Reactive oxygen species (ROS) as pleiotropic physiological signalling agents

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Abstract | 'Reactive oxygen species' (ROS) is an umbrella term for an array of derivatives of molecular oxygen that occur as a normal attribute of aerobic life. Elevated formation of the different ROS leads to molecular damage, denoted as 'oxidative distress'. Here we focus on ROS at physiological levels and their central role in redox signalling via different post-translational modifications, denoted as 'oxidative eustress'. Two species, hydrogen peroxide (H_2O_2) and the superoxide anion radical ($O_2^{\cdot-}$), are key redox signalling agents generated under the control of growth factors and cytokines by more than 40 enzymes, prominently including NADPH oxidases and the mitochondrial electron transport chain. At the low physiological levels in the nanomolar range, H_2O_2 is the major agent signalling through specific protein targets, which engage in metabolic regulation and stress responses to support cellular adaptation to a changing environment and stress. In addition, several other reactive species are involved in redox signalling, for instance nitric oxide, hydrogen sulfide and oxidized lipids. Recent methodological advances permit the assessment of molecular interactions of specific ROS molecules with specific targets in redox signalling pathways. Accordingly, major advances have occurred in understanding the role of these oxidants in physiology and disease, including the nervous, cardiovascular and immune systems, skeletal muscle and metabolic regulation as well as ageing and cancer. In the past, unspecific elimination of ROS by use of low molecular mass antioxidant compounds was not successful in counteracting disease initiation and progression in clinical trials. However, controlling specific ROS-mediated signalling pathways by selective targeting offers a perspective for a future of more refined redox medicine. This includes enzymatic defence systems such as those controlled by the stress-response transcription factors NRF2 and nuclear factor-κB, the role of trace elements such as selenium, the use of redox drugs and the modulation of environmental factors collectively known as the exposome (for example, nutrition, lifestyle and irradiation).

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In biology and medicine, several types of 'reactive species' have attracted interest. They are named according to the nature of the reactive atom, that is, oxygen, nitrogen or sulfur: reactive oxygen species (ROS), reactive nitrogen species and reactive sulfur species. ROS encompass a group of molecules derived from molecular oxygen, which are formed by reduction–oxidation (redox) reactions or by electronic excitation (BOX 1). The chemical reactivity of the various ROS molecules is vastly different, spanning up to 11 orders of magnitude in their respective second-order rate constants with specific targets[1–3]. Obviously, 'ROS' is a term, not a molecule, and speaking of ROS therefore is not chemically precise. However, because of difficulties in discerning between individual ROS compounds, common practice in redox biology has been up to now to use 'ROS' as an umbrella term. Importantly, methodological advances in the study of specific ROS molecules by chemical detection and by non-invasive imaging techniques permit better characterization of the individual species, thereby resulting in the recommendation to restrict the use of the term[4,5]. A wealth of information has been accumulated on the chemistry of the different ROS molecules, and we can now better appreciate their biological significance. Specifically, it is now clear that ROS are fundamentally important for physiology as functional signalling entities.

Hydrogen peroxide (H_2O_2) is recognized as the major ROS in redox regulation of biological activities[6–15]. Like calcium (Ca^{2+})[16,17], H_2O_2 is a versatile

Box 1 | **Reactive oxygen species and the usage of the term**

Reactive oxygen species (ROS) derive from molecular oxygen and are formed by redox reactions or by electronic excitation. They can be divided into non-radical and free radical (with at least one free electron) species. We present here basic information on selected ROS that are discussed in this article; for more comprehensive list, see REFS[1–3,21].

Two-electron (non-radical) ROS include:

- Hydrogen peroxide (H_2O_2). H_2O_2 is produced from O_2 mainly by NADPH oxidases in conjunction with superoxide dismutases, by the mitochondrial electron transport chain and by numerous other enzymes (TABLE 1). H_2O_2 is a strong two-electron oxidant, but its high activation energy confines its reactivity to a few biological targets. H_2O_2 is relatively stable. It reacts very slowly with glutathione, cysteine and methionine, but its reactivity towards cysteine in specific proteins can increase greatly to $10^7\ M^{-1}s^{-1}$ (which is approximately 10^6-fold over that for average cysteines in proteins) depending on the particular protein structure and environment, providing a basis for selectivity and specificity of H_2O_2 in redox signalling. H_2O_2 reacts moderately with iron–sulfur (Fe–S) clusters and loosely bound metals. H_2O_2 can react with CO_2/bicarbonate to form peroxymonocarbonate (HCO_4^-), an oxidant that reacts with biological targets with a second-order rate constant about two to three orders of magnitude greater than that for H_2O_2 (REF.[138]).
- Organic hydroperoxides (ROOH). These ROS include hydroperoxides formed enzymatically and non-enzymatically (lipid peroxidation) from polyunsaturated fatty acids (PUFAs) and sterols (for example, cholesterol). They function in cell signalling, especially in the immune system, and are implicated in cell death via ferroptosis.
- Singlet molecular oxygen ($^1O_2$). $^1O_2$ is an electronically excited form of O_2 (REF.[296]). Its generation by photoexcitation makes $^1O_2$ particularly important in light-exposed tissues such as the skin and the eye. By chemiexcitation it is also formed in enzyme reactions in the dark[297].
- Electronically excited carbonyl. Electronically excited (photoexcitation or chemiexcitation) forms of hydrocarbons containing a carbonyl (R–C=O)[298]. They can transfer energy to O_2 to create $^1O_2$.
- Ozone (O_3). O_3 is a very reactive oxidant that poses a health concern in some geographic areas, especially with air pollution and certain atmospheric conditions, because it causes oxidative stress in the lungs and other exposed tissues.
- Hypochlorous acid and hypobromous acid (HOCl and HOBr). HOCl and HOBr are produced from H_2O_2 by myeloperoxidase in the phagocytic vacuole in neutrophils for pathogen defence[61].

Free radical ROS include:

- Superoxide anion radical ($O_2^{\cdot-}$). $O_2^{\cdot-}$ dismutates spontaneously or catalysed by superoxide dismutases to H_2O_2 and O_2, serving as a major source of H_2O_2. Owing to high electrostatic attraction, $O_2^{\cdot-}$ oxidizes Fe–S clusters at a high rate, releasing iron, but its negative charge makes it less apt for redox signalling via thiols. The perhydroxyl radical ($HO_2^{\cdot}$) is the protonated form of $O_2^{\cdot-}$; it is uncharged and can diffuse in lipids, and it can produce a carbon-centred radical of polyunsaturated lipids. $O_2^{\cdot-}$ reacts efficiently with other radicals, notably nitric oxide (NO), forming peroxynitrite ($ONOO^-$), a tyrosine-nitrating agent.
- Hydroxyl radical (·OH). ·OH is the most reactive ROS. It oxidizes biomolecules at a diffusion-controlled rate (that is, it is an unspecific oxidant). It is formed from H_2O_2 by reduction in metal-catalysed Fenton chemistry, involving free iron (Fe^{2+})[282,283]. Because ·OH reacts directly with the nearest neighbour at the site of its generation, the location of Fe^{2+} determines the site of ·OH toxicity. ·OH is an initiator of lipid peroxidation.
- Peroxyl radical (ROO·). Formed following initiation of free radical chain reaction of PUFAs in lipid peroxidation. Peroxyl radicals propagate free radical chain reactions by abstracting a proton from another PUFA, thereby creating a lipid hydroperoxide and another carbon radical.
- Alkoxyl radical (RO·). Formed as intermediates in lipid peroxidation by metal-catalysed decomposition of lipid hydroperoxides and can amplify lipid peroxidation chain reactions.

In biology and medicine, the term 'reactive oxygen species' is in widespread use, with increasing trend. At the beginning of 2020, the Web of Science had 204,000 entries for 'reactive oxygen species', with more than 15,000 new ones per year (which corresponds to one new ROS publication about every half hour). Obviously, the term is engraved in researchers' minds, and it is likely here to stay.

However, the use of the term 'reactive oxygen species' is problematic because 'ROS' is not a defined chemical compound, it is not a molecule. Rather, 'reactive oxygen species' is a collective term for a number of related molecules with vastly divergent reactivity — the second-order rate constants for the different 'ROS' spans up to 11 orders of magnitude. Therefore, whenever possible, the specific ROS molecule should be mentioned, and the use of the term 'reactive oxygen species' should be restricted as much as possible (see REFS[1,2,4,5]).

Pleiotropic
From Greek *pleion*, meaning 'more', and *tropos*, meaning 'way', in biology originally denoting that one gene can influence two or more seemingly unrelated phenotypic traits. In current usage, the term describes multiple actions exerted by a given agent. If the actions generate opposing effects (for example, both harmful and beneficial to an organism), it is antagonistic pleiotropy.

Redox signalling
Response of a cell to an oxidant or reductant, or to an alteration in redox status of a cellular component, that leads to a variety of downstream effects on cell state directly or via an essential redox relay from a source to a target.

pleiotropic physiological signalling agent. H_2O_2 was first shown, now 50 years ago, to occur physiologically at a low steady-state level in normally respiring eukaryotic cells[18]. Similarly to Ca^{2+}, the intracellular concentration of H_2O_2 is maintained in the low nanomolar range (approximately 1–100 nM), being under tight control: the generation of H_2O_2 is stimulated by metabolic cues or by various stressors, such as growth factors, chemokines or physical stressors[19], while its removal is achieved by efficient reducing systems. Steady-state physiological flux of H_2O_2 to specific protein targets leads to reversible oxidation, thereby altering protein activity, localization and interactions, which contributes to orchestration of various processes in cells and organs, including cell proliferation, differentiation, migration and angiogenesis[3,20,21]. This state of low-level H_2O_2 maintenance and its associated physiological redox signalling is called 'oxidative eustress'[22,23] (FIG. 1). The overall cellular concentration of the superoxide anion radical ($O_2^{\cdot-}$) is maintained at about 10^{-11} M, much lower than that of H_2O_2, at 10^{-8} M (REF.[24]). These numbers should serve only for rough orientation, because the local subcellular concentration of any reactive species varies depending on the activity of generator and removal systems.

The major mechanism by which H_2O_2 attains specificity to mediate biological signalling effects is through oxidation of sulfur (thiolate groups) in target proteins (BOX 2), which show rates of reaction with H_2O_2 several orders of magnitude higher than those of other protein thiols[15,25]. Redox signalling can also occur through reversible methionine oxidation[26], through selenoproteins[27], through oxidation of protein metal centres[28] and through oxidized lipids[29], but these aspects of redox signalling will not be covered in detail here.

The ultimate target of the oxidant signal can be addressed directly or, alternatively, through an intervening carrier of the oxidant message in what is known as redox relay, which provides a means for spatiotemporal precision and specificity of signalling by directed and confined interactions[30]. Overall, physiological targets of oxidants serve as molecular redox switches in signal transduction acting at various levels of cell regulation in

Oxidative eustress
Term describing the physiological oxidative challenge (Greek *eu*, meaning 'good', 'well', 'positive'), essential in redox signalling. Supraphysiological oxidative challenge is denoted as 'oxidative distress'.

Thiolate
Anion formed from thiol by dissociation of a proton ($RSH \rightarrow RS^- + H^+$).

Selenoproteins
Proteins with selenocysteine, the 21st amino acid, in the primary structure. Selenomethionine can sometimes substitute for methionine during protein synthesis, but unlike selenocysteine, this is not encoded within the RNA message and is generally without functional significance.

Hormesis
From Greek *hormesis* 'rapid motion, eagerness', describing a biphasic dose-response phenomenon: low-dose exposure (stimulation, preconditioning) and high-dose exposure (inhibition), J-shaped or inverted U-shaped dose-response curve.

response to stresses or other external perturbations. In line with this important role of oxidants as signalling agents, it has been observed that moderately elevated levels of mitochondrial oxidants improve systemic defence by inducing adaptive responses that support health and longevity, a concept referred to as 'mitohormesis', as an extension of the broader term 'hormesis'[31–33] and its role in redox homeostasis[34].

In contrast to physiological levels of H_2O_2 that are important for signalling, supraphysiological concentration of H_2O_2 (roughly estimated to be above 100 nM) leads to unspecific oxidation of proteins and altered response patterns as well as to reversible and irreversible damage to biomolecules, causing growth arrest and cell death, with associated pathological states, a state referred to as 'oxidative distress' (FIG. 1). Oxidative distress causes damage to all classes of macromolecules, thereby impairing their function[35]. In addition, products of this damage can serve as secondary oxidant signals. For instance, 4-hydroxynonenal and other reactive aldehydes are generated during lipid peroxidation and can signal through reaction with proteins[36]. An array of protein oxidation products have been elucidated, and relevant biological responses have been characterized[2]. Oxidative DNA damage has also been extensively characterized in mutagenesis and cancer development[37], DNA methylation and chromatin structure[38]. Evidence is also accumulating for oxidative damage to RNA[39], but the potential functional impact has not yet been fully elucidated.

This inherent duality of purposeful beneficial functions of oxidants, on the one hand, and deleterious products of oxidants that tend to accumulate over time, on the other hand, represents antagonistic pleiotropy[40].

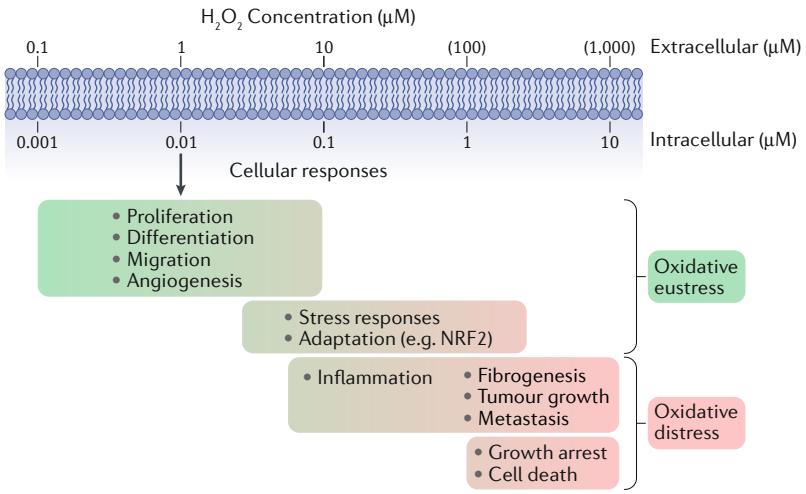


Fig. 1 | **Estimated ranges of H_2O_2 concentration with regard to cellular responses: oxidative eustress and oxidative distress.** The physiological intracellular range spans up to approximately 100 nM. Stress responses and adaptation occur at higher concentrations. Even higher exposure leads to inflammatory response, growth arrest and cell death by various mechanisms. Green and red colouring denotes predominantly beneficial (eustress) or deleterious responses (distress), respectively. An estimated 100-fold concentration gradient from extracellular to intracellular is given for rough orientation; it would be 500-fold if one considers 5 μM H_2O_2 concentration in blood plasma[68]. Experimental high extracellular H_2O_2 exposure is given in parentheses. The gradient will vary with the cell type, location inside the cells and activity of enzymatic sinks (see the main text). For further detailed coverage, see REF.[22]. Adapted from REF.[23], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

Thus, similarly to Ca^{2+}, the pleiotropic character of oxidants impacts numerous fundamental processes simultaneously, with widespread consequences in health and disease (see REF.[41]).

