadSci U S A. 104:8119-24.

30. Saitoh F, Tian QB, Okano A, Sakagami H, Kondo H, Suzuki T. NIDD, (2004) a novel DHHC-containing protein, targets neuronal nitric-oxide synthase

(nNOS) to the synaptic membrane through a PDZ-dependent interaction and regulates nNOS activity. J Biol Chem. 279:29461-8.

31. Izumi Y, Zorumski CF. (1993) Nitric oxide and long-term synaptic depression in the rat hippocampus. Neuroreport. 4:1131-4.

32. Toda N, Ayajiki K, Okamura T. (2009) Control of systemic and pulmonary blood pressure by nitric oxide formed through neuronal nitric oxide synthase. J Hypertens. 27:1929-40.

33. Förstermann U. (2000) Regulation of nitric oxide synthase expression and activity. In: Mayer B, editor. Handbook of Experimental Pharmacology—Nitric Oxide. Berlin: Springer; pp. 71–91.

34. Moncada S, Higgs EA. (2006) Nitric oxide and the vascular endothelium. HandbExp Pharmacol.176:213-54.

35. Ginnan R, Guikema BJ, Halligan KE, Singer HA, Jourd'heuil D. (2008) Regulation of smooth muscle by inducible nitric oxide synthase and NADPH oxidase in vascular proliferative diseases. Free RadicBiol Med. 44:1232-45.

36. Soskić SS, Dobutović BD, Sudar EM, Obradović MM, Nikolić DM, Djordjevic JD, Radak DJ, Mikhailidis DP, Isenović ER. (2011) Regulation of Inducible Nitric Oxide Synthase (iNOS) and its Potential Role in Insulin Resistance, Diabetes and Heart Failure. Open Cardiovasc Med J. 5:153-63.

37. Green SJ, Mellouk S, Hoffman SL, Meltzer MS, Nacy CA. (1990) Cellular mechanisms of nonspecific immunity to intracellular infection: cytokine-induced synthesis of toxic nitrogen oxides from L-arginine by macrophages and hepatocytes. Immunol Lett. 25:15-9.

38. Brown GC, Neher JJ. (2010) Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. MolNeurobiol. 41:242-7.

39. Wong JM, Billiar TR. (1995) Regulation and function of inducible nitric oxide synthase during sepsis and acute inflammation. AdvPharmacol. 34:155-70.

40. Lange M, Enkhbaatar P, Nakano Y, Traber DL. (2009) Role of nitric oxide in shock: the large animal perspective. Front Biosci (Landmark Ed). 14:1979-89.

41. Lisanti MP, Scherer PE, Tang Z, Sargiacomo M. (1994) Caveolae, caveolin and caveolin-rich membrane domains: a signalling hypothesis. Trends Cell Biol. 4:231-5.

42. Hemmens B, Mayer B. (1998) Enzymology of nitric oxide synthases. Methods Mol Biol.100:1-32.

43. Fulton D, Babbitt R, Zoellner S, Fontana J, Acevedo L, McCabe TJ, Iwakiri Y, Sessa WC. (2004) Targeting of endothelial nitric-oxide synthase to the cytoplasmic face of the Golgi complex or plasma membrane regulates Akt-versus calcium-dependent mechanisms for nitric oxide release. J Biol Chem. 279:30349-57.

44. García-Cardeña G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC. (1998) Dynamic activation of endothelial nitric oxide synthase by Hsp90. Nature. 392:821-4.

45. Zimmermann K, Opitz N, Dedio J, Renne C, Muller-Esterl W, Oess S. (2002) NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. Proc Natl AcadSci U S A. 99:17167-72.

46. Fleming I, Busse R. (2003) Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am J PhysiolRegulIntegr Comp Physiol. 284:R1-12.

47. McCabe TJ, Fulton D, Roman LJ, Sessa WC. (2000) Enhanced electron flux and reduced calmodulin dissociation may explain "calcium-independent" eNOS activation by phosphorylation. J Biol Chem. 275:6123-8.

48. Smart EJ, Graf GA, McNiven MA, Sessa WC, Engelman JA, Scherer PE, Okamoto T, Lisanti MP. Caveolins, (1999) liquid-ordered domains, and signal transduction. Mol Cell Biol. 19:7289-304.

49. Ignarro LJ, Harbison RG, Wood KS, Kadowitz PJ. (1986) Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: stimulation by acetylcholine, bradykinin and arachidonic acid. J Pharmacol Exp Ther. 237:893-900.

50. Arndt H, Smith CW, Granger DN. (1993) Leukocyte-endothelial cell adhesion in spontaneously hypertensive and normotensive rats. Hypertension. 21:667-73.

51. Dimmeler S, Zeiher AM. (1999) Nitric oxide-an endothelial cell survival factor. Cell Death Differ. 6:964-8.

52. Radomski MW, Martin JF, and Moncada S. (1991) Synthesis of nitric oxide by the haemocytes of the american horseshoe crab (*Limulus polyphemus*) Philos. Trans. R. Soc. Lond. (Biol) 334:129-133.

53. Jacklet JW. (1995) Nitric oxide is used as an orthogradecotransmitter at identified histaminergic synapses. J. Neurophysiol. 74:891-895.

54. Elofsson R, Carlberg M, Moroz L, Nezlin L, Sakharov D. (1993) Is nitric oxide (NO) produced by invertebrate neurones? Neuroreport. 4:279-282.

55. Moroz LL, and Gillette R. (1996) NADPH diaphorase localization in the CNS and peripheral tissues of the predatory sea-slug *Pleurobranchaeacalifornica*. J. Comp. Neurol. 367:607-622.

56. González-Domenech CM, Muñoz-Chápuli R. (2010) Molecular evolution of nitric oxide synthases in metazoans. Comp BiochemPhysiol Part D Genomics Proteomics. 5:295-301.

57. Ascenzi P, Gradoni L. (2002) Nitric oxide limits parasite development in vectors and in invertebrate intermediate hosts. IUBMB Life. 53:121-3.

58. Luckhart S, Li K. (2001) Transcriptional complexity of the Anopheles stephensi nitric oxide synthase gene. Insect BiochemMol Biol. 31:249-56.

59. Hillyer JF, Estévez-Lao TY. (2010) Nitric oxide is an essential component of the hemocyte-mediated mosquito immune response against bacteria. Dev Comp Immunol. 34:141-9.

60. Chen Y, Rosazza JP. (1994) A bacterial nitric oxide synthase from a Nocardia species. Biochem Biophys Res Commun. 203:1251-8.

61. White O, Eisen JA, Heidelberg JF, Hickey EK, Peterson JD, Dodson RJ, Haft DH, Gwinn ML, Nelson WC, Richardson DL, Moffat KS, Qin H, Jiang L, Pamphile W, Crosby M, Shen M, Vamathevan JJ, Lam P, McDonald L,

Utterback T, Zalewski C, Makarova KS, Aravind L, Daly MJ, Minton KW, Fleischmann RD, Ketchum KA, Nelson KE, Salzberg S, Smith HO, Venter JC, Fraser CM. (1999) Genome sequence of the radioresistant bacterium Deinococcusradiodurans R1. Science. 286:1571-7.

62. Sudhamsu J, Crane BR. (2009) Bacterial nitric oxide synthases: what are they good for? Trends Microbiol. 17:212-8.

63. Choi DW, Oh HY, Hong SY, Han JW, Lee HW. (2000) Identification and characterization of nitric oxide synthase in *Salmonella typhimurium*. Arch Pharm Res. 23:407-12.

64. Pant K, Bilwes AM, Adak S, Stuehr DJ, Crane BR. (2002) Structure of a nitric oxide synthase heme protein from *Bacillus subtilis*. Biochemistry. 41:11071-9.

65. Bird LE, Ren J, Zhang J, Foxwell N, Hawkins AR, Charles IG, Stammers DK. (2002) Crystal structure of SANOS, a bacterial nitric oxide synthase oxygenase protein from Staphylococcus aureus. Structure. 10:1687-96.

66. Agapie T, Suseno S, Woodward JJ, Stoll S, Britt RD, Marletta MA. (2009) NO formation by a catalytically self-sufficient bacterial nitric oxide synthase from *Sorangiumcellulosum*. Proc Natl AcadSci U S A. 106:16221-6.

67. Holden JK, Li H, Jing Q, Kang S, Richo J, Silverman RB, Poulos TL. (2013) Structural and biological studies on bacterial nitric oxide synthase inhibitors. Proc Natl AcadSci U S A. 110:18127-31.

68. Wang Y, Golledge J. (2013) Neuronal nitric oxide synthase and sympathetic nerve activity in neurovascular and metabolic systems. CurrNeurovasc Res. 10:81-9.

69. Kinkel TL, Ramos-Montañez S, Pando JM, Tadeo DV, Strom EN, Libby SJ, Fang FC. (2016) An essential role for bacterial nitric oxide synthase in *Staphylococcus aureus* electron transfer and colonization. Nat Microbiol. 2:16224.

70. Holden JK, Kang S, Beasley FC, Cinelli MA, Li H, Roy SG, Dejam D, Edinger AL, Nizet V, Silverman RB, Poulos TL. (2015) Nitric Oxide Synthase as a

Target for Methicillin-Resistant Staphylococcus aureus. Chem Biol. 22:785-92.

71. Holden JK, Lewis MC, Cinelli MA, Abdullatif Z, Pensa AV, Silverman RB, Poulos TL. (2016) Targeting Bacterial Nitric Oxide Synthase with Aminoquinoline-Based Inhibitors. Biochemistry. 55:5587-5594.

72. Kanadia RN, Kuo WN, Mcnabb M, Botchway A. (1998) Constitutive nitric oxide synthase in Saccharomyces cerevisiae. BiochemMolBiol Int. 45:1081-7.

73. Castello PR, David PS, McClure T, Crook Z, Poyton RO. (2006) Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. Cell Metab. 3:277-87.

74. Kig C, Temizkan G. (2009) Nitric oxide as a signaling molecule in the fission yeast *Schizosaccharomycespombe*. Protoplasma. 238:59-66.

75. Astuti RI, Nasuno R, Takagi H. (2016) Nitric oxide signaling in yeast. Appl Microbiol Biotechnol. 100:9483-9497.

76. Yoshikawa Y, Nasuno R, Kawahara N, Nishimura A, Watanabe D, Takagi H. (2016) Regulatory mechanism of the flavoprotein Tah18-dependent nitric oxide synthesis and cell death in yeast. Nitric Oxide. 57:85-91.

77. Vieira AL, Linares E, Augusto O, Gomes SL. (2009) Evidence of a Ca(2+)-(*)NO-cGMP signaling pathway controlling zoospore biogenesis in the aquatic fungus *Blastocladiella emersonii*. Fungal Genet Biol. 46:575-84.

78. Samalova M, Johnson J, Illes M, Kelly S, Fricker M, Gurr S. (2013) Nitric oxide generated by the rice blast fungus *Magnaporthe oryzae* drives plant infection. New Phytol. 197:207-222.

79. Zumft WG. (1993) The biological role of nitric oxide in bacteria. Arch Microbiol. 160:253-64.

80. Miyamoto T, Petrus MJ, Dubin AE, Patapoutian A. (2011) TRPV3 regulates nitric oxide synthase-independent nitric oxide synthesis in the skin. Nat Commun. 2:369.

81. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, Gladwin MT (2003) Nitrite reduction to nitric oxide by deoxyhemoglobinvasodilates the human circulation. Nat Med. 9:1498–1505,

82. Shiva S, Huang Z, Grubina R, Sun J, Ringwood LA, MacArthur PH, Xu X, Murphy E, Darley-Usmar VM, Gladwin MT (2007) Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. Circ Res. 100:654–661.

83. Tiso M, Tejero J, Basu S, Azarov I, Wang X, Simplaceanu V, Frizzell S, Jayaraman T, Geary L, Shapiro C (2011) Human neuroglobin functions as a redox-regulated nitrite reductase. J Biol Chem. 286:18277–89.

84. Li H, Hemann C, Abdelghany TM, El-Mahdy MA, Zweier JL (2012) Characterization of the mechanism and magnitude of cytoglobin-mediated nitrite reduction and nitric oxide generation under anaerobic conditions. J Biol Chem. 287:36623–36633.

85. Tiso M, Tejero J, Kenney C, Frizzell S, Gladwin MT (2012) Nitrite reductase activity of nonsymbiotichemoglobins from *Arabidopsis thaliana*. Biochem. 51:5285–5292

86. Godber BLJ, Doel JJ, Sapkota GP, Blake DR, Stevens CR, Eisenthal R, Harrison R (2000) Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. J Biol Chem. 275:7757–7763.

87. Millar TM, Stevens CR, Benjamin N, Eisenthal R, Harrison R, Blake DR (1998) Xanthine oxidoreductase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions. FEBS Lett. 427:225–228.

88. Shiva S (2013) Nitrite: a physiological store of nitric oxide and modulator of mitochondrial function. Redox Biol. 1:40–44.

89. L'hirondel J, L'hirondel JL (2002) Nitrate and man: toxic, harmless or beneficial? CABI Publishing, Oxon

90. Miles AM, Wink DA, Cook JC, Grisham MB (1996) Determination of nitric oxide using fluorescence spectroscopy. Methods Enzymol. 268:105–120.

91. Weitzberg E, Lundberg J (1998) Nonenzymatic nitric oxide production in humans. Nitric Oxide 2:1–7.

92. Evans HJ, McAuliffe C (1956) Identification of NO, N_2O, and N_2 as products of the nonenzymatic reduction of nitrite by ascorbate or reduced diphosphopyridine nucleotide. In: McElroy WD, Glass B (eds) Inorganic nitrogen metabolism. Johns Hopkins Press, Baltimore, pp 189–197

93. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl AcadSci U S A. 84:9265-9.

94. Lincoln TM, Wu X, Sellak H, Dey N, Choi CS. (2006) Regulation of vascular smooth muscle cell phenotype by cyclic GMP and cyclic GMP-dependent protein kinase. Front Biosci. 11:356-67.

95. Noguchi A, Takada M, Nakayama K, Ishikawa T. (2008) cGMP-independent anti-apoptotic effect of nitric oxide on thapsigargin-induced apoptosis in the pancreatic beta-cell line INS-1. Life Sci. 83:865-70.

96. Walter U, Gambaryan S. (2009) cGMP and cGMP-dependent protein kinase in platelets and blood cells. Hand bExp Pharmacol. 191:533-48.

97. Korkmaz S, Loganathan S, Mikles B, Radovits T, Barnucz E, Hirschberg K, Li S, Hegedüs P, Páli S, Weymann A, Karck M, Szabó G. (2013) Nitric oxide- and heme-independent activation of soluble guanylate cyclase attenuates peroxynitrite-induced endothelial dysfunction in rat aorta. J Cardiovasc Pharmacol Ther. 18:70-7.

98. Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP. (2000) Molecular mechanism of cGMP-mediated smooth muscle relaxation. J Cell Physiol. 184:409-20.

99. Word RA, Tang DC, Kamm KE. (1994) Activation properties of myosin light chain kinase during contraction/relaxation cycles of tonic and phasic smooth muscles. J Biol Chem. 269:21596-602.

100. Whalen EJ, Foster MW, Matsumoto A, Ozawa K, Violin JD, Que LG, Nelson CD, Benhar M, Keys JR, Rockman HA, Koch WJ, Daaka Y, Lefkowitz RJ,

Stamler JS. (2007) Regulation of beta-adrenergic receptor signaling by *S*-nitrosylation of G-protein-coupled receptor kinase 2. Cell. 129:511-22.

101. Whalen EJ, Johnson AK, Lewis SJ. (2000) Beta-adrenoceptor dysfunction after inhibition of NO synthesis. Hypertension. 36:376-82.

102. Chen Z, Zhang X, Ying L, Dou D, Li Y, Bai Y, Liu J, Liu L, Feng H, Yu X, Leung SW, Vanhoutte PM, Gao Y. (2014) cIMP synthesized by sGC as a mediator of hypoxic contraction of coronary arteries. Am J Physiol Heart Circ Physiol. 307:H328-36.

103. Gao Y, Chen Z, Leung SW, Vanhoutte PM. (2015) Hypoxic Vasospasm Mediated by cIMP: When Soluble Guanylyl Cyclase Turns Bad. J CardiovascPharmacol. 65:545-8.

104. Bruckdorfer R. (2005) The basics about nitric oxide. Mol Aspects Med. 26:3-31.

105. Radomski MW, Palmer RM, Moncada S. (1987) The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br J Pharmacol. 92:639-46.

106. Radomski MW, Palmer RM, Moncada S. (1990) Characterization of the L-arginine:nitric oxide pathway in human platelets. Br J Pharmacol. 101:325-8.

107. Antl M, von Brühl ML, Eiglsperger C, Werner M, Konrad I, Kocher T, Wilm M, Hofmann F, Massberg S, Schlossmann J. (2007) IRAG mediates NO/cGMP-dependent inhibition of platelet aggregation and thrombus formation. Blood. 109:552-9.

108. Dangel O, Mergia E, Karlisch K, Groneberg D, Koesling D, Friebe A. (2010) Nitric oxide-sensitive guanylyl cyclase is the only nitric oxide receptor mediating platelet inhibition. J ThrombHaemost. 8:1343-52.

109. Kim PK, Zamora R, Petrosko P, Billiar TR. (2001) The regulatory role of nitric oxide in apoptosis. Int Immunopharmacol. 1:1421-41.

110. Khan FH, Dervan E, Bhattacharyya DD, McAuliffe JD, Miranda KM, Glynn SA. (2020) The Role of Nitric Oxide in Cancer: Master Regulator or NOt? Int J Mol Sci. 21:9393.

111. Li J, Bombeck CA, Yang S, Kim YM, Billiar TR. (1999) Nitric oxide suppresses apoptosis via interrupting caspase activation and mitochondrial dysfunction in cultured hepatocytes. J Biol Chem. 274:17325-33.

112. Li J, Billiar TR, Talanian RV, Kim YM. (1997) Nitric oxide reversibly inhibits seven members of the caspase family via *S*-nitrosylation. Biochem Biophys Res Commun. 240:419-24.

113. Azad N, Vallyathan V, Wang L, Tantishaiyakul V, Stehlik C, Leonard SS, Rojanasakul Y. (2006) *S*-nitrosylation of Bcl-2 inhibits its ubiquitin-proteasomal degradation. A novel antiapoptotic mechanism that suppresses apoptosis. J Biol Chem. 281:34124-34.

114. De Nadai C, Sestili P, Cantoni O, Lièvremont JP, Sciorati C, Barsacchi R, Moncada S, Meldolesi J, Clementi E. (2000) Nitric oxide inhibits tumor necrosis factor-alpha-induced apoptosis by reducing the generation of ceramide. Proc Natl Acad Sci U S A. 97:5480-5.

115. Brookes PS, Salinas EP, Darley-Usmar K, Eiserich JP, Freeman BA, (2000) Darley-Usmar VM, Anderson PG. Concentration-dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome *c* release. J Biol Chem. 275:20474-9.

116. Nasuno R, Aitoku M, Manago Y, Nishimura A, Sasano Y, Takagi H. (2014) Nitric oxide-mediated antioxidative mechanism in yeast through the activation of the transcription factor Mac1. PLoS One. 9:e113788.

117. Astuti RI, Watanabe D, Takagi H. (2016) Nitric oxide signaling and its role in oxidative stress response in *Schizosaccharomycespombe*. Nitric Oxide. 52:29-40.

118. Osório NS, Carvalho A, Almeida AJ, Padilla-Lopez S, Leão C, Laranjinha J, Ludovico P, Pearce DA, Rodrigues F. (2007) Nitric oxide signaling is disrupted in the yeast model for Batten disease. Mol Biol Cell. 18:2755-67.

119. Kato T, Zhou X, Ma Y. (2013) Possible involvement of nitric oxide and reactive oxygen species in glucose deprivation-induced activation of transcription factor rst2. PLoS One. 8:e78012.

120. Li B, Skinner C, Castello PR, Kato M, Easlon E, Xie L, Li T, Lu SP, Wang C, Tsang F, Poyton RO, Lin SJ. (2011) Identification of potential calorie restriction-mimicking yeast mutants with increased mitochondrial respiratory chain and nitric oxide levels. J Aging Res. 2011:673185.

121. Dedon PC, Tannenbaum SR. (2004) Reactive nitrogen species in the chemical biology of inflammation. Arch Biochem Biophys. 423:12-22.

122. Chen B, Deen WM. (2001) Analysis of the effects of cell spacing and liquid depth on nitric oxide and its oxidation products in cell cultures. Chem Res Toxicol. 14:135-47.

123. Caulfield JL, Singh SP, Wishnok JS, Deen WM, Tannenbaum SR. (1996) Bicarbonate inhibits N-nitrosation in oxygenated nitric oxide solutions. J Biol Chem. 271:25859-63.

124. Lewis RS, Deen WM. (1994) Kinetics of the reaction of nitric oxide with oxygen in aqueous solutions. Chem Res Toxicol. 7:568-74.

125. Licht WR, Tannenbaum SR, Deen WM. (1988) Use of ascorbic acid to inhibit nitrosation: kinetic and mass transfer considerations for an in vitro system. Carcinogenesis. 9:365-72.

126. Caulfield JL, Singh SP, Wishnok JS, Deen WM, Tannenbaum SR. (1996) Bicarbonate inhibits N-nitrosation in oxygenated nitric oxide solutions. J Biol Chem. 271:25859-63.

127. Huie RE, Padmaja S. (1993) The reaction of no with superoxide. Free Radic Res Commun. 18:195-9.

128. Kissner R, Nauser T, Bugnon P, Lye PG, Koppenol WH. (1997) Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. Chem Res Toxicol. 10:1285-92.

129. Nauser T, Koppenol WH. (2002) The Rate Constant of the Reaction of Superoxide with Nitrogen Monoxide: Approaching the Diffusion Limit. J PhysChem A.106:4084-4086.

130. Fielden EM, Roberts PB, Bray RC, Lowe DJ, Mautner GN, Rotilio G, Calabrese L. (1974) Mechanism of action of superoxide dismutase from pulse

radiolysis and electron paramagnetic resonance. Evidence that only half the active sites function in catalysis. Biochem J. 139:49-60.

131. Hughes MN, Nicklin HG. (1968) The Chemistry of Pernitrites. Part 1. Kinetics of Decomposition of Pernitrous Acid. J Chem Soc. 450–52.

132. Gerasimov OV, Lymar SV. (1999) The yield of hydroxyl radical from the decomposition of peroxynitrous acid. Inorg Chem. 38:4317-21.

133. Radi R, Cosgrove TP, Beckman JS, Freeman BA. (1993) Peroxynitrite-induced luminolchemiluminescence. Biochem J. 290:51-7.

134. Lymar SV, Jiang Q, Hurst JK. (1996) Mechanism of carbon dioxide-catalyzed oxidation of tyrosine by peroxynitrite. Biochemistry. 35:7855-61.

135. Augusto O, Bonini MG, Amanso AM, Linares E, Santos CC, De Menezes SL. (2002) Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. Free RadicBiol Med. 32:841-59.

136. Serrano R, Garrido N, Céspedes JA, González-Fernández L, García-Marín LJ, Bragado MJ. (2020) Molecular Mechanisms Involved in the Impairment of Boar Sperm Motility by Peroxynitrite-Induced Nitrosative Stress. Int J Mol Sci. 21:1208.

137. Condeles AL, Gomes F, de Oliveira MA, Soares Netto LE, Toledo Junior JC. (2020) Thiol Peroxidases as Major Regulators of Intracellular Levels of Peroxynitrite in Live *Saccharomyces cerevisiae* Cells. Antioxidants (Basel). 9:434.

138. Zhang Y, Hogg N. (2005) *S*-Nitrosothiols: cellular formation and transport. Free Radic Biol Med. 38:831-8.

139. Smith BC, Marletta MA. (2012) Mechanisms of *S*-nitrosothiol formation and selectivity in nitric oxide signaling. Curr Opin Chem Biol. 16:498-506.

140. Möller MN, Li Q, Vitturi DA, Robinson JM, Lancaster JR Jr, Denicola A. (2007) Membrane "lens" effect: focusing the formation of reactive nitrogen oxides from the $^*NO/O_2$ reaction. Chem Res Toxicol. 20:709-14.

141. Keszler A, Zhang Y, Hogg N. (2010) Reaction between nitric oxide, glutathione, and oxygen in the presence and absence of protein: How are *S*-nitrosothiols formed? Free Radic Biol Med. 48:55-64.

142. Wang PG, Xian M, Tang X, Wu X, Wen Z, Cai T, Janczuk AJ. (2002) Nitric oxide donors: chemical activities and biological applications. Chem Rev. 102:1091-134.

143. Broniowska KA, Diers AR, Hogg N. (2013) *S*-nitrosoglutathione. Biochim Biophys Acta. 1830:3173-81.

144. Klandorf H, Dyke KV (2012) Oxidative and nitrosative stresses: their role in health and disease in man and birds. J Ag & Bio Sci 3: 47-59.

145. Patra SK, Bag PK, Ghosh S. (2017) Nitrosative Stress Response in Vibrio cholerae: Role of *S*-Nitrosoglutathione Reductase. Appl Biochem Biotechnol. 182:871-884.

146. D'Autreaux B, Touati D, Bersch B, Latour JM, Michaud-Soret I. (2002) Direct inhibition by nitric oxide of the transcriptional ferric uptake regulation protein via nitrosylation of the iron. Proc Natl Acad Sci U S A. 99:16619-24.

147. Hausladen A, Privalle CT, Keng T, DeAngelo J, Stamler JS. (1996) Nitrosative stress: activation of the transcription factor OxyR. Cell. 86:719-29.

148. Corpas FJ, Leterrier M, Valderrama R, Airaki M, Chaki M, Palma JM, Barroso JB. (2011) Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. Plant Sci. 181:604-11.

149. Patra SK, Samaddar S, Sinha N, Ghosh S. (2019) Reactive nitrogen species induced catalases promote a novel nitrosative stress tolerance mechanism in Vibrio cholerae. Nitric Oxide. 88:35-44.

150. Reiter TA. (2006) NO* chemistry: a diversity of targets in the cell. Redox Rep. 11:194-206.

151. Wink DA, Mitchell JB. (1998) Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med.25:434-56.

152. Dong M, Wang C, Deen WM, Dedon PC. (2003) Absence of 2'-deoxyoxanosine and presence of abasic sites in DNA exposed to nitric oxide at controlled physiological concentrations. Chem Res Toxicol. 16:1044-55.

153. Dong M, Dedon PC. (2006) Relatively small increases in the steady-state levels of nucleobase deamination products in DNA from human TK6 cells exposed to toxic levels of nitric oxide. Chem Res Toxicol. 19:50-7.

154. Lim KS, Huang SH, Jenner A, Wang H, Tang SY, Halliwell B. (2006) Potential artifacts in the measurement of DNA deamination. Free Radic Biol Med. 40:1939-48.

155. Wink DA, Hanbauer I, Grisham MB, Laval F, Nims RW, Laval J, Cook J, Pacelli R, Liebmann J, Krishna M, Ford PC, Mitchell JB. (1996) Chemical biology of nitric oxide: regulation and protective and toxic mechanisms. Curr Top Cell Regul. 34:159-87.

156. Tretyakova NY, Burney S, Pamir B, Wishnok JS, Dedon PC, Wogan GN, Tannenbaum SR. (2000) Peroxynitrite-induced DNA damage in the supF gene: correlation with the mutational spectrum. Mutat Res. 447:287-303.

157. Yermilov V, Rubio J, Becchi M, Friesen MD, Pignatelli B, Ohshima H. (1995) Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite in vitro. Carcinogenesis. 16:2045-50.

158. Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR. (1999) The chemistry of DNA damage from nitric oxide and peroxynitrite. Mutat Res. 424:37-49.

159. Spencer JP, Wong J, Jenner A, Aruoma OI, Cross CE, Halliwell B. (1996) Base modification and strand breakage in isolated calf thymus DNA and in DNA from human skin epidermal keratinocytes exposed to peroxynitrite or 3-morpholinosydnonimine. Chem Res Toxicol. 9:1152-8.

160. Johansen ME, Muller JG, Xu X, Burrows CJ. (2005) Oxidatively induced DNA-protein cross-linking between single-stranded binding protein and oligodeoxynucleotides containing 8-oxo-7,8-dihydro-2'-deoxyguanosine. Biochemistry. 44:5660-71.

161. Shapiro R, Dubelman S, Feinberg AM, Crain PF, McCloskey JA. (1977) Isolation and identification of cross-linked nucleosides from nitrous acid treated deoxyribonucleic acid. J Am Chem Soc. 99:302-3.

162. Laval F, Wink DA, Laval J. (1997) A discussion of mechanisms of NO genotoxicity: implication of inhibition of DNA repair proteins. Rev Physiol Biochem Pharmacol. 131:175-91.