In this Review, we address ROS generation and its regulation with focus on H_2O_2 and $O_2^{\cdot-}$, their mechanisms of redox signalling, major molecular physiological redox targets and associated cellular stress responses. We also discuss cellular functions and processes affected by ROS as well as deregulation of redox signalling in ageing and some pathological states, with an outlook for prospects of redox medicine.

ROS generation and regulation

ROS are generated by various sources. To maintain their levels at physiological concentrations, various mechanisms to control ROS production and availability, including localized and compartmentalized generation as well as engagement of sinks (detoxifying factors) and redox relays, are in place (FIG. 2).

Endogenous and exogenous ROS sources. Overall, in human cells a total of 41 H_2O_2- and/or $O_2^{\cdot-}$-generating enzymes have been identified[42] (TABLE 1), and this list is increased to well over 50 by inclusion of enzymes generating other ROS such as lipid hydroperoxides or nitric oxide (NO) and hypochlorous acid (Supplementary Table 1). The major endogenous enzymatic sources of $O_2^{\cdot-}$ and H_2O_2 are transmembrane NADPH oxidases (NOXs)[43–45] and the mitochondrial electron transport chain (ETC)[46]. NOXs occupy various cellular localizations (TABLE 1), which contributes to local generation of ROS. NOXs have also been associated with specialized redox-active endosomes (redoxosomes), which form in response to specific extracellular stimuli, such as nutrients, growth factors and cytokines, and allow compartmentalization of H_2O_2 for local redox-mediated regulation (microdomains) or cell signalling from cell-surface receptors[47,48]. In the mitochondrial ETC, complex I and in part complex II release $O_2^{\cdot-}/H_2O_2$ towards the mitochondrial matrix, whereas release from complex III is towards the cristae lumen and the intermembrane space[49,50]. This topological difference has functional significance, which is shown by differences in patterns of redox-modified proteins depending on the source[49]. Apart from NOXs and the ETC, H_2O_2 is also generated by various other oxidases present in subcellular localizations, prominently including the endoplasmic reticulum (ER) and peroxisomes, as well as by several superoxide dismutases (SOD1–SOD3) (TABLE 1), which contribute to the localized production of H_2O_2 from $O_2^{\cdot-}$ (FIG. 2b). It is worth mentioning that beyond the biology of $O_2^{\cdot-}/H_2O_2$, an important area of ROS research concerns lipid-derived ROS, whereby polyunsaturated fatty acids are oxidized, generating lipid hydroperoxides and related radicals, peroxyl and alkoxyl (Supplementary Table 1). Such oxidized lipids have a major impact on redox signalling[51], especially in immune signalling[52–55]. For instance, lipoxygenases and prostaglandin synthases (cyclooxygenases) generate reactive oxidants as intermediates in processes to activate and control inflammatory responses[56].

Box 2 | **Principles of redox signalling**

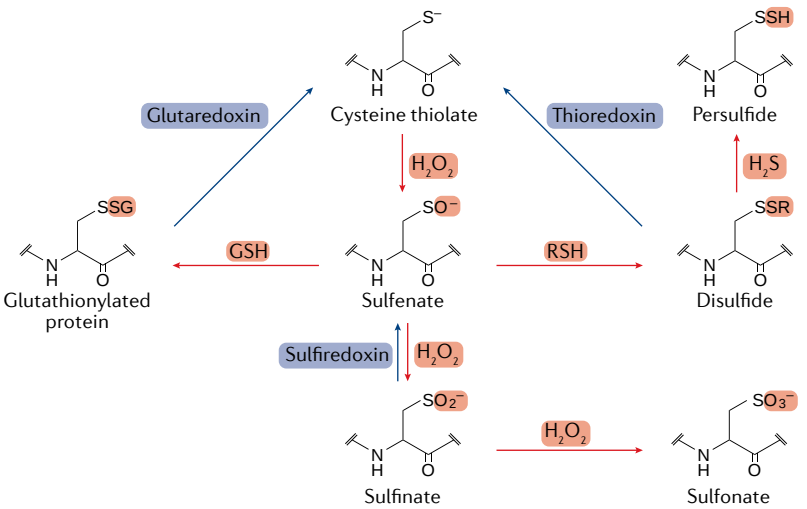
The major mechanism by which reactive oxygen species (ROS) mediate their biological effects in redox regulation is through thiol-based modification of target proteins[25,299]. However, other classes of molecules, such as non-coding RNA[300] or microRNAs[301], have been shown to be redox sensitive (these microRNAs were termed 'redoximiR'[103]) and to contribute to redox signalling. The critical initial step in redox signalling through proteins is that H_2O_2 reacts with a target protein cysteine (Cys) thiolate (S^-) to form the sulfenate (SO^-; see the figure, part **a**; Pr refers to a protein backbone). This in itself can be sufficient to lead to a change in function of the protein, and it can lead to subsequent reactions such as intramolecular or intermolecular disulfide (SS) formation or glutathionylation (SSG) of the reactive cysteine. The superoxide anion radical ($O_2^{\bullet-}$) reacts with Fe–S clusters in proteins, such as in aconitase, affecting function[159]. When $O_2^{\bullet-}$ is generated concomitantly with nitric oxide (NO), peroxynitrite ($ONOO^-$) is formed efficiently, leading to nitration of tyrosine (Tyr) residues in proteins, again causing functional modifications[302] (a prototypical example being the inactivation of SOD2 on nitration of Tyr34 (REF. [303])). NO itself can lead to another cysteine modification, S-nitrosylation. Further, cysteines can be modified by persulfidation (also called 'sulfhydration') with hydrogen sulfide (H_2S) to yield protein persulfides or polysulfides[304,305] (see the figure, part **b**).

Disulfides and glutathionylated, nitrosylated as well as persulfidated cysteines can be reduced back to the original thiol either by the thioredoxin system or by the glutathione (GSH) system, reversibility being an essential feature in redox signalling. Sulfenate can be further oxidized to sulfinate and sulfonate. Sulfinate can be reduced by sulfiredoxin[306,307], whereas sulfonate is not reduced (see the figure, part **b**). These different forms of the oxidized entities of susceptible protein thiols (RSH) provide ample potential for specificity in redox signalling. In human thioredoxin 1 for instance, oxidation of Cys62 and Cys69 inhibits interaction with thioredoxin reductase 1, while oxidation of Cys73 inhibits interaction with peroxiredoxins[308].

a

	Source	Signal	Target	Product
H_2O_2 dependent	NOX, mitochrondria	H_2O_2 +	Pr-(Cys)-S^- →	Pr-(Cys)-SO^- → Pr-(Cys)-S, Pr-(Cys)-S; Pr_1-(Cys)-SS-(Cys)-Pr_2; Pr-(Cys)-SSG
$O_2^{\bullet-}$ dependent	NOX, mitochrondria	$O_2^{\bullet-}$ +	Pr-[4Fe-4S] →	Pr-[3Fe-4S] + Fe^{2+} + H_2O_2
RONS dependent	NOS + NOX: $\cdot NO + O_2^{\bullet-} \rightarrow ONOO^-$;		Pr-(Tyr) + $ONOO^-$ →	Pr-(Tyr)-NO_2

b



NOS, nitric oxide synthase; RNOS, reactive oxygen and nitrogen species; NOX, NADPH oxidase.

4-Hydroxynonenal
A reactive aldehyde produced during free radical chain reaction of polyunsaturated fatty acids.

Lipid peroxidation
Oxidative free radical chain reaction of polyunsaturated fatty acids.

Mitochondrial electron transport chain
(ETC). Series of electron transfer complexes in the mitochondrial inner membrane that support oxidation of metabolic fuels to generate an electrochemical proton gradient for ATP synthesis from ADP and inorganic phosphate. Complexes within this chain are also sources of $O_2^{\bullet-}$ and its product, H_2O_2.

Cristae
Folds of the mitochondrial inner membrane.

A long-standing question is the nature of the predominant intracellular oxidant generators. A recent estimate of the relative contribution from NOXs and mitochondrial ETC sites in resting myoblasts showed that around 40% of net cellular H_2O_2 production was from NOXs and approximately 45% was from the ETC, with the remainder coming from other enzymatic sources[57]. Thus, in this case contributions by NOXs and the ETC are commensurate. Nevertheless, the exact contribution of the different sources to the cellular pool of ROS depends on the context of a given cell and a given metabolic state and hence does vary.

In addition to intracellular sources, oxidants are also generated as a consequence of the cumulative environmental exposure called the 'exposome'[58], which includes molecular factors such as nutrients, drugs, toxicants and pollutants as well as physical stressors (UV, X-ray and other ionizing radiation) and psychological stressors

Peroxiredoxins
Enzymes that catalyse reduction of H_2O_2 with a thioredoxin as the electron donor.

Glutathione
Tripeptide (γ-glutamylcysteinylglycine) that is widespread in biology and, among other functions, supports antioxidant reactions.

Peroxidases
Enzymes which catalyse the reduction of H_2O_2 and other hydroperoxides.

Catalatic reaction
One of the modes of catalysis for catalase, along with peroxidatic reaction. In the overall reaction cycle, the intermediate formed by reaction of catalase haem iron with H_2O_2, termed 'compound I', can be reduced by a second molecule of H_2O_2 (catalatic reaction) or by an alternative hydrogen donor (peroxidatic reaction).

(lifestyle). Because these exposures are highly variable, it is difficult to ascertain the contribution of the exposome to the total pool of oxidants.

Controlling H_2O_2 via sinks and redox relays. Peroxiredoxins[59] and glutathione peroxidases[60] catalyse the removal of H_2O_2. These enzymes have high second-order rate constants in their reaction with H_2O_2, of the order of 10^5–10^8 M^{-1} s^{-1}, contributing to the maintenance of low cellular H_2O_2 concentration in the subcellular spaces where they are located[15]. Catalase, a haem protein, dismutates H_2O_2 to H_2O and O_2 in its catalatic reaction, and in its peroxidatic reaction it reduces H_2O_2 to H_2O by oxidizing hydrogen-donating compounds[24]. Thus, these enzymes are sinks for H_2O_2. Some peroxidases, such as myeloperoxidase, use H_2O_2 to generate other oxidants, such as hypochlorous acid, which is used by neutrophils in defence against pathogens[61], thereby repurposing the ROS for immune function. Peroxiredoxins can transmit the oxidizing equivalents from H_2O_2 to other target proteins, the reaction rate constants with H_2O_2 of which are comparatively low. Examples of such a redox relay were demonstrated for peroxiredoxin 2 and the transcription factor STAT3 (REF.[30]) and for a kinase in the stress-responsive p38 signalling pathway[62].

Mitochondrial nicotinamide nucleotide transhydrogenase (NNT) also plays a role in clearing cellular (including extramitochondrial) H_2O_2 (REF.[63]). This occurs by shifting reducing equivalents from NADH to NADPH, thereby supporting and strengthening the capacity of the thioredoxin system and the glutathione system, which depend on NADPH supply[64]. This serves both defensive functions to protect against physical and chemical causes of oxidative stress and also anabolic and repair functions after injury.

Steady-state gradients and their control. Cellular metabolism is characterized by steady states and by transitions between them in response to changing conditions. It is important to note that there are steady-state gradients across cells and subcellular structures, meaning that at a given steady state in a cell, for the agent in question there is spatial distribution resembling a 'landscape' with hotspots rather than a flat concentration across the cellular space. Analogously, reactive species are maintained at steady-state levels, called the 'redox tone' (which can be defined for individual reactive species, for example, peroxide tone and sulfide tone) owing to tight control of their sources and sinks. For H_2O_2, overall intracellular concentration was estimated to be in the range of 1–10 nM (REFS[24,65]), cytosolic concentration was estimated to be 80 pM (REF.[66]), the concentration in the mitochondrial matrix was estimated to be 5–20 nM (H. Sikes, unpublished results) and the concentration in

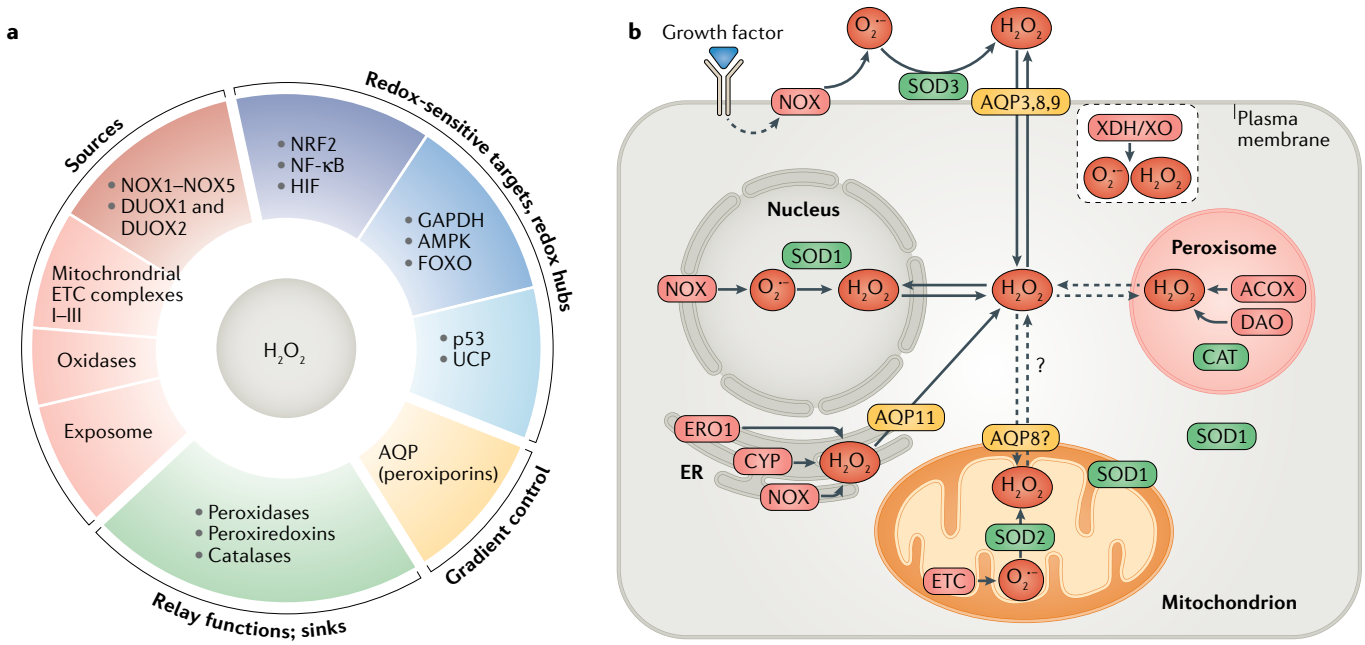


Fig. 2 | **Key modulators and targets of H_2O_2. a** | Shown are H_2O_2 sources (red), redox-sensitive targets and hubs (blue), aquaporins (AQPs)/'peroxiporins' (yellow) and sinks (green). H_2O_2 sources can include oxidases (TABLE 1) and exposome (environmental) sources in addition to NADPH oxidases (NOXs) and mitochondria. Redox-sensitive targets serve as hubs to support biological functions (see FIG. 3). Sinks can include redox relay (electron transfer) reactions in transfer of signalling oxidant. Superoxide dismutases (SODs; not shown) also serve as both a source for H_2O_2 and a sink for the superoxide anion radical ($O_2^{\cdot-}$). **b** | The major reactive oxygen species, $O_2^{\cdot-}$ and H_2O_2, and their subcellular sites of generation. For a list of individual enzymes, see TABLE 1. Key redox systems generating $O_2^{\cdot-}$ and H_2O_2 at various subcellular sites are provided in the text. Diffusion across membranes is limited, and so $O_2^{\cdot-}$ and H_2O_2 exist at different concentrations in different subcellular compartments. Xanthine dehydrogenase/oxidase (XDH) is a liver enzyme that is also found in blood and can have xanthine oxidase (XO) activity, producing both H_2O_2 and $O_2^{\cdot-}$. The substrates hypoxanthine and xanthine are produced from ATP breakdown during ischaemia, with resulting increase in $O_2^{\cdot-}$ and H_2O_2 levels following reperfusion of the heart and other organs. ACOX, acyl-CoA oxidase; AMPK, AMP-activated protein kinase; CAT, catalase; CYP, cytochrome P450-dependent monooxygenases; DAO, D-amino acid oxidase; DUOX, dual oxidase; ER, endoplasmic reticulum; ERO1, endoplasmic reticulum oxidoreductin 1; ETC, electron transport chain; FOXO, forkhead box protein O; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; NRF2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor-κB; UCP, uncoupling protein.