163. Wink DA, Laval J. (1994) The Fpg protein, a DNA repair enzyme, is inhibited by the biomediator nitric oxide in vitro and in vivo. Carcinogenesis. 15:2125-9.

164. Kwon NS, Stuehr DJ, Nathan CF. (1991) Inhibition of tumor cell ribonucleotide reductase by macrophage-derived nitric oxide. J Exp Med. 174:761-7.

165. Lepoivre M, Chenais B, Yapo A, Lemaire G, Thelander L, Tenu JP. (1990) Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. J Biol Chem. 265:14143-9.

166. Hogg N, Kalyanaraman B. (1999) Nitric oxide and lipid peroxidation. Biochim Biophys Acta. 1411:378-84.

167. Bloodsworth A, O'Donnell VB, Freeman BA. (2000) Nitric oxide regulation of free radical- and enzyme-mediated lipid and lipoprotein oxidation. Arterioscler Thromb Vasc Biol. 20:1707-15.

168. Laskey RE, Mathews WR. (1996) Nitric oxide inhibits peroxynitrite-induced production of hydroxyeicosatetraenoic acids and F2-isoprostanes in phosphatidylcholine liposomes. Arch Biochem Biophys. 330:193-8.

169. Goss SP, Hogg N, Kalyanaraman B. (1995) The antioxidant effect of spermine NONOate in human low-density lipoprotein. Chem Res Toxicol. 8:800-6.

170. Hogg N, Struck A, Goss SP, Santanam N, Joseph J, Parthasarathy S, Kalyanaraman B. (1995) Inhibition of macrophage-dependent low density lipoprotein oxidation by nitric-oxide donors. J Lipid Res. 36:1756-62.

171. Malo-Ranta U, Ylä-Herttuala S, Metsä-Ketelä T, Jaakkola O, Moilanen E, Vuorinen P, Nikkari T. (1994) Nitric oxide donor GEA 3162 inhibits

endothelial cell-mediated oxidation of low density lipoprotein. FEBS Lett. 337:179-83.

172. Hayashi K, Noguchi N, Niki E. (1995) Action of nitric oxide as an antioxidant against oxidation of soybean phosphatidylcholine liposomal membranes. FEBS Lett. 370:37-40.

173. Korytowski W, Zareba M, Girotti AW. (2000) Nitric oxide inhibition of free radical-mediated cholesterol peroxidation in liposomal membranes. Biochemistry. 39:6918-28.

174. O'Donnell VB, Chumley PH, Hogg N, Bloodsworth A, Darley-Usmar VM, Freeman BA. (1997) Nitric oxide inhibition of lipid peroxidation: kinetics of reaction with lipid peroxyl radicals and comparison with alpha-tocopherol. Biochemistry. 36:15216-23.

175. Gallon AA, Pryor WA. (1994) The reaction of low levels of nitrogen dioxide with methyl linoleate in the presence and absence of oxygen. Lipids. 29:171-6.

176. Kamel AM, Weiner WD, Felmeister A. (1971) Identification of cholesteryl nitrate as a product of the reaction between NO 2 and cholesterol monomolecular films. Chem Phys Lipids. 6:225-34.

177. Kikugawa K, Beppu M, Okamoto Y. (1995) Uptake by macrophages of low-density lipoprotein damaged by nitrogen dioxide in air. Lipids. 30:313-20.

178. Napolitano A, Camera E, Picardo M, d'Ischia M. (2000) Acid-promoted reactions of ethyl linoleate with nitrite ions: formation and structural characterization of isomeric nitroalkene, nitrohydroxy, and novel 3-nitro-1,5-hexadiene and 1,5-dinitro-1, 3-pentadiene products. J Org Chem. 65:4853-60.

179. Khairutdinov RF, Coddington JW, Hurst JK. (2000) Permeation of phospholipid membranes by peroxynitrite. Biochemistry. 39:14238-49.

180. Khairutdinov RF, Coddington JW, Hurst JK. (2000) Permeation of phospholipid membranes by peroxynitrite. Biochemistry. 39:14238-49.

181. O'Donnell VB, Taylor KB, Parthasarathy S, Kühn H, Koesling D, Friebe A, Bloodsworth A, Darley-Usmar VM, Freeman BA. (1999) 15-Lipoxygenase

catalytically consumes nitric oxide and impairs activation of guanylate cyclase. J Biol Chem. 274:20083-91.

182. Trostchansky A, Batthyány C, Botti H, Radi R, Denicola A, Rubbo H. (2001) Formation of lipid-protein adducts in low-density lipoprotein by fluxes of peroxynitrite and its inhibition by nitric oxide. Arch Biochem Biophys. 395:225-32.

183. Xie Y, Luo X, Li Y, Chen L, Ma W, Huang J, Cui J, Zhao Y, Xue Y, Zuo Z, Ren J. (2018) DeepNitro: Prediction of Protein Nitration and Nitrosylation Sites by Deep Learning. Genomics Proteomics Bioinformatics. 16:294-306.

184. Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS. (2005) Protein *S*-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol. 6:150-66.

185. Hill BG, Dranka BP, Bailey SM, Lancaster JR Jr, Darley-Usmar VM. (2010) What part of NO don't you understand? Some answers to the cardinal questions in nitric oxide biology. J Biol Chem. 285:19699-704.

186. Smith BC, Marletta MA. (2012) Mechanisms of *S*-nitrosothiol formation and selectivity in nitric oxide signaling. Curr Opin Chem Biol. 16:498-506.

187. Martínez-Ruiz A, Cadenas S, Lamas S. (2011) Nitric oxide signaling: classical, less classical, and nonclassical mechanisms. Free Radic Biol Med. 51:17-29.

188. Gould N, Doulias PT, Tenopoulou M, Raju K, Ischiropoulos H. (2013) Regulation of protein function and signaling by reversible cysteine *S*-nitrosylation. J Biol Chem. 288:26473-9.

189. Hess DT, Stamler JS. (2012) Regulation by *S*-nitrosylation of protein post-translational modification. J Biol Chem. 287:4411-8.

190. Kornberg MD, Sen N, Hara MR, Juluri KR, Nguyen JV, Snowman AM, Law L, Hester LD, Snyder SH. (2010) GAPDH mediates nitrosylation of nuclear proteins. Nat Cell Biol. 12:1094-100.

191. Nakamura T, Lipton SA. (2013) Emerging role of protein-protein transnitrosylation in cell signaling pathways. Antioxid Redox Signal. 18:239-49.

192. Sengupta R, Holmgren A. (2013) Thioredoxin and thioredoxin reductase in relation to reversible *S*-nitrosylation. Antioxid Redox Signal. 18:259-69.

193. Martínez-Ruiz A, Araújo IM, Izquierdo-Álvarez A, Hernansanz-Agustín P, Lamas S, Serrador JM. (2013) Specificity in *S*-nitrosylation: a short-range mechanism for NO signaling? Antioxid Redox Signal. 19:1220-35.

194. Lamotte O, Bertoldo JB, Besson-Bard A, Rosnoblet C, Aimé S, Hichami S, Terenzi H, Wendehenne D. (2015) Protein *S*-nitrosylation: specificity and identification strategies in plants. Front Chem. 2:114.

195. Prime TA, Blaikie FH, Evans C, Nadtochiy SM, James AM, Dahm CC, Vitturi DA, Patel RP, Hiley CR, Abakumova I, Requejo R, Chouchani ET, Hurd TR, Garvey JF, Taylor CT, Brookes PS, Smith RA, Murphy MP. (2009) A mitochondria-targeted *S*-nitrosothiol modulates respiration, nitrosates thiols, and protects against ischemia-reperfusion injury. Proc Natl Acad Sci U S A. 106:10764-9.

196. Di Virgilio F, Azzone GF. (1982) Activation of site I redox-driven H+ pump by exogenous quinones in intact mitochondria. J Biol Chem. 257:4106-13.

197. Vinogradov AD. (1998) Catalytic properties of the mitochondrial NADH-ubiquinone oxidoreductase (complex I) and the pseudo-reversible active/inactive enzyme transition. Biochim Biophys Acta. 1364:169-85.

198. Mohr S, Hallak H, de Boitte A, Lapetina EG, Brüne B. (1999) Nitric oxide-induced S-glutathionylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase. J Biol Chem. 274:9427-30.

199. Piantadosi CA. (2012) Regulation of mitochondrial processes by protein *S*-nitrosylation. Biochim Biophys Acta. 1820:712-21.

200. Chen YJ, Lu CT, Su MG, Huang KY, Ching WC, Yang HH, Liao YC, Chen YJ, Lee TY. (2015) dbSNO 2.0: a resource for exploring structural environment, functional and disease association and regulatory network of protein *S*-nitrosylation. Nucleic Acids Res. 43:D503-11.

201. Abello N, Kerstjens HA, Postma DS, Bischoff R. (2009) Protein tyrosine nitration: selectivity, physicochemical and biological consequences,

denitration, and proteomics methods for the identification of tyrosine-nitrated proteins. J Proteome Res. 8:3222-38.

202. Bian K, Gao Z, Weisbrodt N, Murad F. (2003) The nature of heme/iron-induced protein tyrosine nitration. Proc Natl Acad Sci U S A. 100:5712-7.

203. Bhattacharjee A, Majumdar U, Maity D, Sarkar TS, Goswami AM, Sahoo R, Ghosh S. (2009) In vivo protein tyrosine nitration in S. cerevisiae: identification of tyrosine-nitrated proteins in mitochondria. Biochem Biophys Res Commun. 388:612-7.

204. Souza JM, Daikhin E, Yudkoff M, Raman CS, Ischiropoulos H. (1999) Factors determining the selectivity of protein tyrosine nitration. Arch Biochem Biophys. 371:169-78.

205. Radi R. (2013) Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. Acc Chem Res. 46:550-9.

206. Radi R. (2018) Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proc Natl Acad Sci U S A. 5:5839-5848.

207. Sokolovsky M, Riordan JF, Vallee BL. (1967) Conversion of 3-nitrotyrosine to 3-aminotyrosine in peptides and proteins. Biochem Biophys Res Commun. 27:20-5.

208. Heijnen HF, van Donselaar E, Slot JW, Fries DM, Blachard-Fillion B, Hodara R, Lightfoot R, Polydoro M, Spielberg D, Thomson L, Regan EA, Crapo J, Ischiropoulos H. (2006) Subcellular localization of tyrosine-nitrated proteins is dictated by reactive oxygen species generating enzymes and by proximity to nitric oxide synthase. Free Radic Biol Med. 40:1903-13.

209. Ferrer-Sueta G, Campolo N, Trujillo M, Bartesaghi S, Carballal S, Romero N, Alvarez B, Radi R. (2018) Biochemistry of Peroxynitrite and Protein Tyrosine Nitration. Chem Rev. 118:1338-1408.

210. Osoata GO, Yamamura S, Ito M, Vuppusetty C, Adcock IM, Barnes PJ, Ito K. (2009) Nitration of distinct tyrosine residues causes inactivation of histone deacetylase 2. Biochem Biophys Res Commun. 384:366-71.

211. Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, López-Jaramillo J, Padilla MN, Carreras A, Corpas FJ, Barroso JB. (2014)

Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and *S*-nitrosylation. J Exp Bot. 65:527-38.

212. Moreno DM, Martí MA, De Biase PM, Estrin DA, Demicheli V, Radi R, Boechi L. (2011) Exploring the molecular basis of human manganese superoxide dismutase inactivation mediated by tyrosine 34 nitration. Arch Biochem Biophys. 507:304-9.

213. Monteiro HP, Arai RJ, Travassos LR. (2008) Protein tyrosine phosphorylation and protein tyrosine nitration in redox signaling. Antioxid Redox Signal. 10:843-89.

214. Kong SK, Yim MB, Stadtman ER, Chock PB. (1996) Peroxynitrite disables the tyrosine phosphorylation regulatory mechanism: Lymphocyte-specific tyrosine kinase fails to phosphorylate nitrated cdc2(6-20)NH2 peptide. Proc Natl Acad Sci U S A. 93:3377-82.

215. Brito C, Naviliat M, Tiscornia AC, Vuillier F, Gualco G, Dighiero G, Radi R, Cayota AM. (1999) Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxynitrite-driven apoptotic death. J Immunol. 162:3356-66.

216. Brockhaus F, Brüne B. (1999) p53 accumulation in apoptotic macrophages is an energy demanding process that precedes cytochrome *c* release in response to nitric oxide. Oncogene. 18:6403-10.

217. Nishio E, Watanabe Y. (1997) Nitric oxide donor-induced apoptosis in smooth muscle cells is modulated by protein kinase C and protein kinase A. Eur J Pharmacol. 339:245-51.

218. Estévez AG, Crow JP, Sampson JB, Reiter C, Zhuang Y, Richardson GJ, Tarpey MM, Barbeito L, Beckman JS. (1999) Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. Science. 286:2498-500.

219. Hortelano S, Alvarez AM, Boscá L. (1999) Nitric oxide induces tyrosine nitration and release of cytochrome *c* preceding an increase of mitochondrial transmembrane potential in macrophages. FASEB J. 13:2311-7.

220. Almeida B, Buttner S, Ohlmeier S, Silva A, Mesquita A, Sampaio-Marques B, Osório NS, Kollau A, Mayer B, Leão C, Laranjinha J, Rodrigues F, Madeo F, Ludovico P. (2007) NO-mediated apoptosis in yeast. J Cell Sci. 120:3279-88.

221. Hortelano S, Dallaporta B, Zamzami N, Hirsch T, Susin SA, Marzo I, Boscá L, Kroemer G. (1997) Nitric oxide induces apoptosis via triggering mitochondrial permeability transition. FEBS Lett. 410:373-7.

222. Erusalimsky JD, Moncada S. (2007) Nitric oxide and mitochondrial signaling: from physiology to pathophysiology. Arterioscler Thromb Vasc Biol. 27:2524-31.

223. Kwun MS, Lee DG. (2020) An Insight on the Role of Nitric Oxide in Yeast Apoptosis of Curcumin-Treated *Candida albicans*. Curr Microbiol. 77:3104-3113.

224. Brüne B, von Knethen A, Sandau KB. (1998) Nitric oxide and its role in apoptosis. Eur J Pharmacol. 351:261-72.

225. Messmer UK, Brüne B. (1996) Nitric oxide (NO) in apoptotic versus necrotic RAW 264.7 macrophage cell death: the role of NO-donor exposure, NAD+ content, and p53 accumulation. Arch Biochem Biophys. 327:1-10.

226. Liebermann DA, Hoffman B, Steinman RA. (1995) Molecular controls of growth arrest and apoptosis: p53-dependent and independent pathways. Oncogene. 11:199-210.

227. Shankland SJ. (1997) Cell-cycle control and renal disease. Kidney Int. 52:294-308.

228. Ambs S, Hussain SP, Marrogi AJ, Harris CC. (1999) Cancer-prone oxyradical overload disease. IARC Sci Publ. 150:295-302.

229. Moncada S, Higgs A. (1993) The L-arginine-nitric oxide pathway. N Engl J Med. 329:2002-12.

230. Ignarro LJ. (1990) Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol 30:535-60.

231. Jun CD, Oh CD, Kwak HJ, Pae HO, Yoo JC, Choi BM, Chun JS, Park RK, Chung HT. (1999) Overexpression of protein kinase C isoforms protects RAW

264.7 macrophages from nitric oxide-induced apoptosis: involvement of c-Jun N-terminal kinase/stress-activated protein kinase, p38 kinase, and CPP-32 protease pathways. J Immunol. 162:3395-401.

232. Oh-hashi K, Maruyama W, Yi H, Takahashi T, Naoi M, Isobe K. (1999) Mitogen-activated protein kinase pathway mediates peroxynitrite-induced apoptosis in human dopaminergic neuroblastoma SH-SY5Y cells. Biochem Biophys Res Commun. 263:504-9.

233. Steptoe A, Zaninotto P. (2020) Lower socioeconomic status and the acceleration of aging: An outcome-wide analysis. Proc Natl Acad Sci U S A. 117:14911-7.

234. Kaeberlein M, Burtner CR, Kennedy BK. (2007) Recent developments in yeast aging. PLoS Genet. 3:e84.

235. Steinkraus KA, Kaeberlein M, Kennedy BK. (2008) Replicative aging in yeast: the means to the end. Annu Rev Cell Dev Biol. 24:29-54.

236. Dawes IW, Perrone GG. (2020) Stress and ageing in yeast. FEMS Yeast Res. 20:foz085.

237. Fabrizio P, Longo VD. (2003) The chronological life span of *Saccharomyces cerevisiae*. Aging Cell. 2:73-81.

238. Kaeberlein M. (2006) Longevity and aging in the budding yeast. In: Conn PM, editor. Handbook of models for human aging. Boston: Elvesier Press. pp. 109–120

239. Breitenbach M, Laun P, Dickinson JR, Klocker A, Rinnerthaler M, Dawes IW, Aung-Htut MT, Breitenbach-Koller L, Caballero A, Nyström T, Büttner S, Eisenberg T, Madeo F, Ralser M. (2012) The role of mitochondria in the aging processes of yeast. Subcell Biochem. 57:55-78.

240. Kominsky DJ, Thorsness PE. (2000) Expression of the Saccharomyces cerevisiae gene YME1 in the petite-negative yeast Schizosaccharomyces pombe converts it to petite-positive. Genetics. 154:147-54.

241. Mandavilli BS, Santos JH, Van Houten B. (2002) Mitochondrial DNA repair and aging. Mutat Res. 509:127-51.

242. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, Fink GR, Guarente L. (2002) Calorie restriction extends Saccharomyces cerevisiae lifespan by increasing respiration. Nature. 418:344-8.

243. Powers T, Walter P. (1999) Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in *Saccharomyces cerevisiae*. Mol Biol Cell. 10:987-1000.

244. Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, Dang N, Kerr EO, Kirkland KT, Fields S, Kennedy BK. (2005) Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science. 310:1193-6.

245. Smith A, Ward MP, Garrett S. (1998) Yeast PKA represses Msn2p/Msn4p-dependent gene expression to regulate growth, stress response and glycogen accumulation. EMBO J. 17:3556-64.

246. Longo VD. (2003) The Ras and Sch9 pathways regulate stress resistance and longevity. Exp Gerontol. 38:807-11.

247. Takeda E, Jin N, Itakura E, Kira S, Kamada Y, Weisman LS, Noda T, Matsuura A. (2018) Vacuole-mediated selective regulation of TORC1-Sch9 signaling following oxidative stress. Mol Biol Cell. 29:510-522.

248. Trendeleva TA, Zvyagilskaya RA. (2018) Retrograde Signaling as a Mechanism of Yeast Adaptation to Unfavorable Factors. Biochemistry (Mosc). 83:98-106.

249. Dilova I, Chen CY, Powers T. (2002) Mks1 in concert with TOR signaling negatively regulates RTG target gene expression in *S. cerevisiae*. Curr Biol. 12:389-95.

250. Powers T, Dilova I, Chen CY, Wedaman K. (2004) Yeast TOR signaling: a mechanism for metabolic regulation. Curr Top Microbiol Immunol. 279:39-51.

251. Yorimitsu T, Klionsky DJ. (2005) Autophagy: molecular machinery for self-eating. Cell Death Differ. 12:1542-52.

252. Noda T, Ohsumi Y. (1998) Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. J Biol Chem. 273:3963-6.

253. Kamada Y, Sekito T, Ohsumi Y. (2004) Autophagy in yeast: a TOR-mediated response to nutrient starvation. Curr Top Microbiol Immunol. 279:73-84.

254. Turi Z, Lacey M, Mistrik M, Moudry P. (2019) Impaired ribosome biogenesis: mechanisms and relevance to cancer and aging. Aging (Albany NY). 11:2512-2540.

255. Buchwalter A, Hetzer MW. (2017) Nucleolar expansion and elevated protein translation in premature aging. Nat Commun. 8:328.

256. Tiku V, Antebi A. (2018) Nucleolar Function in Lifespan Regulation. Trends Cell Biol. Aug;28:662-672.

257. Helmrich A, Ballarino M, Tora L. (2011) Collisions between replication and transcription complexes cause common fragile site instability at the longest human genes. Mol Cell. 44:966-77.

258. Lill R. (2009) Function and biogenesis of iron-sulphur proteins. Nature. 460:831-8.

259. Lewinska A, Macierzynska E, Grzelak A, Bartosz G. (2011) A genetic analysis of nitric oxide-mediated signaling during chronological aging in the yeast. Biogerontology. 12:309-20.

260. Fabrizio P, Liou LL, Moy VN, Diaspro A, Valentine JS, Gralla EB, Longo VD. (2003) SOD2 functions downstream of Sch9 to extend longevity in yeast. Genetics. 163:35-46.

261. Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, Bohr VA. (2019) Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol. 15:565-581.

262. Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, Stoynov S, Mahamid J, Saha S, Franzmann TM, Pozniakovski A, Poser I, Maghelli N, Royer LA, Weigert M, Myers EW, Grill S, Drechsel D, Hyman AA, Alberti S. (2015) A Liquid-to-Solid Phase Transition of the ALS Protein FUS Accelerated by Disease Mutation. Cell. 162:1066-77.

263. Maiuri T, Suart CE, Hung CLK, Graham KJ, Barba Bazan CA, Truant R. (2019) DNA Damage Repair in Huntington's Disease and Other Neurodegenerative Diseases. Neurotherapeutics. 16:948-956.

264. Mangialasche F, Polidori MC, Monastero R, Ercolani S, Camarda C, Cecchetti R, Mecocci P. (2009) Biomarkers of oxidative and nitrosative damage in Alzheimer's disease and mild cognitive impairment. Ageing Res Rev. 8:285-305.

265. Price DL, Cork LC, Struble RG, Kitt CA, Walker LC, Powers RE, Whitehouse PJ, Griffin JW. (1987) Dysfunction and death of neurons in human degenerative neurological diseases and in animal models. Ciba Found Symp. 126:30-48.

266. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. (2006) Biomarkers of oxidative damage in human disease. Clin Chem. 52:601-23.

267. Shishehbor MH, Aviles RJ, Brennan ML, Fu X, Goormastic M, Pearce GL, Gokce N, Keaney JF Jr, Penn MS, Sprecher DL, Vita JA, Hazen SL. (2003) Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy. JAMA. 289:1675-80.

268. Bloom GS. (2014) Amyloid-β and tau: the trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol. 71:505-8.

269. Goedert M, Spillantini MG, Jakes R, Rutherford D, Crowther RA. (1989) Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron. 3:519-26.

270. Reynolds MR, Berry RW, Binder LI. (2005)Site-specific nitration differentially influences tau assembly in vitro. Biochemistry. 44:13997-4009.

271. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI, Cryns VL. (2003) Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. Proc Natl Acad Sci U S A. 100:10032-7.

272. Chen X, Yan SD. (2006) Mitochondrial Abeta: a potential cause of metabolic dysfunction in Alzheimer's disease. IUBMB Life 58:686-94.

273. Crouch PJ, White AR, Bush AI. (2007) The modulation of metal bio-availability as a therapeutic strategy for the treatment of Alzheimer's disease. FEBS J. 274:3775-83.

274. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR. (1998) Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci. 158:47-52.

275. Moreira PI, Nunomura A, Nakamura M, Takeda A, Shenk JC, Aliev G, Smith MA, Perry G. (2008) Nucleic acid oxidation in Alzheimer disease. Free Radic Biol Med. 44:1493-505.

276. Dröge W. (2002) Free radicals in the physiological control of cell function. Physiol Rev. 82:47-95.

277. Schopfer FJ, Baker PR, Freeman BA. (2003) NO-dependent protein nitration: a cell signaling event or an oxidative inflammatory response? Trends Biochem Sci. 28:646-54.

278. Rizor A, Pajarillo E, Johnson J, Aschner M, Lee E. (2019) Astrocytic Oxidative/Nitrosative Stress Contributes to Parkinson's Disease Pathogenesis: The Dual Role of Reactive Astrocytes. Antioxidants (Basel). 8:265.

279. Muñoz E, Oliva R, Obach V, Martí MJ, Pastor P, Ballesta F, Tolosa E. (1997) Identification of Spanish familial Parkinson's disease and screening for the Ala53Thr mutation of the alpha-synuclein gene in early onset patients. Neurosci Lett. 235:57-60.

280. Tsang AH, Chung KK. (2009) Oxidative and nitrosative stress in Parkinson's disease. Biochim Biophys Acta. 1792:643-50.

281. Chinta SJ, Andersen JK. (2008) Redox imbalance in Parkinson's disease. Biochim Biophys Acta. 1780:1362-7.

282. McBean GJ. (2017) Cysteine, Glutathione, and Thiol Redox Balance in Astrocytes. Antioxidants (Basel). 6:62.

283. Beal MF. (1998) Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. Ann Neurol. 44:S110-4.

284. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM. (2000) Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science. Nov 290:985-9.

285. Chavarría C, Souza JM. (2013) Oxidation and nitration of α-synuclein and their implications in neurodegenerative diseases. Arch Biochem Biophys. 533:25-32.

286. Souza JM, Giasson BI, Chen Q, Lee VM, Ischiropoulos H. (2000) Dityrosine cross-linking promotes formation of stable alpha -synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. J Biol Chem. 275:18344-9.

287. Hodara R, Norris EH, Giasson BI, Mishizen-Eberz AJ, Lynch DR, Lee VM, Ischiropoulos H. (2004) Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. J Biol Chem. 279:47746-53.

288. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, Nemachek C, Ciborowski P, Przedborski S, Mosley RL, Gendelman HE. (2008) Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. PLoS One. 3:e1376.

289. Bassani TB, Vital MA, Rauh LK. (2015) Neuroinflammation in the pathophysiology of Parkinson's disease and therapeutic evidence of anti-inflammatory drugs. Arq Neuropsiquiatr. 73:616-23.

290. Dawson TM, Dawson VL. (2010) The role of parkin in familial and sporadic Parkinson's disease. Mov Disord. 1:S32-9.

291. Chung KK, Thomas B, Li X, Pletnikova O, Troncoso JC, Marsh L, Dawson VL, Dawson TM. (2004) *S*-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. Science. 304:1328-31.

292. Yao D, Gu Z, Nakamura T, Shi ZQ, Ma Y, Gaston B, Palmer LA, Rockenstein EM, Zhang Z, Masliah E, Uehara T, Lipton SA. (2004) Nitrosative stress linked to sporadic Parkinson's disease: *S*-nitrosylation of parkin regulates its E3 ubiquitin ligase activity. Proc Natl Acad Sci U S A. 101:10810-4.

293. Waak J, Weber SS, Waldenmaier A, Görner K, Alunni-Fabbroni M, Schell H, Vogt-Weisenhorn D, Pham TT, Reumers V, Baekelandt V, Wurst W, Kahle PJ. (2009) Regulation of astrocyte inflammatory responses by the Parkinson's disease-associated gene DJ-1. FASEB J. 23:2478-89.

294. Boje KM. (2004) Nitric oxide neurotoxicity in neurodegenerative diseases. Front Biosci. 9:763-76.

295. Deckel AW. (2001) Nitric oxide and nitric oxide synthase in Huntington's disease. J Neurosci Res. 64:99-107.

296. Sugars KL, Rubinsztein DC. (2003) Transcriptional abnormalities in Huntington disease. Trends Genet. 19:233-8.

297. (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell. 72:971-83.

298. Deckel AW, Gordinier A, Nuttal D, Tang V, Kuwada C, Freitas R, Gary KA. (2001) Reduced activity and protein expression of NOS in R6/2 HD transgenic mice: effects of L-NAME on symptom progression. Brain Res. 919:70-81.

299. Pérez-Severiano F, Escalante B, Vergara P, Ríos C, Segovia J. (2002) Age-dependent changes in nitric oxide synthase activity and protein expression in striata of mice transgenic for the Huntington's disease mutation. Brain Res. 951:36-42.

300. Deckel AW, Cohen D. (2000) Increased CBF velocity during word fluency in Huntington's disease patients. Prog Neuropsychopharmacol Biol Psychiatry. 24:193-206.

301. Ghasemi M, Mayasi Y, Hannoun A, Eslami SM, Carandang R. (2018) Nitric Oxide and Mitochondrial Function in Neurological Diseases. Neuroscience. 376:48-71.

302. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, Bird ED, Beal MF. (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol. 41:646-53.

303. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. (1996) Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol. 39:385-9.

304. Mann VM, Cooper JM, Javoy-Agid F, Agid Y, Jenner P, Schapira AH. (1990) Mitochondrial function and parental sex effect in Huntington's disease. Lancet. 336:749.

305. Carrizzo A, Di Pardo A, Maglione V, Damato A, Amico E, Formisano L, Vecchione C, Squitieri F. (2014) Nitric oxide dysregulation in platelets from patients with advanced Huntington disease. PLoS One. 9:e89745.