Table 1 | **H_2O_2-generating and $O_2^{\cdot-}$-generating human enzymes**

Name	Protein abbreviation	Location	Product[a]
Aldehyde oxidase	AOX1	C	H_2O_2
Amine oxidase (flavin-containing) A	AOFA	M	H_2O_2
Amine oxidase (flavin-containing) B	AOFB	M	H_2O_2
D-Amino acid oxidase	OXDA	Px	H_2O_2
L-Amino acid oxidase	OXLA	L	H_2O_2
D-Aspartate oxidase	OXDD	Px	H_2O_2
Amiloride-sensitive amino oxidase (copper containing)	AOC1	S	H_2O_2
Cytochrome P450 3A4	CP3A4	ER	$O_2^{\cdot-}/H_2O_2$
Cytochrome P450 2D6	CP2D6	ER	$O_2^{\cdot-}/H_2O_2$
Cytochrome P450 2E1	CP2E1	ER, M	$O_2^{\cdot-}/H_2O_2$
Cytochrome P450 4A11	CP4AB	ER	$O_2^{\cdot-}/H_2O_2$
ERO1-like protein-α	ERO1A	ER	H_2O_2
ERO1-like protein-β	ERO1B	ER	H_2O_2
FAD-linked sulfhydryl oxidase ALR	ALR	C, M, S	H_2O_2
Hydroxyacid oxidase 1	HAOX1	Px	H_2O_2
Hydroxyacid oxidase 2	HAOX2	Px	H_2O_2
Membrane primary amine oxidase	AOC3	PM	H_2O_2
Peroxisomal N^1-acetylspermine/spermidine oxidase	PAOX	Px, C	H_2O_2
Peroxisomal acyl-CoA oxidase 1	ACOX1	Px	H_2O_2
Peroxisomal acyl-CoA oxidase 3	ACOX3	Px	H_2O_2
Peroxisomal sarcosine oxidase	SOX	Px	H_2O_2
Prenylcysteine oxidase 1	PCYOX	L	H_2O_2
Prenylcysteine oxidase-like	PCYXL	S	H_2O_2
Protein-lysine 6-oxidase	LYOX	S	H_2O_2
Pyridoxine 5′-phosphate oxidase	PNPO	C	H_2O_2
Retina-specific copper amine oxidase	AOC2	PM, C	H_2O_2
Spermine oxidase	SMOX	C, N	H_2O_2
Sulfhydryl oxidase 1	QSOX1	G	H_2O_2
Sulfhydryl oxidase 2	QSOX2	N, PM, S	H_2O_2
Sulfite oxidase, mitochondrial	SUOX	M	H_2O_2
Xanthine dehydrogenase/oxidase	XDH	C, PM, S	H_2O_2
NADPH oxidase 1	NOX1	PM	$O_2^{\cdot-}$
NADPH oxidase 2	NOX2 (also known as CY24B)	PM	$O_2^{\cdot-}$
NADPH oxidase 3	NOX3	PM	$O_2^{\cdot-}$
NADPH oxidase 4	NOX4	ER, PM, N	H_2O_2
NADPH oxidase 5	NOX5	ER	$O_2^{\cdot-}$
Dual oxidase 1	DUOX1	PM	H_2O_2
Dual oxidase 2	DUOX2	PM	H_2O_2
Superoxide dismutase [Cu–Zn]	SOD1	C, N, M	H_2O_2
Superoxide dismutase [Mn], mitochondrial	SOD2	M	H_2O_2
Extracellular superoxide dismutase [Cu–Zn]	SOD3	PM, S	H_2O_2

[a]Enzymes have been characterized in terms of $O_2^{\cdot-}$ and H_2O_2 production, but the analytical methods used are often unable to discriminate the primary product owing to dismutation of $O_2^{\cdot-}$ to produce H_2O_2. Other proteins, such as other cytochrome P450 enzymes and haemoglobin, produce $O_2^{\cdot-}$ or H_2O_2 but are not included because the rates are typically low. Enzymes generating lipid peroxides are included in Supplementary Table 1. C, cytoplasm; ER, endoplasmic reticulum; G, Golgi apparatus; L, lysosome; M, mitochondria; N, nucleus; PM, plasma membrane; Px, peroxisome; S, secreted.

the ER lumen was estimated to be approximately 700 nM (REF.[67]). The last of these concentrations is high because the ER is the site of protein folding involving formation of disulfide bridges, and for each disulfide bond established, one H_2O_2 molecule is formed. The extracellular concentration of H_2O_2 is substantially higher, about 1–5 μM in blood plasma[68] (FIG. 1). Thus, there is a steep gradient of H_2O_2 concentration (100–500-fold) between extracellular and intracellular spaces. The thioredoxin system (see also later) has a predominant role in shaping

Thioredoxin system
A thiol antioxidant system consisting of NADPH, thioredoxin reductase and thioredoxin that supports maintenance of protein thiols and reduction of hydroperoxides.

Glutathione system
A thiol antioxidant system consisting of glutathione peroxidases, glutathione disulfide reductase, glutathione *S*-transferases, glutaredoxin, glutathione synthesis enzymes and glutathione which supports maintenance of protein thiols and reduction of hydroperoxides.

Iron–sulfur (Fe–S) clusters
Redox centres in proteins in which iron is coordinately bonded between cysteinyl residues (thiolates) in protein and sulfide (S^{2-}), for example, Fe_2S_2 and Fe_4S_4.

Sirtuin family
Enzymes that remove acetyl (that is, functioning as deacetylases) or other acyl groups (that is, functioning as desuccinylases, demalonylases, demyristoylases or depalmitoylases) from proteins.

intracellular H_2O_2 gradients[69]. Control of H_2O_2 levels across the cell is also achieved by exchanges between the different sources[70–73], prominently including contacts between ER, mitochondria and peroxisomes[74] (see later).

The dynamics of H_2O_2 metabolism is accessible for analysis using genetically encoded fluorescent protein indicator probes[75]. The first probe using the H_2O_2-sensitive OxyR domain was HyPer[76] (see REFS[77–79] for the current status in this rapidly advancing field and REF.[80] for one recent example of such research). With use of these sensors and other tools, heterogeneity of individual cell responses to oxidants (for example, in the cell cycle) can now be examined[81]. As an uncharged molecule, H_2O_2 is able to traverse biological membranes by passive diffusion at a certain low rate. However, it was found that H_2O_2 was transported at a much higher rate through water channels in the membrane[82]. Indeed, several aquaporins (AQP3, AQP5, AQP8, AQP9 and AQP11) facilitate movement of H_2O_2 across membranes, for which reason they are referred to as 'peroxiporins'[83]. Thus, the pattern of rates of H_2O_2 transfer across cellular membranes contributes to the establishment of steady-state gradients. Work on AQP8 revealed a gating mechanism involving cysteine persulfidation (RSSH), opening the possibility of H_2O_2 gradient control by peroxiporins in a redox-dependent manner[84,85].

Because mitochondria are important sources of H_2O_2, whether and how mitochondria in the intact cell release H_2O_2 as such is a matter of current research. So far, there is no direct demonstration of H_2O_2 release from mitochondria in situ to the cytosol. With use of a novel ultrasensitive genetically encoded indicator for intracellular H_2O_2, HyPer7, H_2O_2 diffusion from the mitochondrial matrix to the cytosol was demonstrable only when the thioredoxin system in the intermembrane space was inhibited, corroborating doubts of a physiological role of direct H_2O_2 release from mitochondria in the intact cell[86]. There is the possibility that the oxidant signal from the ETC, generated as H_2O_2, is converted to an alternative oxidative signal for export to the extramitochondrial space; this requires further research. Likewise, it has been suggested that mitochondrial membranes may harbour aquaporins (AQP8 specifically)[87], but this also appears controversial.

The importance of the maintenance of cellular H_2O_2 gradients can be exemplified by a condition of reductive stress[88]; this could reflect insufficient H_2O_2 to maintain steady-state oxidative eustress. On a systemic level, long-distance communication between tissues and organs by low-abundance oxidants such as H_2O_2 appears unlikely owing to the universal presence of systems for their removal. Thus, short-lived oxidants are mainly physiologically utilized for intracellular signalling, and their production can be coupled to non-reactive substances such as hormones and cytokines, for example, epidermal growth factor (EGF)[48], for intercellular communication. It is nevertheless worth mentioning that lipid hydroperoxides can be transported between tissues bound to lipoproteins, and immune cells with high production rates of H_2O_2 such as granulocytes are highly mobile, which could allow oxidants to be used in intercellular communication.

Targets of redox signalling

Redox signalling affects protein function, leading to changes in signalling outputs, enzyme activity, gene transcription and membrane and genome integrity, to just name a few examples[89] (FIG. 3). As mentioned already, redox signalling can also occur through oxidative modification of RNA. This section highlights the pleiotropy of ROS in physiological signalling. Regarding the total number of cellular cysteines, a calculation showed that about 10–20% thiols of the full 214,000 thiols in the cellular cysteine proteome are readily oxidized under aerobic conditions[90]. These include enzyme, transporter, receptor and transcription factor regulatory sites as well as allosteric and macromolecular interaction sites[91]. Various proteins, including cytoskeletal elements, heat shock proteins, scaffold proteins such as 14-3-3 and many ribonucleoproteins have cysteines that are highly sensitive to oxidation, suggesting the existence of functional protein networks under redox regulation[92]. Cryptic cysteines in proteins can become accessible by alterations in protein structure on growth factor stimulation, as shown for EGF[93]. Other oxidant targets (iron–sulfur (Fe–S) clusters or tyrosine residues; BOX 2) also have potential signalling functions and add to the diversity of redox signalling mechanisms. The list of targets of redox signalling is too extensive to cover exhaustively (for reviews, see REFS[94,95]). In a quantitative tissue-specific landscape of the redox-regulated proteome, called the Oximouse dataset, cysteine oxidation networks have recently become accessible for detailed study[96]. We present here a few prototypical examples of signalling targets.

NRF2–KEAP1 and antioxidant response. The NRF2–KEAP1 system is the paradigm for a physiological thiol-based sensor–effector apparatus responding to oxidant challenge with a role in maintaining redox homeostasis in eukaryotes[97,98]. Its mechanism and function in physiology and pathophysiology is now known to some detail. It is a major sensor for oxidative and electrophilic stresses[19], whereby KEAP1, which functions as an NRF2 inhibitor, harbours several cysteine residues that can be subject to oxidation. This oxidation, importantly including Cys151 disulfide formation in the KEAP1 dimer[99], leads to a conformational change of KEAP1, which prevents NRF2 ubiquitylation, thereby increasing NRF2 stability and allowing its subsequent translocation to the nucleus; there it serves as transcription factor for expression of a number of antioxidant defence proteins. The complexity of thiol-based regulation is illustrated by the multiple cysteine thiol groups of KEAP1, which respond selectively to various oxidants[89,98,100]. The NRF2–KEAP1 system is regulated by thioredoxin reductase 1 (REF.[101]) and by the sirtuin family of deacetylases[102]. Furthermore, redox-sensitive microRNAs modulate the NRF2 system[103].

NF-κB pathway. The transcription factor nuclear factor-κB (NF-κB) is subject to complex regulation[104], with many regulatory molecules involved[105]. NF-κB serves as a master switch of inflammation, which is associated with extensive H_2O_2 production, and hence it is not surprising