306. D'Amico E, Factor-Litvak P, Santella RM, Mitsumoto H. (2013) Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. Free Radic Biol Med. 65:509-527.

307. Carrera-Juliá S, Moreno ML, Barrios C, de la Rubia Ortí JE, Drehmer E. (2020) Antioxidant Alternatives in the Treatment of Amyotrophic Lateral Sclerosis: A Comprehensive Review. Front Physiol. 11:63.

308. Schon EA, Manfredi G. (2003) Neuronal degeneration and mitochondrial dysfunction. J Clin Invest. 111:303-12.

309. Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E. (2012) Mitochondria, oxidative stress and neurodegeneration. J Neurol Sci. 1322:254-62.

310. Bosco DA, Morfini G, Karabacak NM, Song Y, Gros-Louis F, Pasinelli P, Goolsby H, Fontaine BA, Lemay N, McKenna-Yasek D, Frosch MP, Agar JN, Julien JP, Brady ST, Brown RH Jr. (2010) Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat Neurosci. 13:1396-403.

311. Forsberg K, Jonsson PA, Andersen PM, Bergemalm D, Graffmo KS, Hultdin M, Jacobsson J, Rosquist R, Marklund SL, Brännström T. (2010) Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One. 5:e11552.

312. Li Q, Spencer NY, Pantazis NJ, Engelhardt JF. (2011) Alsin and SOD1(G93A) proteins regulate endosomal reactive oxygen species production by glial cells and proinflammatory pathways responsible for neurotoxicity. J Biol Chem. 286:40151-62.

313. Mattiazzi M, D'Aurelio M, Gajewski CD, Martushova K, Kiaei M, Beal MF, Manfredi G. (2002) Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J Biol Chem. 277:29626-33.

314. Pehar M, Beeson G, Beeson CC, Johnson JA, Vargas MR. (2014) Mitochondria-targeted catalase reverts the neurotoxicity of hSOD1G^{93}A astrocytes without extending the survival of ALS-linked mutant hSOD1 mice. PLoS One. 9:e103438.

315. Israelson A, Arbel N, Da Cruz S, Ilieva H, Yamanaka K, Shoshan-Barmatz V, Cleveland DW. (2010) Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. Neuron. 67:575-87.

316. Pickles S, Destroismaisons L, Peyrard SL, Cadot S, Rouleau GA, Brown RH Jr, Julien JP, Arbour N, Vande Velde C. (2013) Mitochondrial damage revealed by immunoselection for ALS-linked misfolded SOD1. Hum Mol Genet. 22:3947-59.

317. Pickles S, Semmler S, Broom HR, Destroismaisons L, Legroux L, Arbour N, Meiering E, Cashman NR, Vande Velde C. (2016) ALS-linked misfolded SOD1 species have divergent impacts on mitochondria. Acta Neuropathol Commun. 4:43.

318. Richardson K, Allen SP, Mortiboys H, Grierson AJ, Wharton SB, Ince PG, Shaw PJ, Heath PR. (2013) The effect of SOD1 mutation on cellular bioenergetic profile and viability in response to oxidative stress and influence of mutation-type. PLoS One. 8:e68256.

319. Golenia A, Leśkiewicz M, Regulska M, Budziszewska B, Szczęsny E, Jagiełła J, Wnuk M, Ostrowska M, Lasoń W, Basta-Kaim A, Słowik A. (2014) Catalase activity in blood fractions of patients with sporadic ALS. Pharmacol Rep. 66:704-7.

320. Abe K, Pan LH, Watanabe M, Konno H, Kato T, Itoyama Y. (1997) Upregulation of protein-tyrosine nitration in the anterior horn cells of amyotrophic lateral sclerosis. Neurol Res. 19:124-8.

321. Casoni F, Basso M, Massignan T, Gianazza E, Cheroni C, Salmona M, Bendotti C, Bonetto V. (2005) Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: possible multifunctional role in the pathogenesis. J Biol Chem. 280:16295-304.

322. Bruijn LI, Beal MF, Becher MW, Schulz JB, Wong PC, Price DL, Cleveland DW. (1997) Elevated free nitrotyrosine levels, but not protein-bound nitrotyrosine or hydroxyl radicals, throughout amyotrophic lateral sclerosis (ALS)-like disease implicate tyrosine nitration as an aberrant in vivo property of one familial ALS-linked superoxide dismutase 1 mutant. Proc Natl Acad Sci U S A. 94:7606-11.

323. Yoshino H, Kimura A. (2006) Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). Amyotroph Lateral Scler. 7:241-5.

324. Nardo G, Pozzi S, Pignataro M, Lauranzano E, Spano G, Garbelli S, Mantovani S, Marinou K, Papetti L, Monteforte M, Torri V, Paris L, Bazzoni G, Lunetta C, Corbo M, Mora G, Bendotti C, Bonetto V. (2011) Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. PLoS One. 6:e25545.

325. Radak Z, Zhao Z, Goto S, Koltai E. (2011) Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA. Mol Aspects Med. 32:305-15.

326. Catalá A. (2006) An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. Int J Biochem Cell Biol. 38:1482-95.

327. Farooqui AA, Horrocks LA. (2006) Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist. 12:245-60.

328. Eckl PM. (2003) Genotoxicity of HNE. Mol Aspects Med. 24:161-5.

329. Doulias PT, Tenopoulou M, Greene JL, Raju K, Ischiropoulos H. (2013) Nitric oxide regulates mitochondrial fatty acid metabolism through reversible protein *S*-nitrosylation. Sci Signal. 6:rs1.

330. Lee YI, Giovinazzo D, Kang HC, Lee Y, Jeong JS, Doulias PT, Xie Z, Hu J, Ghasemi M, Ischiropoulos H, Qian J, Zhu H, Blackshaw S, Dawson VL, Dawson TM. (2014) Protein microarray characterization of the *S*-nitrosoproteome. Mol Cell Proteomics. 13:63-72.

331. Tórtora V, Quijano C, Freeman B, Radi R, Castro L. (2007) Mitochondrial aconitase reaction with nitric oxide, *S*-nitrosoglutathione, and peroxynitrite: mechanisms and relative contributions to aconitase inactivation. Free Radic Biol Med. 42:1075-88.

332. Gupta KJ, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, Bauwe H, (2012) Igamberdiev AU. Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot. 63:1773-84.

333. Yang ES, Richter C, Chun JS, Huh TL, Kang SS, Park JW. (2002) Inactivation of NADP(+)-dependent isocitrate dehydrogenase by nitric oxide. Free Radic Biol Med. 33:927-37.

334. Lee JH, Yang ES, Park JW. (2003) Inactivation of NADP+-dependent isocitrate dehydrogenase by peroxynitrite. Implications for cytotoxicity and alcohol-induced liver injury. J Biol Chem. 278:51360-71.

335. Sun J, Morgan M, Shen RF, Steenbergen C, Murphy E. (2007) Preconditioning results in *S*-nitrosylation of proteins involved in regulation of mitochondrial energetics and calcium transport. Circ Res. 101:1155-63.

336. Chouchani ET, Hurd TR, Nadtochiy SM, Brookes PS, Fearnley IM, Lilley KS, Smith RA, Murphy MP. (2010) Identification of *S*-nitrosated mitochondrial proteins by *S*-nitrosothiol difference in gel electrophoresis (SNO-DIGE): implications for the regulation of mitochondrial function by reversible *S*-nitrosation. Biochem J. 430:49-59.

337. Yarian CS, Rebrin I, Sohal RS. (2005) Aconitase and ATP synthase are targets of malondialdehyde modification and undergo an age-related decrease in activity in mouse heart mitochondria. Biochem Biophys Res Commun. 330:151-6.

338. Elfering SL, Haynes VL, Traaseth NJ, Ettl A, Giulivi C. (2004) Aspects, mechanism, and biological relevance of mitochondrial protein nitration sustained by mitochondrial nitric oxide synthase. Am J Physiol Heart Circ Physiol. 286:H22-9.

339. Zhang Y, Lu N, Gao Z. (2009) Hemin-H2O2-NO2(-) induced protein oxidation and tyrosine nitration are different from those of SIN-1: a study on glutamate dehydrogenase nitrative/oxidative modification. Int J Biochem Cell Biol. 41:907-15.

340. Chen Z, Zhong C. (2013) Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. Prog Neurobiol. 108:21-43.

341. Gibson GE, Kingsbury AE, Xu H, Lindsay JG, Daniel S, Foster OJ, Lees AJ, Blass JP. (2003) Deficits in a tricarboxylic acid cycle enzyme in brains from patients with Parkinson's disease. Neurochem Int. 43:129-35.

342. Naseri NN, Xu H, Bonica J, Vonsattel JP, Cortes EP, Park LC, Arjomand J, Gibson GE. (2015) Abnormalities in the tricarboxylic Acid cycle in Huntington disease and in a Huntington disease mouse model. J Neuropathol Exp Neurol. 74:527-37.

343. Veyrat-Durebex C, Corcia P, Piver E, Devos D, Dangoumau A, Gouel F, Vourc'h P, Emond P, Laumonnier F, Nadal-Desbarats L, Gordon PH, Andres CR, Blasco H. (2016) Disruption of TCA Cycle and Glutamate Metabolism Identified by Metabolomics in an In Vitro Model of Amyotrophic Lateral Sclerosis. Mol Neurobiol. 53:6910-6924.

344. Auger C, Lemire J, Cecchini D, Bignucolo A, Appanna VD. (2011) The metabolic reprogramming evoked by nitrosative stress triggers the anaerobic utilization of citrate in Pseudomonas fluorescens. PLoS One. 6:e28469.

345. Aquilano K, Baldelli S, Ciriolo MR. (2014) Glutathione: new roles in redox signaling for an old antioxidant. Front Pharmacol. 5:196.

346. Dickinson DA, Forman HJ. (2002) Cellular glutathione and thiols metabolism. Biochem Pharmacol. 64:1019-26.

347. Atakisi O, Erdogan HM, Atakisi E, Citil M, Kanici A, Merhan O, Uzun M. (2010) Effects of reduced glutathione on nitric oxide level, total antioxidant and oxidant capacity and adenosine deaminase activity. Eur Rev Med Pharmacol Sci. 14:19-23.

348. Sies H, Sharov VS, Klotz LO, Briviba K. (1997) Glutathione peroxidase protects against peroxynitrite-mediated oxidations. A new function for selenoproteins as peroxynitrite reductase. J Biol Chem. 272:27812-7.

349. Beatrice MC, Stiers DL, Pfeiffer DR. (1984) The role of glutathione in the retention of Ca2+ by liver mitochondria. J Biol Chem. 259:1279-87.

350. Savage MK, Jones DP, Reed DJ. (1991) Calcium- and phosphate-dependent release and loading of glutathione by liver mitochondria. Arch Biochem Biophys. 290:51-6.

351. Savage MK, Reed DJ. (1994) Release of mitochondrial glutathione and calcium by a cyclosporin A-sensitive mechanism occurs without large amplitude swelling. Arch Biochem Biophys. 315:142-52.

352. de la Asuncion JG, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, Sastre J, Viña J. (1996) Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. FASEB J. 10:333-8.

353. Esteve JM, Mompo J, Garcia de la Asuncion J, Sastre J, Asensi M, Boix J, Vina JR, Vina J, Pallardo FV. (1999) Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies in vivo and in vitro. FASEB J. 13:1055-64.

354. Chernyak BV, Bernardi P. (1996) The mitochondrial permeability transition pore is modulated by oxidative agents through both pyridine nucleotides and glutathione at two separate sites. Eur J Biochem. 238:623-30.

355. Scarlett JL, Packer MA, Porteous CM, Murphy MP. (1996) Alterations to glutathione and nicotinamide nucleotides during the mitochondrial permeability transition induced by peroxynitrite. Biochem Pharmacol. 52:1047-55.

356. Klatt P, Molina EP, De Lacoba MG, Padilla CA, Martinez-Galesteo E, Barcena JA, Lamas S. (1999) Redox regulation of c-Jun DNA binding by reversible S-glutathiolation. FASEB J. 13:1481-90.

357. Wu AL, Moye-Rowley WS. (1994) GSH1, which encodes gamma-glutamylcysteine synthetase, is a target gene for yAP-1 transcriptional regulation. Mol Cell Biol. 14:5832-9.

358. Chaudhuri B, Ingavale S, Bachhawat AK. (1997) apd1+, a gene required for red pigment formation in ade6 mutants of Schizosaccharomyces pombe, encodes an enzyme required for glutathione biosynthesis: a role for glutathione and a glutathione-conjugate pump. Genetics. 145:75-83.

359. Miyagi H, Kawai S, Murata K. (2009) Two sources of mitochondrial NADPH in the yeast Saccharomyces cerevisiae. J Biol Chem. 284:7553-60.

360. Xiao W, Wang RS, Handy DE, Loscalzo J. (2018) NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. Antioxid Redox Signal. 28:251-272.

361. Kurutas EB. (2016) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J. 15:71.

362. Wang X, Quinn PJ. (2000) The location and function of vitamin E in membranes (review). Mol Membr Biol. 17:143-56.

363. Kojo S. (2004) Vitamin C: basic metabolism and its function as an index of oxidative stress. Curr Med Chem. 11:1041-64.

364. Packer L, Roy S, Sen CK. (1997) Alpha-lipoic acid: a metabolic antioxidant and potential redox modulator of transcription. Adv Pharmacol. 38:79-101.

365. Senoglu M, Nacitarhan V, Kurutas EB, Senoglu N, Altun I, Atli Y, Ozbag D. (2009) Intraperitoneal Alpha-Lipoic Acid to prevent neural damage after crush injury to the rat sciatic nerve. J Brachial Plex Peripher Nerve Inj. 4:22.

366. Reiter RJ, Acuña-Castroviejo D, Tan DX, Burkhardt S. (2001) Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. Ann N Y Acad Sci. 939:200-15.

367. Fiedor J, Burda K. (2014) Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 6:466-88.

368. Rice-Evans CA, Miller NJ, Paganga G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 20:933-56.

369. Lindemann C, Lupilova N, Müller A, Warscheid B, Meyer HE, Kuhlmann K, Eisenacher M, Leichert LI. (2013) Redox proteomics uncovers peroxynitrite-sensitive proteins that help Escherichia coli to overcome nitrosative stress. J Biol Chem. 288:19698-714.

370. Gardner PR. (2005) Nitric oxide dioxygenase function and mechanism of flavohemoglobin, hemoglobin, myoglobin and their associated reductases. J Inorg Biochem. 99:247-66.

371. Gardner PR. (2012) Hemoglobin: a nitric-oxide dioxygenase. Scientifica (Cairo).; 2012:683729.

372. Ullmann BD, Myers H, Chiranand W, Lazzell AL, Zhao Q, Vega LA, Lopez-Ribot JL, Gardner PR, Gustin MC. (2004) Inducible defense mechanism against nitric oxide in Candida albicans. Eukaryot Cell. 3:715-23.

373. Hromatka BS, Noble SM, Johnson AD. (2005) Transcriptional response of Candida albicans to nitric oxide and the role of the YHB1 gene in nitrosative stress and virulence. Mol Biol Cell. 16:4814-26.

374. Gardner PR, Gardner AM, Martin LA, Dou Y, Li T, Olson JS, Zhu H, Riggs AF. (2000) Nitric-oxide dioxygenase activity and function of flavohemoglobins. sensitivity to nitric oxide and carbon monoxide inhibition. J Biol Chem. 275:31581-7.

375. Helmick RA, Fletcher AE, Gardner AM, Gessner CR, Hvitved AN, Gustin MC, Gardner PR. (2005) Imidazole antibiotics inhibit the nitric oxide dioxygenase function of microbial flavohemoglobin. Antimicrob Agents Chemother. 49:1837-43.

376. Lewinska A, Bartosz G. (2006) Yeast flavohemoglobin protects against nitrosative stress and controls ferric reductase activity. Redox Rep. 11:231-9.

377. Brown GC. (2007) Nitric oxide and mitochondria. Front Biosci. 12:1024-33.

378. Rocco NM, Carmen JC, Klein BS. (2011) Blastomyces dermatitidis yeast cells inhibit nitric oxide production by alveolar macrophage inducible nitric oxide synthase. Infect Immun. 79:2385-95.

379. Cassanova N, O'Brien KM, Stahl BT, McClure T, Poyton RO. (2005) Yeast flavohemoglobin, a nitric oxide oxidoreductase, is located in both the cytosol and the mitochondrial matrix: effects of respiration, anoxia, and the mitochondrial genome on its intracellular level and distribution. J Biol Chem. 280:7645-53.

380. Liu L, Hausladen A, Zeng M, Que L, Heitman J, Stamler JS. (2001) A metabolic enzyme for *S*-nitrosothiol conserved from bacteria to humans. Nature. 410:490-4.

381. Xu S, Guerra D, Lee U, Vierling E. (2013) *S*-nitrosoglutathione reductases are low-copy number, cysteine-rich proteins in plants that control multiple developmental and defense responses in Arabidopsis. Front Plant Sci. 4:430.

382. Tillmann AT, Strijbis K, Cameron G, Radmaneshfar E, Thiel M, Munro CA, MacCallum DM, Distel B, Gow NA, Brown AJ. (2015) Contribution of Fdh3 and Glr1 to Glutathione Redox State, Stress Adaptation and Virulence in Candida albicans. PLoS One. 10:e0126940.

383. Guerra D, Ballard K, Truebridge I, Vierling E. (2016) *S*-Nitrosation of Conserved Cysteines Modulates Activity and Stability of *S*-Nitrosoglutathione Reductase (GSNOR). Biochemistry. 55:2452-64.

384. Jensen DE, Belka GK, Du Bois GC. (1998) *S*-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. Biochem J. 331:659-68.

385. Lee U, Wie C, Fernandez BO, Feelisch M, Vierling E. (2008) Modulation of nitrosative stress by *S*-nitrosoglutathione reductase is critical for thermotolerance and plant growth in Arabidopsis. Plant Cell. 20:786-802.

386. Luebke JL, Giedroc DP. (2015) Cysteine sulfur chemistry in transcriptional regulators at the host-bacterial pathogen interface. Biochemistry. 54:3235-49.

387. Li B, Skinner C, Castello PR, Kato M, Easlon E, Xie L, Li T, Lu SP, Wang C, Tsang F, Poyton RO, Lin SJ. (2011) Identification of potential calorie

restriction-mimicking yeast mutants with increased mitochondrial respiratory chain and nitric oxide levels. J Aging Res. 2011:673185.

388. Sahoo R, Dutta T, Das A, Sinha Ray S, Sengupta R, Ghosh S. (2006) Effect of nitrosative stress on Schizosaccharomyces pombe: inactivation of glutathione reductase by peroxynitrite. Free Radic Biol Med. 40:625-31.

389. Sies H. (2015) Oxidative stress: a concept in redox biology and medicine. Redox Biol. 4:180-3.

390. Sahoo R, Bhattacharjee A, Majumdar U, Ray SS, Dutta T, Ghosh S. (2009) A novel role of catalase in detoxification of peroxynitrite in S. cerevisiae. Biochem Biophys Res Commun. 385:507-11.

391. Lushchak OV, Lushchak VI. (2008) Catalase modifies yeast Saccharomyces cerevisiae response towards *S*-nitrosoglutathione-induced stress. Redox Rep. 13:283-91.

392. Carlberg I, Mannervik B. (1975) Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem. 250:5475-80.

393. Song JY, Cha J, Lee J, Roe JH. (2006) Glutathione reductase and a mitochondrial thioredoxin play overlapping roles in maintaining iron-sulfur enzymes in fission yeast. Eukaryot Cell. 5:1857-65.

394. Tuggle CK, Fuchs JA. (1985) Glutathione reductase is not required for maintenance of reduced glutathione in *Escherichia coli* K-12. J Bacteriol. 162:448-50.

395. Russel M, Holmgren A. (1988) Construction and characterization of glutaredoxin-negative mutants of *Escherichia coli*. Proc Natl Acad Sci U S A. 85:990-4.

396. Sheng Y, Abreu IA, Cabelli DE, Maroney MJ, Miller AF, Teixeira M, Valentine JS. (2014) Superoxide dismutases and superoxide reductases. Chem Rev. Apr 9;114(7):3854-918.

397. Molavian H, Madani Tonekaboni A, Kohandel M, Sivaloganathan S. (2015) The Synergetic Coupling among the Cellular Antioxidants Glutathione Peroxidase/Peroxiredoxin and Other Antioxidants and its Effect on the Concentration of H_2O_2. Sci Rep. 5:13620.

398. Mohammadi S, Saberidokht B, Subramaniam S, Grama A. (2015) Scope and limitations of yeast as a model organism for studying human tissue-specific pathways. BMC Syst Biol. 9:96.

399. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG. (1996) Life with 6000 genes. Science. 274:563-7.

400. Lashkari DA, DeRisi JL, McCusker JH, Namath AF, Gentile C, Hwang SY, Brown PO, Davis RW. (1997) Yeast microarrays for genome wide parallel genetic and gene expression analysis. Proc Natl Acad Sci U S A. 94:13057-62.

401. DeRisi JL, Iyer VR, Brown PO. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. Science. 278:680-6.

402. Cho RJ, Campbell MJ, Winzeler EA, Steinmetz L, Conway A, Wodicka L, Wolfsberg TG, Gabrielian AE, Landsman D, Lockhart DJ, Davis RW. (1998) A genome-wide transcriptional analysis of the mitotic cell cycle. Mol Cell. 2:65-73.

403. Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Bidlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M. Global analysis of protein activities using proteome chips. (2001) Science. 293:2101-5.

404. Villas-Bôas SG, Moxley JF, Akesson M, Stephanopoulos G, Nielsen J. (2005) High-throughput metabolic state analysis: the missing link in integrated functional genomics of yeasts. Biochem J. 388:669-77.

405. Jewett MC, Hofmann G, Nielsen J. (2006) Fungal metabolite analysis in genomics and phenomics. Curr Opin Biotechnol. 17:191-7.

406. Mitchison JM. (1990) The fission yeast, Schizosaccharomyces pombe. Bioessays. 12:189-91. doi: 10.1002/bies.950120409.

407. Mitchison JM, Nurse P. (1985) Growth in cell length in the fission yeast Schizosaccharomyces pombe. J Cell Sci. 75:357-76.

408. Mitchison JM. (1957) The growth of single cells. I. *Schizosaccharomyces pombe.* Exp Cell Res. 13:244-62.

409. Fantes PA. (1977) Control of cell size and cycle time in Schizosaccharomyces pombe. J Cell Sci. 24:51-67.

410. Wood V, *et al*. (2002) The genome sequence of Schizosaccharomyces pombe. Nature. 415:871-80.

411. Hoyt MA, Totis L, Roberts BT. (1991) *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. Cell. 66:507-17.

412. al-Khodairy F, Carr AM. (1992) DNA repair mutants defining G2 checkpoint pathways in *Schizosaccharomyces pombe*. EMBO J. 11:1343-50.

413. Guaragnella N, Palermo V, Galli A, Moro L, Mazzoni C, Giannattasio S. (2014) The expanding role of yeast in cancer research and diagnosis: insights into the function of the oncosuppressors p53 and BRCA1/2. FEMS Yeast Res.14:2-16.

414. Ferreira R, Limeta A, Nielsen J. (2019) Tackling Cancer with Yeast-Based Technologies. Trends Biotechnol. 37:592-603.

415. Miller-Fleming L, Giorgini F, Outeiro TF. (2008) Yeast as a model for studying human neurodegenerative disorders. Biotechnol J. 3:325-38.

416. Cervelli T, Lodovichi S, Bellè F, Galli A. (2020) Yeast-based assays for the functional characterization of cancer-associated variants of human DNA repair genes. Microb Cell. 7:162-174.

417. Pérez-Torres I, Manzano-Pech L, Rubio-Ruíz ME, Soto ME, Guarner-Lans V. (2020) Nitrosative Stress and Its Association with Cardiometabolic Disorders. Molecules. 25:2555.

418. Chatterji A, Banerjee D, Billiar TR, Sengupta R. (2021) Understanding the role of *S*-nitrosylation/nitrosative stress in inflammation and the role of cellular denitrosylases in inflammation modulation: Implications in health and diseases. Free Radic Biol Med. 172:604-621.

419. Lushchak OV, Inoue Y, Lushchak VI. (2010) Regulatory protein Yap1 is involved in response of yeast Saccharomyces cerevisiae to nitrosative stress. Biochemistry (Mosc). 75:629-64.

420. Merhej J, Delaveau T, Guitard J, Palancade B, Hennequin C, Garcia M, Lelandais G, Devaux F. (2015) Yap7 is a transcriptional repressor of nitric oxide oxidase in yeasts, which arose from neofunctionalization after whole genome duplication. Mol Microbiol. 96:951-72.

421. Merhej J, Thiebaut A, Blugeon C, Pouch J, Ali Chaouche Mel A, Camadro JM, Le Crom S, Lelandais G, Devaux F. (2016) A Network of Paralogous Stress Response Transcription Factors in the Human Pathogen Candida glabrata. Front Microbiol. 7:645.

422. Sellam A, Tebbji F, Whiteway M, Nantel A. (2012) A novel role for the transcription factor Cwt1p as a negative regulator of nitrosative stress in Candida albicans. PLoS One. 7:e43956.

423. Anam K, Nasuno R, Takagi H. (2020) A Novel Mechanism for Nitrosative Stress Tolerance Dependent on GTP Cyclohydrolase II Activity Involved in Riboflavin Synthesis of Yeast. Sci Rep. 10:6015.

424. Kar P, Biswas P, Patra SK, Ghosh S. (2018) Transcription factors Atf1 and Sty1 promote stress tolerance under nitrosative stress in *Schizosaccharomyces pombe*. Microbiol Res. 206:82-90.

Objectives of the study...

Objectives of the study:

Reactive oxygen species (ROS) can be generated in the yeast cells due to the physiochemical activities of it and subsequently reactive nitrogen species (RNS) are formed. Nitric oxide (NO) reacts with ROS or molecular oxygen (O_2) and RNS like peroxynitrite ($ONOO^-$), nitrogen trioxide (N_2O_3), nitrite (NO_2^-) and nitrate (NO_3^-) are formed. Accumulation of such RNS, may lead to damage of the important macromolecules like enzymes, proteins, lipids, DNA etc., this hostile condition is known as nitrosative stress, in analogy with the 'oxidative stress'. Due to the alteration of the structure of important macromolecules and redox homeostasis, electron transport chain as well as metabolism may be affected. Thus, the work was planned to characterize the effect of nitrosative stress on ethanol production using *Saccharomyces cerevisiae*, one of the best system to study the effect of nitrosative stress as its genome sequence has been well characterized and an excellent background on nitrosative stress studies. Hence, the work was performed with the following objectives:

- Determination of the cell viability and growth of *Saccharomyces cerevisiae* under nitrosative stress.
- Characterization of physiochemical properties of *Saccharomyces cerevisiae* under nitrosative stress.
- Quantification and analysis of the ethanol production by *Saccharomyces cerevisiae* under nitrosative stress.
- Optimization of ethanol production using immobilized stressed *Saccharomyces* cells.

Materials and methods...

1. Strain and media:

Sachharomyces cerevisiae Y190 (ATCC 96400) was used in this study. The strain was grown in YPD medium (2% W\V Yeast extract [HiMedia], 2% W\V peptone [HiMedia] and 2% W\V dextrose [Merck]), YPG medium (2% W\V yeast extract, 2% W\V peptone, and 3% V/V glycerol) and minimal media containing different concentration of molasses and ammonium sulphate was used for the experiment based on CCRD-RSM. Strains were grown at 30˚C under shaking condition (80 RPM). The strain was preserved in 50% glycerol stocks at -20°C freezer. The glycerol stock was used for the preparation of preinoculum. 200 µL from the glycerol stock was inoculated in a fresh YPD broth and incubated overnight at 30°C. Then, streak plating was performed on YPD agar plate using the overnight grown culture and incubated overnight at 30°C to isolate single colonies. After that, the culture was checked for contamination by phase contrast microscopy. Following that, pre-inoculum was prepared by inoculating single isolated colony in YPD broth and again incubated overnight at 30°C. The overnight grown *S. cerevisiae* cells were then used as inoculum for further experiments and initial $O.D._{600}$~0.05 was maintained for each of the samples.

2. Preparation of acidified sodium nitrite:

100 mM acidified sodium nitrite (ac. $NaNO_2$) stock solution was prepared by mixing dissolved $NaNO_2$ (Sigma-Aldrich) in double distilled (DdH_2O) with concentrated HCl in a 1:1 ratio (V/V) [1]. Effective concentrations (0.5 mM, 1 mM, and 3 mM) of acidified sodium nitrite was prepared from the stock solution for the experiments.