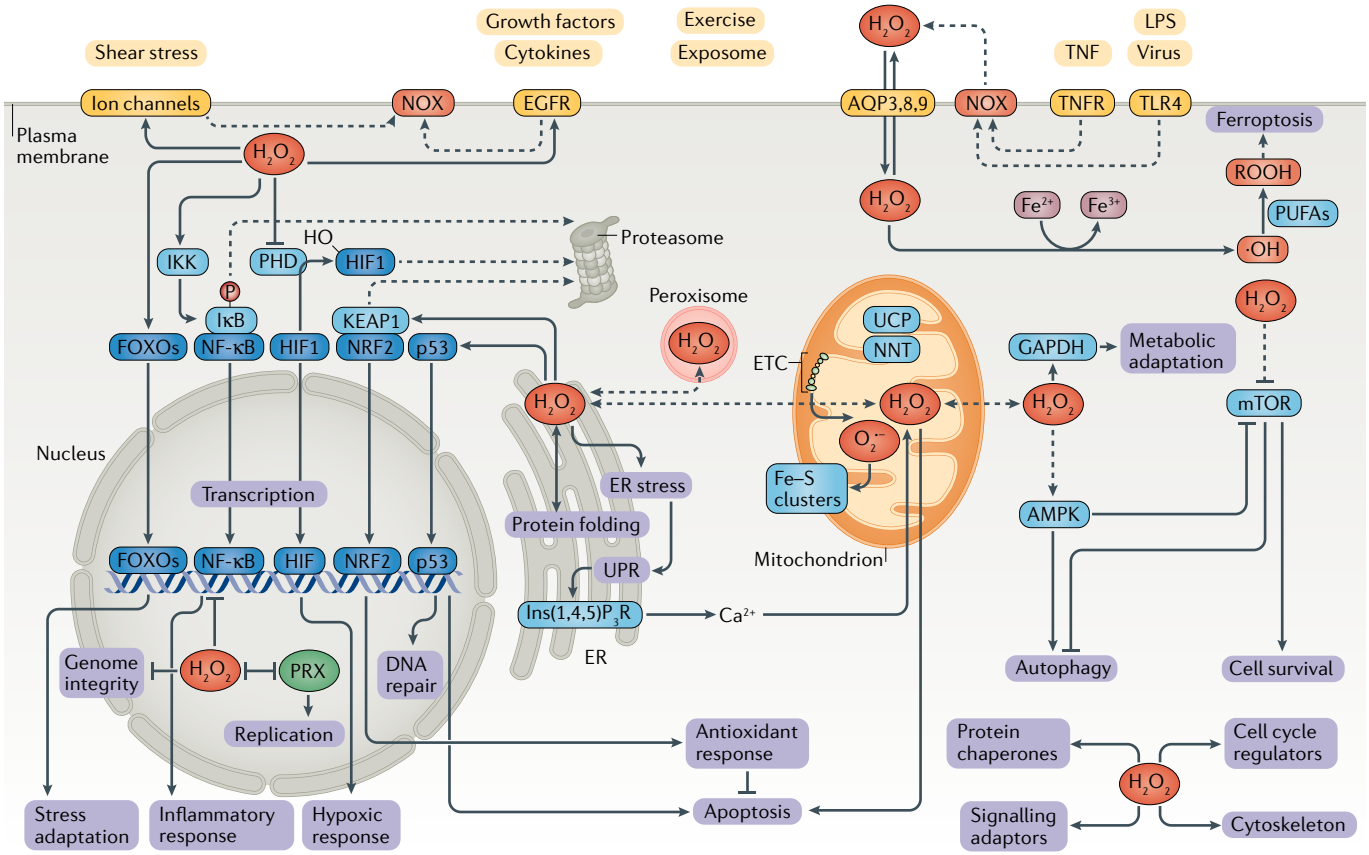


Fig. 3 | **Pleiotropy of redox signalling in cell biology.** Cells respond to a plethora of exogenous stimuli (pale yellow) via various receptors and channels (dark yellow) in the plasma membrane, initiating generation of $O_2^{\cdot-}$ and H_2O_2 (red; see also FIG. 2 for an overview of sources of reactive oxygen species), which subsequently act on a plethora of cellular targets (blue), thereby eliciting diverse biological activities (purple). These activities are highly pleiotropic, including the regulation of stress adaptation (including antioxidant response), inflammatory response, cell death and metabolic adaptation. For details the reader is referred to the main text. Also, environmental inputs (such as diet and lifestyle; known as the exposome) and exercise (yellow) contribute to cellular generation of $O_2^{\cdot-}$ and H_2O_2, contributing to redox signalling. Dashed arrows indicate indirect processes. AMPK, AMP-activated protein kinase; AQP, aquaporin; EGFR, epidermal growth factor receptor; ER; endoplasmic reticulum; ETC, electron transport chain; FOXO, forkhead box protein O; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; IκB, inhibitor of nuclear factor-κB; IKK, inhibitor of nuclear factor-κB kinase; Ins(1,4,5)P_3R, inositol 1,4,5-trisphosphate receptor; Keap1, kelch-like ECH associated protein 1; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; NNT, nicotinamide nucleotide transhydrogenase; NOX, NADPH oxidase; NRF2, nuclear factor erythroid-2-related factor 2; PHD, prolyl hydroxylase; PRX, peroxiredoxin; PUFAs, polyunsaturated fatty acids; TLR4, Toll-like receptor 4; TNF, tumour necrosis factor; TNFR, tumour necrosis factor receptor; UCP, uncoupling protein; UPR, unfolded protein response.

that NF-κB is subject to redox regulation. H_2O_2 can have both stimulatory and inhibitory roles in NF-κB function depending on the context[106]. Cytosolic H_2O_2 can activate the NF-κB pathway[107], and this occurs via H_2O_2-mediated oxidation and activation of the inhibitor of NF-κB (IκB) kinases, which negatively control the stability of IκB. H_2O_2 can also directly modulate NF-κB owing to the presence of oxidizable cysteines in the DNA-binding region of NF-κB. In this context, increased nuclear H_2O_2 generation inhibited NF-κB DNA binding, reducing its transcriptional activity, while increase in the nuclear content of the H_2O_2 scavenger peroxiredoxin 1 stimulated activity[108].

Hypoxia-inducible factor and response to hypoxia. Hypoxia-inducible factor (HIF) is a transcription factor that serves as the master regulator of transcriptional responses to decreased oxygen levels[109,110]. Hypoxia (oxygen deficiency) has been associated with an increase in $O_2^{\cdot-}$ (and subsequent H_2O_2) generation due to inhibition of the mitochondrial ETC[111]. In line with this, chronic intermittent hypoxia, a life-threatening condition that occurs in many different diseases, including sleep apnoea (disturbed breathing during sleep), has been shown to activate redox signalling, which contributes to several systemic and cellular responses (which include changes in blood pressure, increased release of neurotransmitters and neurotrophic factors, and alteration of sleep and cognitive behaviours) that were associated with activation of second-messenger pathways and transcription regulators of hypoxia[112]. Oxidants help stabilize HIF during hypoxia, thereby helping to mount a hypoxic response[113,114]. HIF prolyl hydroxylases, which sense oxygen availability and drive HIF hydroxylation and subsequent proteasomal degradation, are modulated by oxidants. Even in normoxia, oxidant generation interferes with Fe^{2+} availability in the catalytic site of prolyl hydroxylase, inhibiting their activity and promoting HIF-mediated transcription (see REFS[115,116]). In this way, oxidants affect HIF pathways also under non-hypoxic conditions, thereby driving a stress-adaptive response.

Autophagy
A process for removal of damaged cellular structures that contributes to maintenance of cellular homeostasis.

Glutathionylation
A post-translational protein modification functioning in redox signalling comprising the reversible formation of an *S*-glutathione adduct of a cysteinyl residue in proteins. Glutaredoxins are enzymes that catalyse the formation and removal of S-glutathionylated proteins.

Src kinase
A member of a family of non-receptor tyrosine kinases with many protein targets and functions in differentiation and regulation of cell growth.

Regulation of stress sensors. The theme of homeostatic adaptation mediated by redox signalling is also reflected by the responsiveness of various stress sensors to oxidants. For instance, the forkhead box protein O (FOXO) family of transcription factors contributes to maintenance of cellular and organismal homeostasis through integrating redox signals with other signalling cues. As observed with other redox signalling systems, this occurs via direct cysteine oxidation in FOXO members as well as via oxidant-mediated tuning of upstream regulatory mechanisms[117,118].

There are also strong links between oxidants and p53, a transcription factor that governs responses to a variety of stresses associated with genomic instability and the deregulation of which is strongly associated with cancer. p53 is under oxidant control, whereby H_2O_2 modulates selective transactivation of p53 target genes, which occurs indirectly, via modulation of signalling networks, and perhaps also directly, by oxidation of p53 cysteine residues. Reciprocally, p53 maintains the cellular redox balance by regulating the expression of antioxidant genes[119]. This role could support its tumour-suppressor function — in the tumour niche, which is typically proinflammatory and characterized by oxidative stress, p53 could act to limit oxidative stress-induced DNA damage and tumour progression[120].

Regulation of energy stress mediated by AMP-activated protein kinase (AMPK)[121] is also under redox control. However, even though AMPK harbours cysteine residues which could be subject to redox regulation, it was recently shown that AMPK activity in response to redox changes is not due to direct oxidation on AMPK itself but is a secondary consequence of redox effects on other processes converging on mitochondrial respiration and leading to decreased levels of energy generation[122].

Finally, redox signalling is coupled to nutrient stress. Both nutrient excess and nutrient deprivation are associated with increased oxidant formation and modulation of the function of key nutrient sensors. These include AMPK (discussed above), which senses glucose availability, as well as mTOR[123], which responds to amino acid availability by stimulating cell growth by promoting proliferation and inhibiting autophagy. Furthermore, autophagy is regulated by ROS in both an mTOR-dependent fashion and an mTOR-independent fashion[124].

Glyceraldehyde phosphate dehydrogenase and metabolic adaptation. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a central enzyme in glycolysis, is an interesting ROS target as it can acquire non-glycolytic functions on oxidation. For example, it translocates to the nucleus on oxidation and fulfils functions in DNA repair. Further non-glycolytic functions include a role in tRNA export and ER-to-Golgi transport of secretory cargo[125]. GAPDH is also inhibited by H_2O_2, which reacts with a conserved catalytic cysteine that is required for its sensitivity towards its glycolytic substrate, glyceraldehyde 3-phosphate. The sensitivity of this cysteine to H_2O_2 versus glyceraldehyde 3-phosphate is determined by a proton relay with a neighbouring cysteine, which was found to be required for metabolic adaptation and cell survival in response to increased H_2O_2 levels[126].

Uncoupling proteins and regulation of mitochondrial energetics. Mitochondrial uncoupling is a process whereby the flux through the ETC is decoupled from ATP synthesis. This uncoupling results in proton leak and consequent dissipation of the electrochemical proton gradient as heat, which is a key principle of thermogenesis in thermogenic fat (brown/beige adipocytes) and has been attributed to an uncoupling protein[127], now referred to as UCP1 (REF.[128]). There are five UCPs, UCP1–USP5, and their role extends beyond brown/beige fat tissue, with different isoforms being expressed in various tissues[129,130]. UCP uncoupling activity is promoted by $O_2^{\cdot-}$[131], which causes mild mitochondrial uncoupling. This has been shown to limit the activity of the ETC, thereby providing negative feedback on mitochondrial oxidant production. In addition, UCP2 and UCP3 are maintained in an inactive state by glutathionylation. Slight increases in mitochondrial oxidant production cause deglutathionylation and thereby activation of these UCPs[132]. In addition, UCP2 activity involves redox-activated mitochondrial phospholipase, the activity of which releases free fatty acids that make possible UCP2-mediated uncoupling[130].

Protein-tyrosine phosphorylation and dephosphorylation. Protein kinases mediate distinct cellular processes ranging from proliferation and differentiation to decisions on survival or cell death/apoptosis by protein phosphorylation. Protein-tyrosine phosphorylation is impacted by direct redox-based regulation of protein-tyrosine phosphatases and protein-tyrosine kinases[133,134]. On the one hand, oxidation of reactive cysteine in protein-tyrosine phosphatases causes their inactivation, resulting in an enhanced level of tyrosine phosphorylation; one example here is protein-tyrosine phosphatase 1B (PTP1B), the oxidation-dependent inactivation of which was shown to depend on its interaction with protein 14-3-3 (REF.[135]). On the other hand, protein-tyrosine kinases may be activated by H_2O_2. For example, EGFR is a target of EGF signal-derived H_2O_2, and oxidation of active site cysteine to sulfenate enhances its kinase activity[136]. Likewise, cysteine sulfenylation drives activation of Src kinase[137]. CO_2/bicarbonate was found to be essential for oxidation of PTP1B, a target in EGF-dependent H_2O_2 signalling, demonstrating a role for HCO_4^- (REF.[138]), and likewise CO_2/bicarbonate increased H_2O_2-mediated hyperoxidation of peroxiredoxin 1 (REF.[139]).

In addition to enzymes, signalling adaptors can also be modulated by redox signalling. For example, GRB2-associated-binding protein 1 (GAB1), a multifunctional adaptor protein with a key role in tyrosine kinase signalling pathways, has recently been identified as a novel redox target of NOX4, with Cys374 and Cys405 as major target sites[140].

Regulation of conductance through ion channels. Redox signalling is also a common regulatory principle for ion channels[141,142]. At the plasma membrane, the prototypical example is provided by voltage-gated K^+ channels[143], with early observation that inactivation of mammalian fast $I_{K(A)}$ channels is regulated by cysteine oxidation[144]. Furthermore, transient receptor potential channels,

Xanthine oxidase
A form of xanthine dehydrogenase that generates a relatively high proportion of $O_2^{\cdot-}$ instead of H_2O_2 during oxidation of hypoxanthine to xanthine or xanthine to uric acid.

Aconitase
An enzyme in the citric acid cycle that has an Fe–S cluster and is sensitive to inactivation by $O_2^{\cdot-}$.

ORAI calcium channels, voltage-gated calcium channels and purinergic receptors in the plasma membrane are all subject to redox signalling[141]. Inositol 1,4,5-trisphosphate receptor ($Ins(1,4,5)P_3R$) Ca^{2+} channels in the ER are also important targets of redox signalling and will be discussed in the following section.

Cellular roles of redox signalling

In light of the many targets of ROS, redox signalling has diverse cellular functions. These different downstream effects are strongly linked between subcellular compartments (FIG. 3) and show extensive crosstalk with other signalling modes, establishing intricate signalling networks[145]. Redox modifications of proteins also interplay with other post-translational protein modifications. One example here is the interrelation between protein S-glutathionylation and O-GlcNAcylation — the covalent addition of O-linked β-*N*-acetylglucosamine (*O*-GlcNAc) to hydroxyl groups of serine or threonine residues in cytosolic and nuclear proteins[146]. The reciprocal interplay between this nutrient-sensitive form of glycosylation and redox signalling is an active field of current study, and the term 'glyco-redox' has been coined to capture this linkage[147]. Here we highlight key cellular hubs of redox signalling and demonstrate the functional pleiotropy of ROS in cell biology (FIG. 3).

Signal transduction at the plasma membrane. The plasma membrane is a key platform for cell signalling, integrating and transmitting signals between the extracellular space and the intracellular space. This includes generation and transmission of redox signals (see REF.[148]). The plasma membrane is a major site of oxidant generation (mediated by NOXs, xanthine oxidase and so on) and transport (via aquaporins) (FIG. 2b). It is also the site where a plethora of signalling receptors (importantly including receptor tyrosine kinases) and ion channels localize, which as discussed earlier, are subject to redox regulation. Reciprocally, oxidant generation at the plasma membrane can be regulated by signalling pathways downstream of various cues, including cytokines (for example, tumour necrosis factor) and pathogen products (via Toll-like receptor 4) in the context of inflammation, growth factors (such as EGF) and shear stress (via mechanoresponsive ion channels)[148]. Hence, the plasma membrane is an important hub of redox signalling that supports its regulation by microenvironmental and intercellular communication.

In the context of membranes, oxidants also target lipids. The resulting lipid peroxidation generates lipid hydroperoxides and lipid-derived second messengers, which then can attack key regulatory proteins (for example, the NRF2 system) or heat shock response pathways, generating lipid–protein adducts in a process known as lipoxidation[149]. These events can have physiological functions contributing to redox signalling but may also lead to disease. This is exemplified by a form of regulated cell death, known as ferroptosis, which is characterized by iron-dependent accumulation of lipid hydroperoxides to lethal levels[150]. The membrane-associated phospholipid hydroperoxide glutathione peroxidase (GPX4) has been shown to be required to prevent hydroperoxide-induced ferroptosis by preventing accumulation of lipid hydroperoxides[151].

Redox signalling in the nucleus. Signals from the plasma membrane and other cellular sites control activation of gene transcription, many of which are aimed at homeostasis maintenance and are regulated by oxidants via master switches such as NRF2 and NF-κB as discussed earlier. The overall redox environment in the nucleus is more reduced than in the cytosol, and gene expression is sensitive to oxidants, as is DNA replication. Specifically, peroxiredoxin 2 has been shown to be an important, redox-sensitive regulator of replication forks: at low oxidant levels, peroxiredoxin 2 is oligomerized and promotes replication fork progression; H_2O_2 at an elevated concentration oxidizes and dissociates peroxiredoxin 2 into its subunits with lower affinity to chromatin, which leads to replication fork slowdown. As increase in the levels of oxidants is linked to the suppression of synthesis of deoxynucleoside triphosphate molecules — the building blocks of DNA — this redox signalling via peroxiredoxin 2 has been proposed as a general means of adaptation of DNA replication to changing metabolic conditions[152].

Another function of peroxiredoxins in the nucleus was demonstrated for telomere length maintenance, whereby peroxiredoxin 1 was shown to counteract the oxidative damage of telomeric DNA mediated by H_2O_2 and to work together with 7,8-dihydro-8-oxoguanine triphosphatase (MTH1) — an enzyme 'cleaning' oxidized nucleotide pools — to promote telomere elongation by telomerase[153]. Furthermore, redox signalling fine-tunes the cell cycle, which occurs through cysteine sulfenylation in CDC25 proteins — dual-specificity phosphatases that are critical for cell cycle phase transitions[154,155].

Finally, oxidants have modulatory effects on DNA damage response, integrating DNA damage response signalling[156], transcriptional regulation, DNA repair, cell cycle and DNA replication regulation.

ROS in mitochondria. As mentioned already, mitochondria are key cellular sources of $O_2^{\cdot-}$ and H_2O_2 (REFS[46,157]). The physiological role of mitochondrial redox metabolism spans numerous fundamental aspects beyond energy capture, as diverse as participating in anabolic and catabolic pathways and having a role in epigenetic cell regulation[158]. Mitochondria themselves respond to oxidant exposure with functional consequences. Several mitochondrial proteins, importantly including ETC components, contain Fe–S clusters, which are highly reactive towards $O_2^{\cdot-}$, which, as shown for aconitase in the matrix[159], leads to a change in the function of the enzyme. Also, the assembly of Fe–S clusters is redox sensitive[160], and hence changes in oxidant availability will inevitably impact mitochondrial functions in metabolism, including respiration and subsequent oxidant generation. There is also a tight bidirectional interplay between oxidants and mitochondrial dynamics (fusion and fission events as well as cristae remodelling that regulate the morphology of the mitochondrial network and mitochondrial functions)[161–163]. In this process, the morphology of the mitochondrial network impacts

Mitochondrial permeability transition pore
A pore formed from structural changes in mitochondrial inner membrane proteins that allow influx of solutes into the mitochondrial matrix. Activation of the pore causes high-amplitude swelling of the mitochondria and activates cell death mechanisms.

Unfolded protein response
(UPR). A cell stress response triggered by disruption of protein processing within the endoplasmic reticulum.

ER stress
Perturbation of functions of the endoplasmic reticulum (ER), for example abnormal folding and processing of proteins.

mitochondrial respiration and oxidant generation, while reciprocally, oxidants lead to modifications in the expression and/or activity of the proteins implicated in the mitochondrial dynamics. Furthermore, downstream of oxidant level-regulated AMPK lies the mitochondrial biogenesis factor peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), thereby further connecting redox signalling to the regulation of mitochondrial networks[121]. Oxidants also regulate mitochondrial quality control via autophagy, which permits removal of dysfunctional mitochondria and thus has an important role in maintaining cellular homeostasis. In this case, low oxidant levels trigger primarily selective removal of mitochondria by mitophagy (in a fission-dependent manner), whereas higher oxidant levels lead to non-selective autophagy (macroautophagy)[164]. Higher oxidant levels also open the mitochondrial permeability transition pore, which stimulates further oxidant formation, termed 'ROS-induced ROS release', and generally has been linked to apoptosis[165]. Nevertheless, transient opening of the mitochondrial permeability transition pore has been proposed as a mechanism to initiate extramitochondrial adaptive responses, whereby oxidants activate local pools of redox-sensitive enzymes involved in protective signalling pathways[165].

Peroxisomal H_2O_2 in redox homeostasis and signalling. The role of peroxisomes in the metabolism of lipids and H_2O_2[166] has led to interesting new perspectives in redox signalling[70]. Peroxisomes contain a number of H_2O_2-generating oxidases (TABLE 1), such as fatty acyl-CoA oxidase and D-amino acid oxidase, as well as H_2O_2-reducing enzymes, such as catalase and peroxiredoxin 5. While peroxisomal H_2O_2 metabolism obviously relates to regulation of peroxisomal functions, it also addresses extraperoxisomal redox targets such as FOXO3 or PTEN[70,71]. In addition, peroxisomal catalases are able to modulate oxidative stress at the cellular level[167], and catalase can even be secreted, which has been associated with malignant transformation[168]. The abundance and distribution of peroxisomes is highly variable among cell types, raising the possibility of an important role in cell-specific redox signalling.

Role in protein folding in the ER and ER stress. In the context of protein metabolism, a major process is the formation of disulfide bridges, which for proteins that enter the secretory route occurs in the ER during oxidative protein folding. For every disulfide formed in a reaction catalysed by protein disulfide isomerases, there is production of one oxidizing equivalent, H_2O_2, resulting from reoxidation of protein disulfide isomerases catalysed by endoplasmic oxidoreductin 1 (ERO1). Hence, H_2O_2 is a by-product of protein folding. The ERO1-generated H_2O_2 can be subsequently used by glutathione peroxidase 7, which utilizes this H_2O_2 to further promote oxidative folding, at the same time reducing the oxidative burden on the ER[169]. Different cell types have different secretory products dependent on these ER systems. For instance, plasma cells use H_2O_2 to support antibody production[170], and pancreatic islets use H_2O_2 to support insulin production[171].

Deregulation of ER redox balance has been linked to the activation of the unfolded protein response (UPR), which is generally aimed at restoring ER homeostasis on ER stress. Specifically, increase in the levels of both oxidizing agents or reducing agents in the ER can lead to ER stress and UPR activation. In turn, activation of the UPR has been shown to lead to further increase in ROS levels by affecting mitochondrial function[172,173]. In line with this interplay between oxidative stress and ER stress, oxidative stress-induced endothelial dysfunction was shown to be counteracted by targeting the UPR[174].

H_2O_2 and interorganellar signalling. Substantial crosstalk occurs between calcium signalling and H_2O_2 signalling[175–177]. Mobilization of Ca^{2+} from the ER in response to stimulation by growth factors and hormones as well as in response to ER stress (see earlier) occurs via Ins(1,4,5)P_3R channels, which are themselves redox targets, and the functionally relevant cysteine residues in Ins(1,4,5)P_3R1 have now been identified[178]. Notably, ER establishes close connections (membrane contact sites) with other organelles, including mitochondria, with functional consequences; ER–mitochondria contact sites have been importantly linked to Ca^{2+} influx into mitochondria. This Ca^{2+} influx has been shown to occur at H_2O_2 mitochondrial nanodomains, which are induced by Ca^{2+} signalling at the interface between ER and mitochondria[179,180]. In addition to mitochondria, ER also establish contact sites with peroxisomes, and these three organelles were suggested to form a 'redox triangle' that would support exchange of oxidants and regulate redox activities in the different organelles, thereby functioning as a focal point in redox messaging[74].

Oxidants in pathophysiology

Given the role of oxidants in cellular processes as described in the preceeding sections, it comes as no surprise that dysregulated redox homeostasis is a common pathophysiological condition, denoted as the transition from oxidative eustress to oxidative distress[23,181,182]. In an exemplary fashion, we here illustrate redox signalling in health and disease (see also REFS[183,184]). In the following subsections, we present the involvement of oxidants, notably H_2O_2, in several organs in terms of their physiology and in dysregulated disease processes, highlighting their pleiotropic, often antagonistic effects. Importantly, at the current state of knowledge, it can be difficult to delineate a distinction between beneficial and harmful signalling (eustress versus distress). Thus, large-scale systematic studies will be needed to map out the signal–response relationships in each context to better understand the complex roles of oxidants in pathophysiology.

Developmental processes. The course of development of an embryo entails considerable changes in redox state[185]. H_2O_2 is crucially involved in those redox signalling events and has far-reaching implications for morphogenesis and cell differentiation[186]. One prominent example is the nervous system, where ROS impact neuronal polarity, regulation of connectivity, synaptic transmission and the tuning of neuronal networks[187]. For instance, there

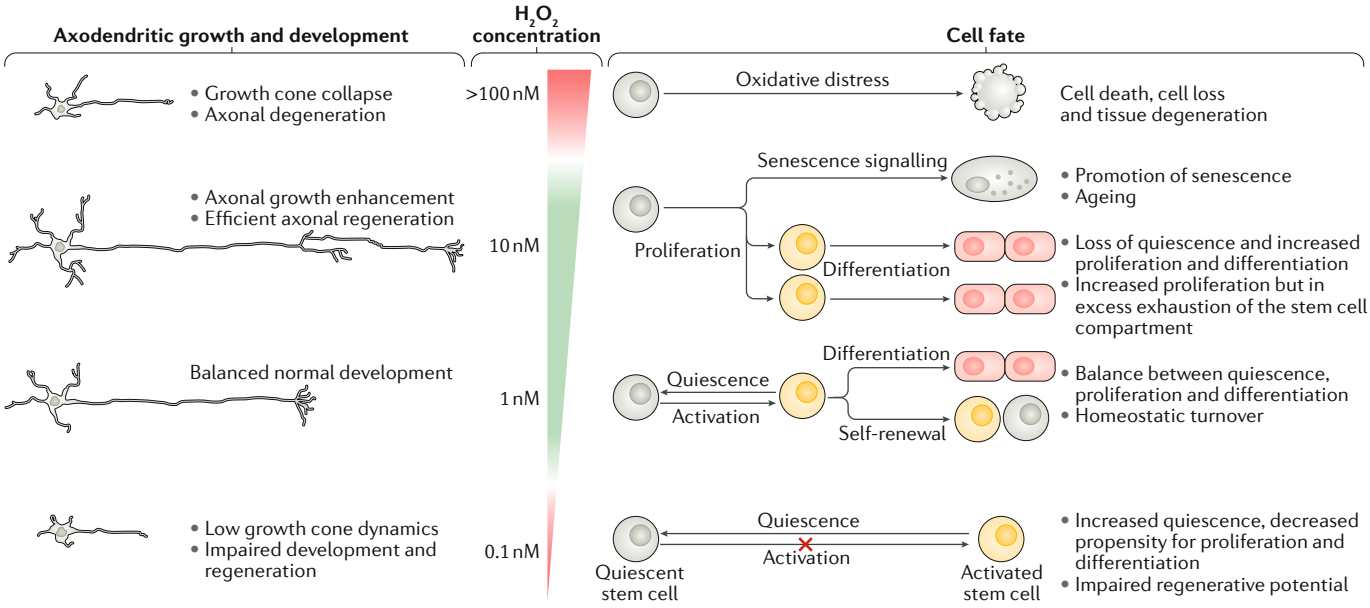


Fig. 4 | **H_2O_2 drives different cellular outcomes depending on its concentrations.** Left-hand side: in neurons, levels of H_2O_2 within the physiological range (1–10 nM), promote both axonal growth and dendritic growth (oxidative eustress), which is associated with normal, balanced neural development. A moderate increase in H_2O_2 concentration (up to about 100 nM) has been associated with further promotion of dendritic growth, which is important in the context of axonal regeneration after injury. Abnormally high H_2O_2 concentration (greater than 100 nM) favours oxidative distress, leading to the collapse of the axonal growth cone and subsequent degeneration. Abnormally low H_2O_2 concentration (less than 0.1 nM) impairs neuronal development. Right-hand side: relation between oxidant level and stem cell potential. Generally, stem cells maintain a low basal level of oxidants (mainly H_2O_2) in quiescence and during self-renewal, which increases with differentiation. Hence, physiological levels of H_2O_2 determine the balance between proliferation, self-renewal and differentiation. Lower levels of H_2O_2 lead to impaired exit from quiescence and reduced proliferation, which can negatively impact regeneration. Higher levels of H_2O_2 favour increased exit from quiescence and increased proliferation with subsequent differentiation. This can be beneficial in the context of regeneration, but when uncontrolled can lead to the depletion of the stem cell pool. Elevation of H_2O_2 concentration has also been associated with replicative senescence of stem cells. Finally, further increase of H_2O_2 concentration will lead to oxidative distress and cell death. Left panel modified with permission from REF.[188], Elsevier.

is ample evidence for an essential contribution of H_2O_2 produced by NOXs to axonal growth cone pathfinding, whereby neuronal growth and guidance depend on physiological amounts of H_2O_2. Insufficient ROS generation was linked to spatial memory deficits in mice, whereas a pathological increase in ROS level causes growth cone collapse and axonal degeneration[187,188] (FIG. 4, left-hand side). The physiological range of concentrations for H_2O_2 was estimated to be between 1–10 nM. However, these values will depend on several parameters, including cell type and developmental stage[188]. In line with these roles of H_2O_2 in the growth cone dynamics, ROS have been implicated in cytoskeletal organization[189].

Stem cell biology is also tightly linked to redox homeostasis[190] (FIG. 4, right-hand side). Stem cells maintain a low basal level of oxidants (mainly H_2O_2) in quiescence, which increases with differentiation. These physiological levels are thought to preserve stem cell function, allowing the achievement of proper balance between quiescence, proliferation and differentiation, and to regulate stem cell activity in response to stress. Lower oxidant levels lead to impaired cell cycle entry, thereby interfering with proliferation and differentiation, whereas increased levels cause hyperproliferation and induce cell senescence, consequently leading to stem cell exhaustion. Even higher exposure to oxidants will inevitably cause damage to macromolecules, inducing cell death. Long-term survival of stem cells is particularly important in the context of adult stem cells, and hence changes in the redox balance will ultimately affect tissue regeneration potential.

Circadian rhythms. In eukaryotic cells, reversible redox transitions of proteins are known to occur in a circadian fashion[191,192]. These oscillatory redox changes together with diurnal changes in energy levels are important signals to cellular clocks to adapt to temporal tissue-specific needs[193]. For example, diurnal oscillations of endogenous H_2O_2 are sustained by p66Shc — a redox enzyme that accomplishes oxygen reduction using reducing equivalents from the respiratory chain — resulting in rhythmic redox control of a master regulator of circadian rhythms, the transcription activator CLOCK[194]. In flies, the sleep–wake rhythm was connected to oxidative modulation of a potassium channel in sleep-regulating neurons[195]. Because circadian rhythms are key to animal homeostasis, diurnal redox regulation has far-reaching implications for pathophysiology[196]. Importantly, inclusion of temporal variation into research protocols greatly increases the complexity of the design, but failure to consider such fluctuations can obscure understanding and potentially cause important contributions of oxidant signalling to be overlooked.

Central nervous system. As a tissue that is highly dependent on O_2, the central nervous system (CNS) is particularly sensitive to changes in O_2 levels, and NOXs play a

Axonal growth cone
Site of growth at the tip of a dendrite or an axon of neurons, containing cytoplasm and actin formed in the leading edge of the cell during neuronal development and regeneration.