3. Preparation of *S*-nitrosoglutathione:

GSNO was prepared as per the method of Hart with slight modifications [2]. In brief, 0.5 M GSNO was obtained by mixing 1 M of $NaNO_2$ (Sigma-Aldrich) in DdH_2O and 1 M GSH (Himedia) in 1 N HCl in cold (1:1 V/V). The concentration of GSNO was determined spectrophotometrically (ThermoScientific MultiskanGO) at 335 nm (ε = 922 $M^{-1}cm^{-1}$).

4. Cell viability assay:

S. cerevisiae cells were grown in YPD medium and treated with different concentrations of ac. $NaNO_2$ (0.5 mM, 1 mM, 3 mM)) and GSNO (0.25 mM, 0.5 mM, 1 mM) at the early log phase ($O.D._{600}$~0.3). Following an overnight incubation, 1 ml of culture from each sample was serially diluted and plated on YPD agar medium for viable cell count. As a control, a culture without GSNO or ac. $NaNO_2$ treatment was used. The growth curve was created by recording the O.D. at 600 nm for 11 hours at 60-min intervals [3]. The growth curve was used to calculate the specific growth rate using $\mathbf{Y=Ae^{BX}}$ formula where Y= Final cell count, A= Initial cell count, B=growth rate, X= O.D. value.

5. Preparation of cell-free extracts (CFE) and estimation of total protein:

Cell-free extract (CFE) of treated and control or untreated cultures were prepared for different enzymatic assays. Overnight grown cultures of treated and untreated samples were centrifuged, and the supernatants were discarded. The cell pellets were lysed by using glass beads, 425-600 μM diameter (Sigma) and lysis buffer containing 100 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1 mM SDS, 2 mM EDTA, 0.1% protease inhibitor cocktail (Sigma-Aldrich), and 1 mM PMSF [4]. CFEs were used for all the enzymatic assays. The concentration of protein was estimated as per the Bradford protocol. The standard curve for estimation of protein concentration was prepared by using BSA [5].

6. Estimation of reduced and oxidized glutathione:

The concentrations of GSH (reduced glutathione) and GSSG (oxidized glutathione) were determined using the method described by Akerboom *et al.* [6]. CFEs (from both treated and untreated samples) were first deproteinized with 2 M $HCLO_4$ (Merck), 2 M EDTA (Himedia), and then neutralized with 2 M KOH (Himedia), 0.3 M HEPES (Himedia) to pH 7. After centrifuging one portion of the neutralized samples at 5000 *g* for 5 min, the supernatants were collected to determine the total *in vivo* thiol concentration (GSH+GSSG) using Glutathione Reductase (GR) dependent DTNB (Himedia) reduction. Another portion of the sample was treated with 2-vinylpyridine (50:1 V/V) for 60 min and used to mask GSH and determine the concentration of

GSSG. Time scan was done at 412 nm for 3 min using spectrophotometer. Both GSH and GSSG concentrations were expressed in nmol/mg of protein.

7. Detection of reactive nitrogen species (RNS) and reactive oxygen species (ROS):

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) were detected by using confocal microscopy and FACS.

7.1. *Confocal microscopy:*

Confocal microscopy (Leica TCS SP8) was used to detect reactive nitrogen species (RNS) and reactive oxygen species (ROS). RNS and ROS were detected using the Invitrogen protocol, with some modifications. In brief, $2x10^6$ cells were washed and resuspended in PBS pH 7.4 before being fixed with absolute ethanol. The dyes (H_2DCFDA [Invitrogen] specific for ROS and DAF-FM [Invitrogen] specific for RNS) were then added at a final concentration of 1.5 μM and incubated in the dark for 20 minutes. Excitation was set to 495 nm and emission to 515 nm for confocal microscopy. Experiments for RNS and ROS were repeated independently at least three times, and micrographs (45X) were taken. The intensity of fluorescence was measured using the Leica LAS X software.

7.2. *FACS*:

FACS (BD LSRFortessa) analysis for ROS and RNS was done from IICB, Kolkata. Samples were prepared as mentioned for the confocal microscopy. Dye free cell preparation was used as the blank for FACS analysis. The photomultiplier tube voltage was kept at 190 mV for FITC channel and flow rate was set at12 μl/min for all the experiments.10^4 events were recorded for each sample and histograms were prepared by plotting the cell count against fluorescence in the FITC channel. For the FACS analysis excitation was fixed at 495 nm and emission at 515 nm (as per the protocol of Invitrogen).

8. Estimation of ethanol production:

Ethanol was estimated by the modified potassium dichromate method [7] and potassium permanganate method [8] with slight modifications. In case of potassium

dichromate method, overnight grown untreated and treated *S. cerevisiae* broth cultures were centrifuged at 5000 *g*, and supernatants were collected. 1 ml supernatant of each sample was then mixed with a reaction mixture containing 0.25 M potassium dichromate ($K_2Cr_2O_7$) [Himedia], 0.1 M silver nitrate ($AgNO_3$) [Himedia], and 6 N sulfuric acid (H_2SO_4) [Himedia] and incubated for 10 min. The samples were then diluted, and the O.D. was recorded at 560 nm. Reaction mixture without supernatant was taken as blank.

In case of potassium permanganate method, supernatants of overnight grown untreated and treated *S. cerevisiae* broth cultures were mixed with equal volume of 20% TCA at room temperature for 5 min and then centrifuged at 10000 *g*. The supernatants were then treated with 1/5 volume of 20% CTAB at 65°C for 10 min and again centrifuged at 10000 *g*. These pretreated samples were then diluted 100-fold for ethanol estimation. Pretreated samples were mixed with 10 mM $KMnO_4$ solution and incubated at 40°C for 90 min. Initial and final O.D. were recorded at 526 nm. The 10-fold diluted pretreated sample was mixed with DNS solution for the estimation of sugar concentration. The final ethanol concentration of the pretreated sample was determined by subtracting the concentration of reducing sugar contributing in A526 from the concentration of the ethanol determined by using $KMnO_4$ method. Standard curves for the estimation of ethanol and reducing sugar concentration were prepared by using EMSURE absolute ethanol (Merck) and glucose (Merck) respectively. Further, ethanol yield and productivity were determined as mentioned by Mithra *et al.* with slight modification [9]. The volumetric productivity and yield were expressed as g/L/h and g/g of glucose. Percentage of the theoretical ethanol yield was calculated as follows:

Ethanol concentration X 100/ (Theoretical maximum ethanol yield/g of sugar i.e. 0.511 X concentration of consumed reducing sugar).

9. Enzymatic assay:

Enzymatic assays were done with the CFE or pure protein. 1 enzymatic unit (1 U) is defined as the 1 mg of protein that catalyzes the conversion of one micromole of substrate per minute under the specified conditions of the assay method.

9.1. *Glutathione reductase assay:*

The glutathione reductase assay was performed according to the protocol of Carlberg and Mannervik with slight modification [10]. In brief, 2 mM GSSG (Himedia), 3 mM DTNB, and 2 mM NADPH (Himedia) were mixed with an assay buffer containing 1 mM EDTA and CFE. Time scan was done at 412 nm for 3 min using spectrophotometer. Reaction mixture without CFE was taken as a blank. Specific activity was expressed in mU/mg of protein.

9.2. *Catalase assay:*

Catalase activity was assayed according to the method of Aebi with slight modification [11]. In brief, H_2O_2 degradation was measured at 240 nm for 2 min using spectrophotometer. The reaction mixture contained 0.1 M potassium phosphate buffer at pH 7.5, 50 mM EDTA, H_2O_2 (Sigma-Aldrich), and CFE. Reaction mixture without CFE was taken as a blank. Specific activity was expressed in mU/mg of protein.

9.3. S-*nitrosoglutathione reductase (GSNOR) assay:*

GSNO Reductase assay was performed according to the protocol of Sahoo *et al.* with slight modifications [12]. In brief, 100 mM GSNO, 0.2 mM NADH (Himedia), and 0.5 mM EDTA were mixed in 20 mM Tris-Cl pH 8.0 with CFE. The conversion of NADH to NAD was recorded at 340 nm for 5 min. Reaction mixture without CFE was taken as a blank. Specific activity was expressed in mU/mg of protein.

9.4. *Alcohol dehydrogenase assay:*

Alcohol dehydrogenase activity was determined as per the protocol of Walker with some modifications [13]. In brief, the reaction mixture contained 50 mM sodium phosphate buffer at pH 8.8, 95% V/V acetaldehyde (Sigma-Aldrich), 50 mM β-NADH, and diluted CFE. The O.D. was recorded at 340 nm for 6 min to determine the formation of β-NAD from β-NADH. Reaction mixture without CFE was taken as a blank. Specific activity was expressed in mU/mg of protein.

In a different set, the effect of GSNO or Ac. $NaNO_2$ was studied by directly adding the nitrosative stress agent to CFE. Cells were first grown under the previously mentioned conditions, and CFE was prepared. The CFE was then treated directly with

0.25 mM GSNO or ac. $NaNO_2$ for 60 min. Following that, the ADH activity of treated and untreated samples was determined, as previously stated [13]. The experiment was repeated with pure ADH (Sigma-Aldrich).

9.5. *Aconitase assay:*

Aconitase assay was performed according to the protocol of Castro *et al.* [14] with slight modifications. In brief, the formation of isocitrate (Sigma-Aldrich) from cis-aconitate was determined spectrophotometrically at 240 nm for 3 min. The reaction mixture contained 500 mM cis-aconitate, 100 mM Tris-Cl pH 8 with CFE. Reaction mixture without CFE was taken as a blank. Specific activity was expressed in mU/mg of protein.

9.6. *Aldehyde dehydrogenase assay:*

Aldehyde dehydrogenase (ALDH) activity was assayed spectrophotometrically by measuring the increase in NADH concentration at 340 nm for 3 min [15]. Reaction mixture contained final concentration of 1 M Tris-Cl buffer pH 8, 20 mM ß-Nicotinamide adenine dinucleotide (ß-NAD), 0.1 M acetaldehyde, 3 M potassium Chloride, 1 M 2-Mercaptoethanol and CFE was added to start the reaction. Specific activity was expressed in mU/mg of protein.

9.7. *Pyruvate dehydrogenase assay:*

Pyruvate dehydrogenase activity was assayed spectrophotometrically by measuring the decrease in NADH concentration at 340 nm for 5 min [16]. In short, reaction mixture contained 150 mM MOPS pH 7.4, 12 mM magnesium chloride, 0.6 mM calcium chloride, 18 mM TPP, 0.75 mM coenzyme A, 20 mM NAD^+, 15.6 mM L-cysteine, 75 mM pyruvic acid as the final concentration along with CFE. Reaction mixture without CFE was taken as blank. Specific activity was expressed in mU/mg of protein.

9.8. *Isocitare dehydrogenase assay:*

Isocitrate dehydrogenase assay was performed as per the protocol of Bergmeyer *et al.* with slight modification [16]. In short, the reaction mixture contained 70 mM glycylglycine pH 7.4, 0.5 mM isocitric acid, 1 mM β-nicotinamide adenine dinucleotide phosphate 0.5 mM manganese chloride as the final concentration. Assay reaction was

started with CFE. The conversion of NAD to NADH was measured spectrophotometrically at 340 nm for 3 min. Specific activity was expressed in mU/mg of protein.

9.9. *Pyruvate carboxylase assay:*

Pyruvate carboxylase activity was assayed spectrophotometrically by measuring the decrease in NADH concentration at 340 nm for 3 min as per the protocol of Payne and Morris with slight modification [17]. The reaction mixture contained 1 M Tris-Cl pH 8, 1 M magnesium sulfate, 0.1 M pyruvic acid, 0.1mM acetyl coenzyme A (Sigma), 0.1 M adenosine 5'-triphosphate, 0.5 M potassium bicarbonate as the final concentration. Assay reaction was started with CFE. Specific activity was expressed in mU/mg of protein.

9.10. *Pyruvate decarboxylase assay:*

Pyruvate decarboxylase assay was performed as per the protocol of Gounaris *et al.* with some modifications [18]. The reaction mixture contained 187 mM citric acid, 33 mM sodium pyruvate, 0.11 mM ß-nicotinamide adenine dinucleotide, reduced form, 10 unit of alcohol dehydrogenase (Sigma-Aldrich) as the final concentration. Reaction was started with CFE. The conversion of NADH to NAD was measured spectrophotometrically at 340 nm for 3 min. Specific activity was expressed in mU/mg of protein.

9.11. *Malate dehydrogenase assay:*

Malate dehydrogenase activity was assayed spectrophotometrically by measuring the decrease in NADH concentration at 340 nm for 3 min [16]. The reaction mixture contained 100 mM potassium phosphate, 0.13 mM β-nicotinamide adenine dinucleotide, 0.25 mM oxaloacetic acid as the final concentration. Assay reaction was started with CFE. Specific activity was expressed in mU/mg of protein.

9.12. *Citrate synthase assay:*

Citrate synthase assay was performed as per the protocol of Srere [19]. The final concentrations of the reagents were 100 mM Tris-Cl pH 8, 0.3 mM 5,5'-dithiobis-(2-nitrobenzoate), 0.2 mM acetyl coenzyme A, and 0.2 mM oxaloacetate. Assay reaction

was started with CFE and O.D. was recorded for 2 min at 412 nm. Specific activity was expressed in mU/mg of protein.

9.13. *Malate dehydrogenase (Decarboxylating) assay:*

Malate dehydrogenase (Decarboxylating) assay was performed as per the protocol of Geer *et al.* with some modifications [20]. The final concentrations of the reagents were 65 mM triethanolamine buffer (HiMedia), 3.3 mM L-malic acid, 0.3 mM β-nicotinamide adenine dinucleotide phosphate, 5 mM manganese chloride. Assay reaction was started with CFE. The conversion of NADP to NADPH was measured spectrophotometrically at 340 nm for 3 min. Specific activity was expressed in mU/mg of protein.

9.14. *Malate synthase assay:*

Malate synthase assay was performed as per the protocol of Chell *et al.* with some modifications [21]. The reaction mixture contained 30 mM imidazole Buffer, pH 8.0, 10 mM magnesium chloride, 0.25 mM acetyl-CoA, 1 mM glyoxylic acid, 0.2 mM DTNB. Assay reaction was started with CFE and O.D. was recorded for 2 min at 412 nm. Specific activity was expressed in mU/mg of protein.