Cell senescence
A state of cells with arrested cell division and resistance to cell proliferation signals and uncontrolled cell growth. Accumulation of senescent cells is a hallmark of ageing; it contributes to decline in organ function and some age-related diseases.

role in the CNS[197]. Deregulation of redox balance (oxidative distress) is strongly linked to neurodegeneration, including major long-term degenerative diseases such as Alzheimer disease, Parkinson disease, Huntington disease and amyotrophic lateral sclerosis[198–200].

High oxidant levels can cause cell death as discussed already, and hence NOX levels are maintained low in the resting cells of the CNS, ensuring a low steady state of H_2O_2 flux. Maintenance of brain redox homeostasis also requires adequate function of antioxidant selenoproteins, which involves an interplay between uptake of selenoprotein P from blood plasma into astrocytes (the main glial cell type of the brain) and subsequent supply of newly synthesized selenoproteins to neurons to increase their antioxidant capacity[201]. Insults to the CNS, such as accumulation of protein aggregates associated with neurodegenerative diseases, cause upregulation of NOX activity and oxidant generation — involving both neurons and microglia (the main phagocytes in the brain that serve as neuron-supporting cells). Neuronal cell death can occur in a cell-autonomous manner and a non-autonomous manner due to these interactions of astrocytes and microglia. Other types of CNS insult can also activate microglia, upregulate NOX2 and increase ROS generation. Generally, this response is meant to be protective by clearing debris and supporting neuronal survival. However, in some cases microglia become overactivated and overproduce and secrete ROS and reactive nitrogen species, thereby leading to neuroinflammation and impeding neuronal and oligodendroglial survival[197,202]. Furthermore, ischaemic conditions lead to enhanced NOX4 activity in both neurons and endothelial cells, which, in turn, causes neuronal autotoxicity and breakdown of the blood–brain barrier[203].

As discussed already, H_2O_2 is an important factor supporting axonal growth and regulating cell proliferation. It is thus not surprising that after injury or amputation, nerves crucially increase the production of oxidants, foremost H_2O_2, which facilitates axon regrowth and tissue regeneration[204]. A novel aspect in the promoting role of oxidants in regeneration after axonal injury concerns the delivery of the H_2O_2-producing NOX2 to the site. NOX2 is delivered by macrophages via exosomes, which are subsequently incorporated into injured axons via endocytosis, ultimately leading to axonal regeneration[205].

Further highlighting the pleiotropy of ROS functions and the fine balance between oxidative eustress and distress, recent findings highlight the importance of mitochondrial H_2O_2 production for maintaining proper function of neurons. Specifically, transcriptomic, metabolomic, biochemical, immunohistochemical and behavioural analyses showed that decreasing H_2O_2 production in astrocytes by overexpressing catalase caused alterations to brain redox, carbohydrate, lipid and amino acid metabolic pathways associated with neuronal function[206].

Pertaining to the action of ROS in the CNS, there is also evidence that oxidants are mediators of psychological stress responses. An early finding was a mechanism converting psychosocial stress into mononuclear cell activation via NF-κB activation[207]. Furthermore, chronic psychological stress exposure promotes oxidative damage of nucleic acids and lipids (which could contribute to stress-induced ageing; see also the discussion on ageing later)[208]. As shown in rats, psychosocial stress induces early elevation of NOX2-derived oxidative stress in the hypothalamus, ultimately leading to altered behaviour. Mild psychological stress in humans may enhance psychobiological resilience to oxidative damage[208,209]. A well-studied type of psychological stress is noise stress. The damaging effects of noise on vascular function and inflammatory responses include specific activation of NOX3 in the inner ear[210,211], opening avenues for therapy for hearing loss[212]. Also, the causes of mental (neuropsychiatric) disorders include mitochondrial malfunction, suggesting a role of redox signalling in these disorders[213].

Immune system, inflammation and wound repair. Oxidative mechanisms are important in immune system function, not only in protecting against infectious agents but also in critical stepwise functions required for complex processes, such as wound healing. The role of redox reactions in inflammation and in immunology has been widely studied[214,215], and only a few highlights are summarized here. In response to a cut in the skin, NO and $O_2^{\cdot-}$ generation contribute to vasoconstriction and changes in vascular permeability; eicosanoids, including reactive oxidized lipids, function in platelet recruitment and activation; redox signalling controls fibroblast proliferation and differentiation; neutrophils are also recruited by oxidants, and then generate more potent oxidants to kill microbes; and during the wound repair process, redox signals cause proliferation of endothelial cells and keratinocytes. In such processes, phagocytes, neutrophils in particular, are major sources of $O_2^{\cdot-}$ and H_2O_2 (REF.[61]). In neutrophils, these oxidants are produced by NOX2 and are utilized by peroxidases such as myeloperoxidase and eosinophil peroxidase to generate hypochlorous acid and other oxidants that are used by these professional phagocytes to kill pathogens. These oxidants are also implicated in the formation of neutrophil extracellular traps, which support the capture and killing of bacteria[216,217]. There is an extensive range of examples of ROS functioning in the immune system, and we provide only a few additional examples. In antibody production by plasma cells, oxidants are essential for proper folding and maturation of immunoglobulins[218]. There is also involvement of mitochondrial oxidants in innate immune signalling[219]. As one example, there is substantial adjustment in the respiratory chain supercomplexes in macrophages on transition from the resting state to the activated state, leading to increased oxidant levels. These, in turn, affect downstream signalling which involves the NLRP3 inflammasome and cytokine and chemokine release[220]. ROS have also been implicated in the communication between the host and the gut microbiota. Specifically, it was shown that probiotic gut bacteria contribute to H_2O_2 generation, both directly and by stimulating H_2O_2 production in enterocytes on contact. This H_2O_2 generation could contribute to pathogen defence and promote redox signalling in enterocytes, including NRF2 signalling, which confers

Exosomes
Nanometre-sized vesicles released by cells, a means for cell–cell communication. Exosomes possess characteristics of cells of origin and can be used as biomarkers of disease.

Neutrophil extracellular traps
Extracellular fibre networks created by neutrophils to trap and kill invading microbes. They contain DNA from extruded chromatin and antimicrobial proteins such as neutrophil elastase and cathepsin G.

NLRP3 inflammasome
NLRP3 is protein complex in cells that activates inflammatory processes. 'NLR' refers to 'nucleotide-binding oligomerization domain, leucine-rich repeat'. The complex senses a broad range of microbial and environmental stressors.

cytoprotective and reparative responses, for example in the event of inflammation[221,222].

Inflammation is a first step in the wound healing process and, not surprisingly, oxidants are important mediators of this process. ROS function in pathogen defence, stimulation of angiogenesis and proliferation and in myofibroblasts, which remodel the extracellular matrix to seal the wound[223]. In line with these important functions of oxidant signals, the process of wound healing is characterized by redox gradients[224]. Experiments involving *Xenopus laevis* tadpole tail amputation (using Hyper)[225] and zebrafish tail fin amputation (using Hyper7)[86] demonstrated that H_2O_2 forms tissue-scale gradients on tissue wounding and subsequent regeneration. Redox gradients occur due to physical separation of sources and sinks of oxidants; such gradients provide polarity for orderly processing of complex sequences of biomolecular reactions. In migrating cells, such as fibroblasts, H_2O_2 production occurs at the leading edge of the cell, with the H_2O gradient determining the stability of cellular protrusions by influencing dynamics of the actin filament network[86]. Overall, impairment of oxidant generation impedes wound healing[226]. However, overproduction of oxidants — oxidative distress — in the wound site inevitably impairs wound healing, and hence a balance between ROS production and ROS scavenging is key to ensure unperturbed wound closure. This is of particular importance in diabetes, which is associated with increased oxidant production in various tissues. In the wounds of patients with diabetes, several pathological mechanisms contribute to the accumulation of ROS, leading to wound healing complications in such patients[227].

Cardiovascular system. The vascular endothelium, the single cell layer lining the lumen of blood vessels, is pivotal in maintaining vascular function, and endothelial dysfunction is a major initial cause of cardiovascular disease. Regulation of endothelial function has an important redox component, and oxidative stress and inflammation are major contributors to cardiovascular disease[228–230]. Reflecting the pleiotropy of ROS, NOX4 has both beneficial and detrimental activities in endothelial redox regulation[44]. The formation of new capillaries in angiogenesis involves multiple redox control mechanisms[231]. On the one hand, keeping physiological low levels of H_2O_2 by NOX4 contributes to vasodilation, maintenance of endothelial function and lower blood pressure and vascular remodelling. On the other hand, supraphysiological flux through NOX4 leads to vasoconstriction, endothelial dysfunction, hypertension and a proinflammatory state through secretion of cytokines and chemokines, contributing to atherosclerosis[232] and aneurysm[233]. The NOX1, NOX2 and NOX5 isoforms also play roles in cardiovascular disease. These enzymes produce $O_2^{\cdot-}$, which reacts with NO to form peroxynitrite. Protein-tyrosine nitration involving peroxynitrate is a process of potentially harmful consequence, so coordinated expression and control of endothelial NO synthase (eNOS) with NOX enzymes is mandatory. In some conditions, for example in patients with diabetes, eNOS can become a generator of $O_2^{\cdot-}$ in a process known as uncoupling: eNOS activity is switched to support simultaneous production of $O_2^{\cdot-}$, thereby promoting production of peroxynitrate.

Also of interest, redox regulation of vasculature involves interorgan communication and specifically signals from the adipose tissue. Adipose tissue secretes hormones (adipokines), which regulate vascular redox state and provide a link between obesity and cardiovascular disease[234]. Extensive earlier research also documented the contribution of ROS to cardiovascular disease as key mediators of atherosclerotic plaque formation by promoting oxidation of low-density lipoprotein (LDL), generating oxidized LDL (ox-LDL)[235]. Generation of ROS occurs at the sites of endothelial damage, which are caused by ox-LDL itself as well as by physical or chemical forces due to disturbed flow and infection, with endothelial cells, macrophages and smooth muscle cells contributing to the ROS load. Ox-LDL then damages endothelial cells, causing inflammation that leads to the recruitment of macrophages, which then take up ox-LDL and transform into plaque-forming foam cells[235].

Skeletal muscle. Redox signalling has important roles in muscle function, exercise adaptation and decline in muscle function in frailty. For instance, redox signalling supports neuromuscular development[187], blood flow for immediate physical activity[236] and long-term remodelling and adaptation to contractile activity[237]. It was also shown that skeletal muscle regeneration is under redox control, whereby physiological ROS — acting through the many redox-sensitive signalling pathways — regulate the activity of muscle stem cells[238]. However, like in other tissues, increased ROS load is detrimental to muscle function, and skeletal muscle frailty, the general decline in muscle mass and function commonly associated with ageing, is a phenotypic manifestation of underlying oxidative stress[239]. This skeletal muscle decline is thought to occur owing to perturbed physiological adaptation to H_2O_2 and products of oxidative damage to biomolecules[240]. As expected, on the basis of the role of constant progressive physical activity in boosting resilience and increasing ROS detoxifying mechanisms[227], exercise is able to counteract age-related muscle loss[241,242]. There is also recent evidence that disruption of redox balance and impairment of redox signalling specifically in motor neurons are implicated in muscle loss[243].

Insulin sensitivity and pathogenesis of diabetes. It is now well established that oxidants have various inputs into regulating insulin signalling, so much so that type 2 diabetes is being considered as a 'redox disease'[244]. As mentioned already, oxidation is required for protein folding in the ER, and hence insufficient supply of oxidants will lead to UPR activation, which compromises insulin sensitivity in muscle and liver and at the same time can induce apoptosis of pancreatic β-cells, leading to decreased insulin secretion. Furthermore, several of the targets and regulators of insulin receptor signalling[245] are redox sensitive, such as AKT, FOXO, PTEN, PTP1B and JUN amino-terminal kinase (JNK), providing ample nodes for redox regulation of insulin-derived

Schiff base
A chemical structure with a double bond between nitrogen and carbon formed by reaction of an amine with a carbonyl group.

Michael addition
An addition reaction of a nucleophilic chemical with a chemical having an electrophilic α,β-conjugated carbonyl structure. The reaction can be important in pathological processes because DNA and other macromolecules contain nucleophiles, and α,β-conjugated carbonyls are major products of lipid peroxidation of polyunsaturated fatty acids.

signals[246]. Generally, physiological low oxidant levels increase insulin sensitivity by inhibiting the activity of PTP1B — which dephosphorylates and deactivates the insulin receptor — through their ability to modulate stress-response kinases. Nevertheless, increased ROS production potently promotes insulin resistance. As a prominent mechanism, high levels of oxidants favour activation of JNK, which is the most studied effector of insulin resistance[247].

ROS in ageing and lifespan regulation. Early studies on free radical mechanisms provided a basis for a free radical theory of ageing[248]. This theory invoked ROS as key reactants in uncontrolled processes involving reactions with all classes of macromolecules. Indeed, metabolites generated by redox reactions have the capacity to spontaneously modify macromolecules over time, and cumulative macromolecular damage can, in principle, contribute to many mechanisms of ageing, including non-enzymatic modification via Schiff base formation or Michael addition[249]. However, such changes can be the result of the inherent activity of many reactive molecules, not just ROS[182,249].

The damaging roles of oxidants are consistent with the hallmarks of ageing[250], which include mitochondrial dysfunction, protein denaturation and aggregate formation, altered cell membranes and intercellular communication, loss of regenerative cell populations owing to cell death and senescence, and genomic instability. All of these hallmarks have a redox-regulated component. Thus, an unresolved question is whether oxidant signals are a cause or a consequence of these hallmarks. Ageing is characterized by declining cellular homeostatic responses and diminished removal of damaged macromolecules, organelles and cells (impaired quality control), which results in weakened resilience. The extent of weakening resilience to challenges will inevitably determine the transition from healthy ageing to premature ageing.

Importantly, however, redox signalling is central to adaptive homeostasis mechanisms[251], as discussed earlier, and target systems of ROS-mediated signalling, including NRF2 activity[252], NF-κB[253], AMPK[254], uncoupling proteins[255], proteostasis[256–258] and mitochondrial signalling[259], are all subject to age-dependent decline. As documented with advanced redox proteomic tools, ageing rewires tissue-specific redox signalling networks[96]. Accumulating evidence now suggests contribution of oxidants to the extension of lifespan[260,261]. Specifically, mitochondrial oxidant signalling was found to be essential in extending lifespan in the process of mitohormesis, which has been shown to be a prolongevity mechanism in response to caloric restriction, exposure to mild hypoxia, temperature stress caused by elevated or decreased body temperature and physical activity[32,262]. Furthermore, work with *Caenorhabditis elegans* revealed that early-life exposure to ROS impacts stress resistance later in life, extending lifespan. Mechanistically, H_2O_2 was found to cause oxidant-sensitive epigenetic changes (global reduction of histone H3 Lys4 trimethylation) that increase stress resistance and lifespan[263]. Thus, redox processes in early development ultimately prolong lifespan.

Overall, as with other ROS functions, redox regulation of ageing seems to involve antagonistic pleiotropy[264].

Cancer. As with ageing, the hallmarks of cancer[265] include redox perspectives of considerable complexity[266,267]. This large area of research is not covered here in detail, but oxidant generation is strongly linked to initiation, progression, bystander effects in the tumour microenvironment and the biology of metastasis. Cancer cells increase ROS production[268], which, by controlling various signalling pathways and transcription factors, helps boost proliferation, helps rewire cancer cell metabolism and allows cell adaptation to nutritional and hypoxic stresses[45,269,270]. Exposome influence, encompassing various environmental exposures, psychological stress, diet and lifestyle, and so on, is particularly important in malignant transformation, potentially by impacting ROS-mediated mechanisms in cancer. In light of this dependence of cancer cell adaptation on ROS, it has been postulated that supplementation with selenium or application of antioxidants such as vitamin E or vitamin C could be a strategy to prevent malignant transformation. Nevertheless, data on the benefits of ROS-scavenging approaches for cancer prevention are inconsistent[270,271]. Some tumours increase their activity of antioxidant systems[269], indicating that a careful balance between oxidants and antioxidants is required for malignancy. This heightened antioxidant capacity of cancer cells is frequently associated with resistance to chemotherapy, as several currently used anticancer drugs are known to drive cytotoxicity at least partially via oxidant generation[272]. Overall, successful anticancer therapy, with the choice to use oxidant-producing chemotherapeutics to induce oxidative distress or antioxidants to perturb redox balance required for cancer progression, should be tailored to the particular case, including consideration of the stage and type of the tumour, oxidant levels in the tumour niche and the endogenous antioxidant capacity of the tumour[270,273,274]. High-dose (pharmacological) ascorbate is currently being investigated in tumour therapy as means of sensitizing cancer cells to radiochemotherapy[275,276].

Prospects for redox medicine

In light of the extensive impact of ROS on health, there has been continued interest in targeting ROS for therapeutic benefit in the development of redox medicine. However, the expanding knowledge of pleiotropy of ROS signalling demands that redox medicine use strategies with selectivity to address disease-relevant mechanisms while avoiding disruption of other important signalling processes. Trials with non-selective low molecular mass antioxidants at high doses have generally failed in prevention or treatment of disease processes, likely owing to disturbed redox signalling. In general, these trials were designed before the physiological importance of H_2O_2 signalling was recognized. With expanding knowledge of the mechanisms of ROS, therapeutic interventions focused on disease-relevant sources and targets of ROS are now becoming possible and are entering clinical trials[277].

Depending on the ROS source, disease or pathological conditions, therapeutic applications targeting ROS

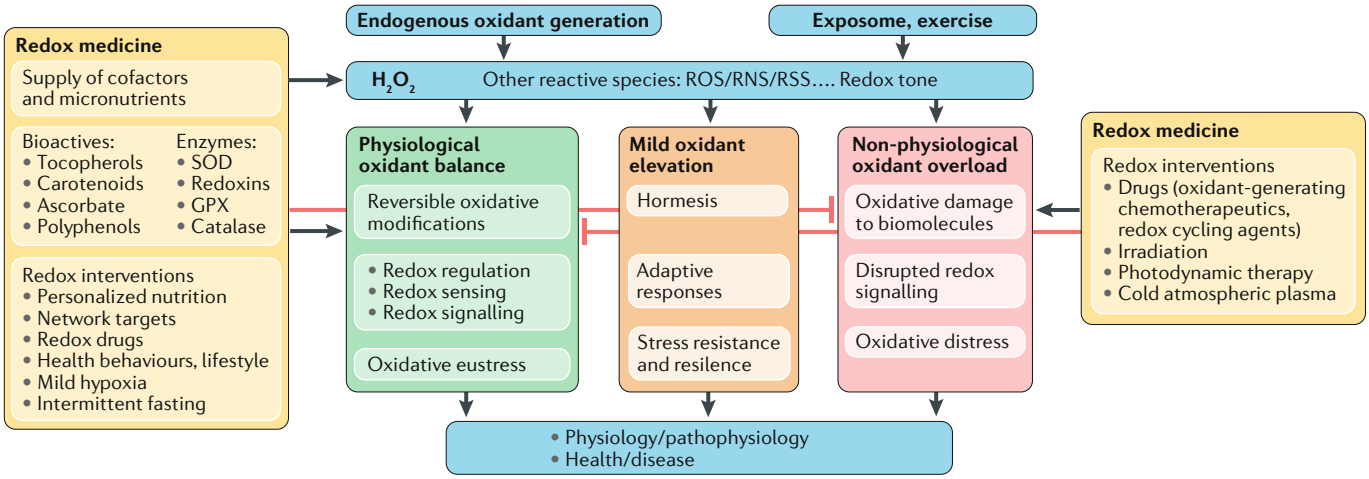


Fig. 5 | **Prospects for redox medicine.** As discussed in the main text, reactive oxygen species (ROS) and other active species are generated via various endogenous sources that are coupled to each other and to cellular signalling networks (see FIGS 2,3; TABLE 1). It is also now well established that the totality of exposures — to various chemical and biologic agents, radiation, psychosocial components, nutrition, exercise and lifestyle — throughout the lifetime, the so-called exposome, has an important input into oxidant levels. We also extensively discussed the importance of the proper redox balance for homeostasis maintenance and for physiological cellular and organismal function. Physiological levels of oxidants support physiology (oxidative eustress), while excessive oxidant exposure causes damage (oxidative distress). Mild elevation of oxidant levels leads to adaptation to stress and resilience (a concept known as hormesis). Redox medicine has the potential to modulate levels of oxidants for therapeutic benefit. It is desirable to control excessive levels of oxidants to prevent toxicity associated with oxidative distress (leading to cell death and tissue degeneration) and maintain proper redox balance. Applications of these strategies include use in (neuro) degenerative disorders and disorders associated with pathological inflammation. ROS scavenging has also been proposed as a strategy for lifespan extension and also in cancer prevention (as elevated ROS levels have been associated with cancer cell pathology). One possibility to control ROS is to supply redox cofactors (importantly nicotinamide nucleotides and flavins), which are essential for most redox reactions and hence are required for proper redox balance. An additional approach is to supply antioxidants, including various small bioactive molecules, such as vitamins, micronutrients and metal ions, as building blocks and components of enzymes, such as redoxins (thioredoxin, glutaredoxin and peroxiredoxin), glutathione peroxidase (GPX) and superoxide dismutases (SODs). There is also potential to use various redox interventions, including lifestyle changes and appropriate nutrition, but also redox-active drugs. Furthermore, exposure to hypoxia can have beneficial effects on redox balance and has been associated with increased resilience to stresses, including oxidative stress. In some circumstances, such as cancer and other types of aberrant tissue formation as well as to boost immune responses and pathogen defence, for example in the context of wound healing, it might be beneficial to induce elevated ROS generation to cause oxidative distress and promote cell killing. These approaches can be realized with the use of various chemotherapeutics, irradiation and redox cycling drugs and also with physical methods such as photodynamic therapy or the use of cold atmospheric plasma. RNS, reactive nitrogen species; RSS reactive oxygen species.

can involve upregulation of certain antioxidant systems, such as the NRF2 system[278], supplying low molecular mass antioxidants, such as vitamins, carotenoids and even bacterial metabolites, supplementation with trace elements and micronutrients (in the case of their deficiency)[279], and environmental interventions (nutrition, lifestyle and exercise) (FIG. 5). There is also potential for the application of more targeted inhibitors of ROS, such as specific suppressors of $O_2^{\cdot-}$ production at mitochondrial sites[280,281]. Control of free iron is another promising target in controlling ROS, as this controls the site and extent of generation of the highly aggressive hydroxyl radical[282,283] (BOX 1). Such approaches could be beneficial for the treatment of diseases caused by oxidative distress, including neurodegenerative and cardiovascular diseases related to chronic oxidative stress and in the treatment of patients with inflammatory disorders, viral diseases and sepsis. The response to hypoxia provides another therapeutic opportunity to target aberrant ROS generation, in this case associated with mitochondria[284]. In line with this, genetic or small-molecule activation of the response to hypoxia was found to be protective against mitochondrial dysfunction in cell culture and the zebrafish model, while chronic hypoxia led to an improvement in survival, physiology and disease biomarkers in a genetic mouse model of Leigh syndrome, which is a common paediatric manifestation of a mitochondrial disease. These beneficial effects of hypoxia on mitochondrial toxicity were likely driven at least in part by modulating oxidants[285]. Furthermore, successful ROS-targeting therapeutic strategies have involved application of oxidative distress (FIG. 5) to induce cell death in treatment of cancers (ionizing radiation, coupled with redox cycling agents as oxidant-producing chemotherapeutics) and skin disorders, such as acne and psoriasis (generating singlet oxygen in photodynamic therapy)[286]. An interesting application of oxidative distress is the use of cold atmospheric plasma, a mixture of ROS and other oxidants, which has been successfully applied to support antibacterial defence in wound healing as well as in cancer cell treatment[287].

In addition to modulation of oxidative distress — inhibition to counteract pathological cell loss and induction to promote cell death of pathological cells — an interesting avenue in redox medicine is modulation of redox eustress to regulate redox signalling and associated cell functions. This would require information on patient-related status; the Bioenergetic Health Index in human monocytes has been formulated as a sensitive measure of oxidative stress[288]. Such concepts

Redox cycling agents
Chemicals that undergo enzymatic one-electron reduction, generating a transient radical, which is reoxidized by molecular oxygen, reducing it to $O_2^{\cdot-}$.

Photodynamic therapy
A therapy using photoexcitable agents and light to generate toxic reactive oxygen species, predominantly singlet molecular oxygen.

in redox metabolism offer new insights into developing metabolism-based clinical tests[289]. In the context of the process of tumorigenesis, which as described earlier relies on elevated levels of oxidants to drive malignancy, there is a therapeutic avenue of using drugs inhibiting redox signalling, and a number of such approaches have been evaluated[290,291].

On the new front in redox medicine, the emerging field of ROS-based nanomedicine, involving nanomaterials with ROS-regulating properties, holds promise for optimized therapeutic efficacies[292,293]. Furthermore, the new tools of network medicine, involving the phenotypic analysis with consideration of intracellular and intercellular connectivity, encompassing the different omic levels and their interconnection, now make possible identification of functional modules leading to disease[294]. In the area of redox regulation, this data-driven approach was used to link the different omics levels to mitochondrial function, thereby providing a systems biology view of mitochondrial macromolecular and metabolic functions, including regulation of mitochondrial H_2O_2 production[295]. Such approaches will be key in developing novel, more targeted therapies for ROS control.

Conclusions and perspective

The signalling and damaging properties of ROS, in particular the most prevalent and best studied cellular oxidant H_2O_2, form the basis for the concept of redox homeostasis, with its components of oxidative eustress and oxidative distress. Redox signalling is universally integrated with the central homeostatic mechanisms at the molecular, organellar, cellular, tissue and organismic levels. The scientific community has learned a great deal about these mechanisms in development, health and disease as well as in ageing. This led to the emergence of a new field of redox medicine that explores the potential of targeting the production and detoxification of oxidants as a medical strategy. However, because of the extensive pleiotropy of ROS, their role as physiological signalling agents remains difficult to explicitly define in terms of simple cause–effect relationships.

Given the recent availability of advanced methods to analyse specific oxidants mentioned already, an important step forward for future research in understanding the complexity of the contribution of oxidants to physiology is to restrict considering 'ROS' — which does not refer a single molecule after all — in favour of examining specific molecular agents such as $O_2^{\bullet-}$, H_2O_2 or $^1O_2$ that have vastly different properties, mechanisms and roles in physiology (BOX 1) and hence may require very different approaches for therapeutic modulation. Furthermore, the borderline between oxidative eustress and oxidative distress in different physiological settings is highly context dependent and needs to be better characterized. Thus, to successfully use redox modulation as a therapeutic strategy, it is of crucial importance to characterize redox balance in the different contexts. Omics and imaging technology will provide access to understanding such global effects. We anticipate that with these advancements, the potential for the emerging field of redox medicine will be better realized.

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1. Halliwell, B., Gutteridge & J. M. C. in *Free radicals in biology and medicine* (Oxford University Press, 2015).
2. Hawkins, C. L. & Davies, M. J. Detection, identification, and quantification of oxidative protein modifications. *J. Biol. Chem.* **294**, 19683–19708 (2019).
3. Sies, H., Berndt, C. & Jones, D. P. Oxidative stress. *Annu. Rev. Biochem.* **86**, 715–748 (2017).
Concept of oxidative stress: physiological (eustress) and supraphysiological (distress).
4. Murphy, M. P. et al. Unraveling the biological roles of reactive oxygen species. *Cell Metab.* **13**, 361–366 (2011).
5. Brandes, R. P., Rezende, F. & Schröder, K. Redox regulation beyond ROS: why ROS should not be measured as often. *Circ. Res.* **123**, 326–328 (2018).
6. Rhee, S. G. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp. Mol. Med.* **31**, 53–59 (1999).
7. Thannickal, V. J. & Fanburg, B. L. Reactive oxygen species in cell signaling. *Am. J. Physiol. Lung Cell Mol. Physiol.* **279**, L1005-L1028 (2000).
8. Sauer, H., Wartenberg, M. & Hescheler, J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol. Biochem.* **11**, 173–186 (2001).
9. Stone, J. R. & Yang, S. Hydrogen peroxide: a signaling messenger. *Antioxid. Redox Signal.* **8**, 243–270 (2006).
10. D'Autreaux, B. & Toledano, M. B. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell Biol.* **8**, 813–824 (2007).
Fundamental perspectives on ROS signalling.
11. Forman, H. J., Maiorino, M. & Ursini, F. Signaling functions of reactive oxygen species. *Biochemistry* **49**, 835–842 (2010).
12. Sies, H. Role of metabolic H_2O_2 generation: redox signaling and oxidative stress. *J. Biol. Chem.* **289**, 8735–8741 (2014).
13. Holmström, K. M. & Finkel, T. Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat. Rev. Mol. Cell Biol.* **15**, 411–421 (2014).
14. Reczek, C. R. & Chandel, N. S. ROS-dependent signal transduction. *Curr. Opin. Cell Biol.* **33**, 8–13 (2015).
15. Winterbourn, C. C. Biological production, detection, and fate of hydrogen peroxide. *Antioxid. Redox Signal.* **29**, 541–551 (2018).
Comprehensive overview on H_2O_2 in biology.
16. Berridge, M. J., Lipp, P. & Bootman, M. D. The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **1**, 11–21 (2000).
17. Bagur, R. & Hajnoczky, G. Intracellular Ca^{2+} sensing: its role in calcium homeostasis and signaling. *Mol. Cell* **66**, 780–788 (2017).
18. Sies, H. & Chance, B. The steady state level of catalase compound I in isolated hemoglobin-free perfused rat liver. *FEBS Lett.* **11**, 172–176 (1970).
First detection of H_2O_2 in normal aerobic eukaryotic metabolism.
19. Parvez, S., Long, M. J. C., Poganik, J. R. & Aye, Y. Redox signaling by reactive electrophiles and oxidants. *Chem. Rev.* **118**, 8798–8888 (2018).
20. Jones, D. P. & Sies, H. The redox code. *Antioxid. Redox Signal.* **23**, 734–746 (2015).
The 'redox code' as a set of principles by which redox biology is organized.
21. Zhang, L. et al. Biochemical basis and metabolic interplay of redox regulation. *Redox Biol.* **26**, 101284 (2019).
22. Sies, H. *Oxidative stress: eustress and distress* (Academic, 2020).
23. Sies, H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: oxidative eustress. *Redox Biol.* **11**, 613–619 (2017).
24. Chance, B., Sies, H. & Boveris, A. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**, 527–605 (1979).
Role and significance of hydroperoxides in metabolism.
25. Zeida, A. et al. Catalysis of peroxide reduction by fast reacting protein thiols. *Chem. Rev.* **119**, 10829–10855 (2019).
Comprehensive review of thiol-based redox chemistry.
26. Kaya, A., Lee, B. C. & Gladyshev, V. N. Regulation of protein function by reversible methionine oxidation and the role of selenoprotein MsrB1. *Antioxid. Redox Signal.* **23**, 814–822 (2015).
27. Brigelius-Flohé, R. & Flohé, L. Selenium and redox signaling. *Arch. Biochem. Biophys.* **617**, 48–59 (2017).
28. Santos, C. X. et al. Targeted redox inhibition of protein phosphatase 1 by Nox4 regulates eIF2alpha-mediated stress signaling. *EMBO J.* **35**, 319–334 (2016).
29. Poli, G., Leonarduzzi, G., Biasi, F. & Chiarpotto, E. Oxidative stress and cell signalling. *Curr. Med. Chem.* **11**, 1163–1182 (2004).
30. Sobotta, M. C. et al. Peroxiredoxin-2 and STAT3 form a redox relay for H_2O_2 signaling. *Nat. Chem. Biol.* **11**, 64–70 (2015).
Prototypical example of redox relay in H_2O_2 signalling.
31. Tapia, P. C. Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: "mitohormesis" for health and vitality. *Med. Hypotheses.* **66**, 832–843 (2006).
32. Ristow, M. & Zarse, K. How increased oxidative stress promotes longevity and metabolic health: the concept of mitochondrial hormesis (mitohormesis). *Exp. Gerontol.* **45**, 410–418 (2010).
33. Ristow, M. & Schmeisser, K. Mitohormesis: promoting health and lifespan by increased levels of reactive oxygen species (ROS). *Dose Response* **12**, 288–341 (2014).
34. Ursini, F., Maiorino, M. & Forman, H. J. Redox homeostasis: the golden mean of healthy living. *Redox Biol.* **8**, 205–215 (2016).
35. Sies, H. Biochemistry of oxidative stress. *Angew. Chem. Int. Ed. Engl.* **25**, 1058–1071 (1986).
36. Zhang, H. & Forman, H. J. 4-Hydroxynonenal-mediated signaling and aging. *Free Radic. Biol. Med.* **111**, 219–225 (2017).