10. Estimation of the concentration of citrate:

Intracellular and extracellular citrate concentration was determined by using citrate assay kit (Sigma-Aldrich). concentration of citrate was expressed in ng/µL.

11. Gene expression analysis quantitative Real Time PCR:

11.1. *RNA isolation:*

RNA was isolated as per the protocol of Dr. KPC Life Sciences, India, using their developed RNA isolation kit. In brief, treated and untreated *S. cerevisiae* cells were centrifuged at 5000 *g*, and then pellets were washed with 1X PBS. Following the addition of buffers, the entire solution containing cell pellets were transferred to prelim column and centrifuged at 10000 *g*. After that, isopropanol was added with cell pellet and then entire solution was transferred to a Chrome Column and centrifuged at 10000 *g*. Following a wash, RNA was extracted finally in nuclease-free water and then quantified using 1% agarose TAE gel.

11.2. *cDNA preparation*:

cDNA was prepared as per the protocol of Dr. KPC Life Sciences, India, using their developed cDNA synthesis kit. In brief, 500 ng of RNA was used as the template for cDNA synthesis for each sample. At first, RNA sample was mixed with 10 mM dNTP and 10 mM random hexamer and denatured at 65°C for 5 min, followed by chilling on ice. Then, diluted reverse transcriptase (RT) enzyme (Thermo Scientific) was then mixed with the chilled reaction mixture and incubated at 42°C for 60 min to synthesize cDNA. The reaction was inactivated by heating at 65°C for 15 min.

11.3. *quantitative Real Time PCR set up:*

SUPERZym qPCR mastermix, manufactured by Dr. KPC Life sciences, India was used to perform Quantitative Real-time PCR (Biorad CFX-96) Synthesized cDNA was used as the template for one reaction (+RT). To set up the (-RT) negative reaction, diluted RNA sample was used as the template. This set up was very important to check any DNA contamination. Negative control (NTC) reaction was set up without adding template. The qPCR set up was a two steps process including denaturation at 95°C for 15 sec, annealing and extension at 60°C for 30 sec. The number of cycle was repeated for 40 times and melt curve was created. All these reactions were performed in triplicate. The primers for the experimental and housekeeping genes were designed from NCBI and enlisted in **Table 1**.

Table 1: List of primers along with their sequences

Primers	Sequences (5′▼ 3′)	~Amplicon lengths (bp)
*ADH3*F	GTTGCCATCTCTGGTGCTGC	
*ADH3*R	ACACCATGAGGGCCACCTTT	300
*ADH1*F	GTTACACCCACGACGGTTCT	
*ADH1*R	ACGGTGGTACCGTTAGCTCT	445
*ADH2*F	CTGTCCTCACGCTGACTTGT	
*ADH2*R	CAACAGTACCGTTCGCCCTA	440
*ACO1*F	AGACCGTAGCACCGTTGAAG	
*ACO1*R	ATGATAGCGAAACCGCCCAA	400
*ACO2*F	TCGCATCTTTGCGATCCTGA	
*ACO2*R	CGCCTGCATTTGGTGTATGG	400
*GAPDH*F1	CGGTAGATTGGTCATGAGAAT	
*GAPDH*R1	TGGTACAAGAAGCGTTGGAAA	400
*GAPDH*F2	AACTGTTTGGCTCCATTGGC	
*GAPDH*R2	CGTTGTCGTACCAGGAAACC	200

12. Functional annotation and network analysis:

The enzymes, with the altered activity in the presence of 0.5 mM acidified sodium nitrite, were subjected for functional enrichment analysis. Initially, the STRING database was used to screen out the interactions among those enzymes in yeast system followed by creating a functionally interacting network [22]. Few closely associated enzymes were added to the network to make more stable for reliable predictions. The networks were analyzed and visualized using Cytoscape (Version 3.7) [23]. Annotation of functionally activated and deactivated enzymes were analyzed using Gene Ontology (GO) analysis by DAVID (Database for Annotation, Visualization and Integrated Discovery) [24]. Gene sets were taken from respective networks, and their annotations classified into biological process (BP), cellular component (CC) and molecular function (MF). For the GO analyses, Bonferroni correction method was used to find out the significant terms associated with the genes and decrease the error rates by removing the false discovery outcomes from any prediction.

13. Condition of stress and assays with pure aconitase and alcohol dehydrogenase:

Pure proteins were subjected to treatment by different concentrations (0.1 mM, 0.3 mM, 0.5 mM) of acidified sodium nitrite and 0.1 mM peroxynitrite (positive control). 200 µg of pure proteins were exposed to acidified sodium nitrite or peroxynitrite treatment for 30 min at room temperature. 80 µg of these treated proteins were used for determining PTN by western blotting. The rest portion of the proteins were used for specific activity determination as mentioned earlier.

14. Western blotting for Protein tyrosine nitration (PTN):

Treated and untreated pure enzymes (Aconitase [Sigma-Aldrich], Alcohol dehydrogenase [Sigma-Aldrich]) were run in 10% SDS-PAGE as per the protocol of Laemmli [25] and then transferred to PVDF membranes using wet transfer apparatus (Biorad) and transfer buffer pH 8.3 containing 25 mM Tris, 190 mM glycine and 20% methanol at 50 V for 60 min in a cold condition. To observe the successful transfer of proteins, PVDF membrane was stained with ponceau-S (HiMedia). After that, PVDF membrane was blocked overnight by using blocking buffer (HiMedia) at 4°C. Then, membranes were washed by TBST buffer (0.019 M Tris, 0.136 M, 0.1% V/V Tween 20) and probed with anti 3-nitrotyrosine monoclonal antibody (Sigma-Aldrich) at 1:1000 dilution in TBST, for 60 min at room temperature. The membranes were then washed in TBST. Following that, membranes were probed with a HRP conjugated goat anti-mouse IgG secondary antibody (Sigma-Aldrich) at 1:10000 dilution in TBST, for 30 min at room temperature. After that membranes were again washed in TBST [26]. Then the immunopositive spots were visualized by using chemilluminiscent reagent (Abcam) as per the direction of the manufacturer. Photographic plates were captured by using DNR bio-imaging system miniBIS Pro (USA) with GelQuant Express Analysis Software.

15. Western blotting analysis for *S*-nitrosylation:

S-nitrosylation was detected using Pierce™ *S*-nitrosylation western blot kit (Thermo-Fisher). At first, pure proteins were subjected to treatment by different concentrations (0.1 mM and 0.25 mM) of GSNO. 200 µg of pure proteins were exposed to GSNO

treatment for 30 min at room temperature. 100 µg of these treated and untreated proteins were used for sample preparation as mentioned in the manual of Pierce™ *S*-nitrosylation western blot kit. In short, GSNO treated proteins were treated with MMTS to block free sulfhydryl group. Following that sodium ascorbate and iodoTMT were added to modify S-NO to S-TMT. After that, proteins were run in 10% SDS-PAGE and anti-TMT monoclonal antibody was used as the primary antibody at 1:1000 ratio. Remaining protocol was same as mentioned earlier. Rest of the proteins were used for specific activity determination as mentioned earlier.

16. Estimation of ethanol production by immobilization of nitrated yeast cells:

16.1. *Immobilization of nitrated yeast cells*:

For the immobilization assay *S. cerevisiae* cells were first grown overnight in specified media in presence of 0.5 mM sodium nitrite. Next, the culture was centrifuged and the cell pellet was resuspended in PBS buffer pH 7.0. Resuspended cells were then added slowly with 1% sodium alginate. After that cells were transferred to 0.5 M $CaCl_2$ solution drop wise with the help of a syringe with the formation of Ca-alginate beads having immobilized yeast cells [27].

16.2. *Estimation of ethanol production*:

To quantify the ethanol concentration produced by immobilized nitrated yeast cells, 20 such beads were inoculated in a broth medium. Ethanol concentration was determined as stated earlier [10].

17. Optimization of ethanol production by central composite rotatable design based (CCRD) response surface methodology (RSM):

Optimization of ethanol production was carried out using central composite rotatable design based (CCRD) response surface methodology (RSM) in order to study the interaction effect between three independent variables viz., molasses concentration (C-source) (A), ammonium sulphate concentration (N-source) (B) and incubation time of yeast (C) in the fermentation broth. Due to the presence of "axial points" around the centre point in the CCRD design curvature of the model is allowed. As suggested by Saha *et al.* [26]. Three independent variables molasses concentration (A), ammonium

sulphate concentration (B) and incubation time of yeast (C) were used in five different coded levels (-α,-1,0,+1,+α). **Table 2** represents the relationship between the coded level and actual values of each variable used in this study to optimize ethanol production. The relation between the coded level of variables and actual values of the variables were explained by the following equation [28].

$$X_\alpha=(Z_\alpha-Z_0)/\Delta Z$$

Where, X_α is the coded value, Z_α is the actual value, Z_o is the actual value at the centre point and ΔZ is the step change of the variables. Total 20 experimental runs were conducted and the ethanol produced by the yeast was analyzed by the second order polynomial regression equation.

$$Y=a_0+a_1x_1+a_2x_2+a_3x_3+a_{11}x_1^2+a_{22}x_2^2+a_{33}x_3^2+a_{12}x_1x_2+a_{13}x_1x_3+a_{23}x_2x_3$$

Table 2: Coded and actual levels of variables used to construct the model

Factor	Unit	Coded levels				
		-α	**-1**	**0**	**+1**	**+ α**
A(Carbon source)	%W/V	0	5	12.5	20	25.11
B(Nitrogen source)	%W/V	0	0.05	1.02	2	2.66
C(Incubation time)	Hours	0	6	15	24	30.14

18. Statistical analysis:

All individual results are expressed as mean ± SD (Standard deviation) of at least three independent experiments for each biological sample, whereever applicable. To analyze the significant difference between control and treated samples, Student T-test, F-test were performed at 0.05 level of significance (p).

References:

1. Heaselgrave W, Andrew PW, Kilvington S. (2010) Acidified nitrite enhances hydrogen peroxide disinfection of Acanthamoeba, bacteria and fungi. J Antimicrob Chemother. 65:1207-14.

2. Hart TW. (1985) Some observations concerning the S-nitroso and S-phenylsulphonyl derivatives of Lcysteine and glutathione. Tetrahedron Lett. 26:2013-16.

3. Stevenson K, McVey AF, Clark IBN, Swain PS, Pilizota T. (2016) General calibration of microbial growth in microplate readers. Sci Rep. 6:38828.

4. Sahoo R, Sengupta R, Ghosh S. (2003) Nitrosative stress on yeast: inhibition of glyoxalase-I and glyceraldehyde-3-phosphate dehydrogenase in the presence of GSNO. Biochem Biophys Res Commun. 302:665-70.

5. Bradford MM. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248–54.

6. Akerboom TP, Sies H. (1981) Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol. 77:373-82.

7. Seo HB, Kim HJ, Lee OK, Ha JH, Lee HY, Jung KH. (2009) Measurement of ethanol concentration using solvent extraction and dichromate oxidation and its application to bioethanol production process. J Ind Microbiol Biotechnol. 36:285-92.

8. Zhang P, Hai H, Sun D, Yuan W, Liu W, Ding R, Teng M, Ma L, Tian J, Chen C. (2019) A high throughput method for total alcohol determination in fermentation broths. BMC Biotechnol. 19:30.

9. Mithra MG, Jeeva ML, Sajeev MS, Padmaja G. (2018) Comparison of ethanol yield from pretreated lignocellulo-starch biomass under fed-batch SHF or SSF modes. Heliyon. 4:e00885.

10. Carlberg I, Mannervik B. (1975) Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem. 250: 5475-80.

11. Aebi H. (1984) Catalase in vitro. Methods Enzymol. 105; 121-26.

12. Sahoo R, Sengupta R, Ghosh S. (2003) Nitrosative stress on yeast: inhibition of glyoxalase-I and glyceraldehyde-3-phosphate dehydrogenase in the presence of GSNO. Biochem Biophys Res Commun. 302:665-70.

13. Walker JRL. (1992) Spectrophotometric determination of enzyme activity: alcohol dehydrogenase (ADH). Biochem. Edu. 20:42-43.

14. Castro L, Rodriguez M, Radi R. (1994) Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. J Biol Chem. 269:29409-15.

15. Bostian KA, Betts GF. (1978) Kinetics and reaction mechanism of potassium-activated aldehyde dehydrogenase from *Saccharomyces cerevisiae*. Biochem J. 173: 787-98.

16. Bergmeyer HU, Gawehn K, Grassl M. (1974) Methods of enzymatic analysis. 2nd edn. Weinheim : Verlag Chemie, Academic Press, NY.

17. Payne J, Morris JG. (1969) Pyruvate carboxylase in *Rhodopseudomonas spheroides*. J Gen Microbiol. 59: 97-101.

18. Gounaris AD, Turkenkopf I, Buckwald S, Young A. (1971) Pyruvate decarboxylase. I. Protein dissociation into subunits under conditions in which thiamine pyrophosphate is released. J Biol Chem. 246: 1302-9.

19. Srere, PA. (1969) Citrate synthase: [EC 4.1.3.7. Citrate oxaloacetate-lyase (CoA-acetylating)]. Methods Enzymol. 13:3-11.

20. Geer, BW, Krochko D, Oliver MJ, Walker VK, Williamson JH. (1980) A comparative study of the NADP-malic enzymes from Drosophila and chick liver. Comp Biochem Physiol. 65:25-34.

21. Chell RM, Sundaram TK, Wilkinson AE. (1978) Isolation and characterization of isocitratelyase from a thermophilic *Bacillus* sp. Biochem J. 173:165-77.

22. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. (2019) STRING v11: protein-protein association networks with increased coverage, supporting

functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47: D607-D613.

23. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13: 2498-504.

24. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. (2003) DAVID: database for annotation, visualization, and integrated discovery. Genome Biol. 4:P3.

25. Laemmli UK. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-5.

26. Bhattacharjee A, Majumdar U, Maity D, Sarkar TS, Goswami AM, Sahoo R, Ghosh S. (2009) In vivo protein tyrosine nitration in S. cerevisiae: identification of tyrosine-nitrated proteins in mitochondria. Biochem Biophys Res Commun. 388:612-7.

27. Agashima M, Azuma M, Noguchi S, Inuzuka K, Samejima H. (1984) Continuous ethanol fermentation using immobilized yeast cells. Biotechnol Bioeng. 26:992-7.

28. Saha SP & Ghosh S. (2014) Optimization of xylanase production by *Penicillium citrinum* xym2 and application in saccharification of agro-residues. Biocatal. Agric. Biotechnol. 3:188–196.

Results and Discussion...