37. Jackson, S. P. & Bartek, J. The DNA-damage response in human biology and disease. *Nature* **461**, 1071–1078 (2009).
38. Kreuz, S. & Fischle, W. Oxidative stress signaling to chromatin in health and disease. *Epigenomics* **8**, 843–862 (2016).
39. Poulsen, H. E. et al. Oxidatively generated modifications to nucleic acids in vivo: measurement in urine and plasma. *Free Radic. Biol. Med.* **145**, 336–341 (2019).
40. Lambeth, J. D. Nox enzymes, ROS, and chronic disease: an example of antagonistic pleiotropy. *Free Radic. Biol. Med.* **43**, 332–347 (2007).
41. Santolini, J., Wootton, S. A., Jackson, A. A. & Feelisch, M. The redox architecture of physiological function. *Curr. Opin. Physiol.* **9**, 34–47 (2019).
42. Go, Y. M., Chandler, J. D. & Jones, D. P. The cysteine proteome. *Free Radic. Biol. Med.* **84**, 227–245 (2015).
43. Bedard, K. & Krause, K. H. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol. Rev.* **87**, 245–313 (2007).
44. Knock, G. NADPH oxidase in the vasculature: expression, regulation and signalling pathways; role in normal cardiovascular physiology and its dysregulation in hypertension. *Free Radic. Biol. Med.* **145**, 385–427 (2019).
45. Parascandolo, A. & Laukkanen, M. O. Carcinogenesis and reactive oxygen species signaling: interaction of the NADPH oxidase NOX1-5 and superoxide dismutase 1-3 signal transduction pathways. *Antioxid. Redox Signal.* **30**, 443–486 (2019).
46. Murphy, M. P. How mitochondria produce reactive oxygen species. *Biochem. J.* **417**, 1–13 (2009). **A comprehensive account of mitochondrial ROS production**.
47. Spencer, N. Y. & Engelhardt, J. F. The basic biology of redoxosomes in cytokine-mediated signal transduction and implications for disease-specific therapies. *Biochemistry* **53**, 1551–1564 (2014).
48. Mishina, N. M. et al. Imaging H_2O_2 microdomains in receptor tyrosine kinases signaling. *Methods Enzymol.* **526**, 175–187 (2013).
49. Bleier, L. et al. Generator-specific targets of mitochondrial reactive oxygen species. *Free Radic. Biol. Med.* **78**, 1–10 (2015).
50. Brand, M. D. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic. Biol. Med.* **100**, 14–31 (2016).
51. Higdon, A., Diers, A. R., Oh, J. Y., Landar, A. & Darley-Usmar, V. M. Cell signalling by reactive lipid species: new concepts and molecular mechanisms. *Biochem. J.* **442**, 453–464 (2012).
52. Hitzel, J. et al. Oxidized phospholipids regulate amino acid metabolism through MTHFD2 to facilitate nucleotide release in endothelial cells. *Nat. Commun.* **9**, 2292–04602 (2018).
53. Kagan, V. E. et al. Redox phospholipidomics of enzymatically generated oxygenated phospholipids as specific signals of programmed cell death. *Free Radic. Biol. Med.* **147**, 231–241 (2020).
54. Spickett, C. M. & Pitt, A. R. Oxidative lipidomics coming of age: advances in analysis of oxidized phospholipids in physiology and pathology. *Antioxid. Redox Signal.* **22**, 1646–1666 (2015).
55. Tyurina, Y. Y. et al. "Only a life lived for others is worth living": redox signaling by oxygenated phospholipids in cell fate decisions. *Antioxid. Redox Signal.* **29**, 1333–1358 (2018).
56. Czapski, G. A., Czubowicz, K., Strosznajder, J. B. & Strosznajder, R. P. The lipoxygenases: their regulation and implication in Alzheimer's disease. *Neurochem. Res.* **41**, 243–257 (2016).
57. Wong, H. S., Benoit, B. & Brand, M. D. Mitochondrial and cytosolic sources of hydrogen peroxide in resting C2C12 myoblasts. *Free Radic. Biol. Med.* **130**, 140–150 (2019).
58. Niedzwiecki, M. M. et al. The exposome: molecules to populations. *Annu. Rev. Pharmacol. Toxicol.* **59**, 107–127 (2019).
59. Rhee, S. G. & Kil, I. S. Multiple functions and regulation of mammalian peroxiredoxins. *Annu. Rev. Biochem.* **86**, 749–775 (2017).
60. Brigelius-Flohé, R. & Flohé,L. Regulatory phenomena in the glutathione peroxidase superfamily. *Antioxid. Redox Signal*. https://doi.org/10.1089/ars.2019.7905 (2019).
61. Winterbourn, C. C., Kettle, A. J. & Hampton, M. B. Reactive oxygen species and neutrophil function. *Annu. Rev. Biochem.* **85**, 765–792 (2016).
62. Barata, A. G. & Dick, T. P. A role for peroxiredoxins in H_2O_2- and MEKK-dependent activation of the p38 signaling pathway. *Redox Biol.* **28**, 101340 (2019).
63. Mailloux, R. J. Mitochondrial antioxidants and the maintenance of cellular hydrogen peroxide levels. *Oxid. Med. Cell Longev.* **2018**, 7857251 (2018).
64. Hanschmann, E. M., Godoy, J. R., Berndt, C., Hudemann, C. & Lillig, C. H. Thioredoxins, glutaredoxins, and peroxiredoxins–molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. *Antioxid. Redox Signal.* **19**, 1539–1605 (2013).
65. Lyublinskaya, O. & Antunes, F. Measuring intracellular concentration of hydrogen peroxide with the use of genetically encoded H_2O_2 biosensor HyPer. *Redox Biol.* **24**, 101200 (2019).
66. Lim, J. B., Huang, B. K., Deen, W. M. & Sikes, H. D. Analysis of the lifetime and spatial localization of hydrogen peroxide generated in the cytosol using a reduced kinetic model. *Free Radic. Biol. Med.* **89**, 47–53 (2015).
67. Gao, C., Tian, Y., Zhang, R., Jing, J. & Zhang, X. Endoplasmic reticulum-directed ratiometric fluorescent probe for quantitive detection of basal H_2O_2. *Anal. Chem.* **89**, 12945–12950 (2017).
68. Forman, H. J., Bernardo, A. & Davies, K. J. What is the concentration of hydrogen peroxide in blood and plasma? *Arch. Biochem. Biophys.* **603**, 48–53 (2016).
69. Mishina, N. M. et al. Which antioxidant system shapes intracellular H_2O_2 gradients? *Antioxid. Redox Signal.* **31**, 664–670 (2019).
70. Fransen, M. & Lismont, C. Redox signaling from and to peroxisomes: progress, challenges, and prospects. *Antioxid. Redox Signal.* **30**, 95–112 (2019).
71. Lismont, C., Revenco, I. & Fransen, M. Peroxisomal hydrogen peroxide metabolism and signaling in health and disease. *Int. J. Mol. Sci.* **20**, ijms20153673 (2019).
72. Appenzeller-Herzog, C. et al. Transit of H_2O_2 across the endoplasmic reticulum membrane is not sluggish. *Free Radic. Biol. Med.* **94**, 157–160 (2016).
73. Bestetti, S. et al. Human aquaporin-11 guarantees efficient transport of H_2O_2 across the endoplasmic reticulum membrane. *Redox Biol.* **28**, 101326 (2019).
74. Yoboue, E. D., Sitia, R. & Simmen, T. Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. *Cell Death. Dis.* **9**, 331 (2018).
75. Dooley, C. T. et al. Imaging dynamic redox changes in mammalian cells with green fluorescent protein indicators. *J. Biol. Chem.* **279**, 22284–22293 (2004).
76. Belousov, V. V. et al. Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nat. Methods* **3**, 281–286 (2006). **First genetically encoded H_2O_2 probe using the OxyR domain**.
77. Bilan, D. S. & Belousov, V. V. In vivo imaging of hydrogen peroxide with HyPer probes. *Antioxid. Redox Signal.* **29**, 569–584 (2018).
78. Morgan, B. et al. Real-time monitoring of basal H_2O_2 levels with peroxiredoxin-based probes. *Nat. Chem. Biol.* **12**, 437–443 (2016).
79. Roma, L. P., Deponte, M., Riemer, J. & Morgan, B. Mechanisms and applications of redox-sensitive green fluorescent protein-based hydrogen peroxide probes. *Antioxid. Redox Signal.* **29**, 552–568 (2018).
80. Fernandez-Puente, E. et al. Expression and functional analysis of the hydrogen peroxide biosensors HyPer and HyPer2 in C2C12 myoblasts/myotubes and single skeletal muscle fibres. *Sci. Rep.* **10**, 871–57821 (2020).
81. Huang, B. K., Ali, S., Stein, K. T. & Sikes, H. D. Interpreting heterogeneity in response of cells expressing a fluorescent hydrogen peroxide biosensor. *Biophys. J.* **109**, 2148–2158 (2015).
82. Henzler, T. & Steudle, E. Transport and metabolic degradation of hydrogen peroxide in Chara corallina: model calculations and measurements with the pressure probe suggest transport of H_2O_2 across water channels. *J. Exp. Bot.* **51**, 2053–2066 (2000). **Discovery of H_2O_2 transport across membranes by aquaporins**.
83. Bienert, G. P. & Chaumont, F. Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochim. Biophys. Acta* **1840**, 1596–1604 (2014). **Groundbreaking work on the role of some aquaporins as peroxiporins**.
84. Bestetti, S. et al. A persulfidation-based mechanism controls aquaporin-8 conductance. *Sci. Adv.* **4**, eaar5770 (2018).
85. Medrano-Fernandez, I. et al. Stress regulates aquaporin-8 permeability to impact cell growth and survival. *Antioxid. Redox Signal.* **24**, 1031–1044 (2016).
86. Pak, V. V. et al. Ultrasensitive genetically encoded indicator for intracellular hydrogen peroxide identifies novel roles for cellular oxidants in cell migration and mitochondrial function. *Cell Metab.* **31**, 642–653 (2020).
87. Tamma, G. et al. Aquaporin membrane channels in oxidative stress, cell signaling, and aging: recent advances and research trends. *Oxid. Med. Cell Longev.* **2018**, 1501847 (2018).
88. Rajasekaran, N. S. et al. Human alpha B-crystallin mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice. *Cell* **130**, 427–439 (2007).
89. Marinho, H. S., Real, C., Cyrne, L., Soares, H. & Antunes, F. Hydrogen peroxide sensing, signaling and regulation of transcription factors. *Redox Biol.* **2**, 535–562 (2014).
90. Jones, D. P. Radical-free biology of oxidative stress. *Am. J. Physiol. Cell Physiol* **295**, C849-C868 (2008).
91. Bak, D. W., Bechtel, T. J., Falco, J. A. & Weerapana, E. Cysteine reactivity across the subcellular universe. *Curr. Opin. Chem. Biol.* **48**, 96–105 (2019).
92. Go, Y. M. & Jones, D. P. The redox proteome. *J. Biol. Chem.* **288**, 26512–26520 (2013).
93. Behring, J. B. et al. Spatial and temporal alterations in protein structure by EGF regulate cryptic cysteine oxidation. *Sci. Signal.* **13**, 13–615 (2020).
94. Brigelius-Flohé, R. & Flohé, L. Basic principles and emerging concepts in the redox control of transcription factors. *Antioxid. Redox Signal.* **15**, 2335–2381 (2011).
95. Young, D. et al. Protein promiscuity in H_2O_2 signaling. *Antioxid. Redox Signal.* **30**, 1285–1324 (2019).
96. Xiao, H., et al. A quantitative tissue-specific landscape of protein redox regulation during aging. *Cell* **180**, 968–983 (2020).
97. Itoh, K. et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem. Biophys. Res. Commun.* **236**, 313–322 (1997). **Discovery of the NRF2–KEAP1 system**.
98. Yamamoto, M., Kensler, T. W. & Motohashi, H. The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiol. Rev.* **98**, 1169–1203 (2018).
99. Fourquet, S., Guerois, R., Biard, D. & Toledano, M. B. Activation of NRF2 by nitrosative agents and H_2O_2 involves KEAP1 disulfide formation. *J. Biol. Chem.* **285**, 8463–8471 (2010).
100. Kobayashi, M. et al. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. *Mol. Cell Biol.* **29**, 493–502 (2009).
101. Cebula, M., Schmidt, E. E. & Arner, E. S. TrxR1 as a potent regulator of the Nrf2-Keap1 response system. *Antioxid. Redox Signal.* **23**, 823–853 (2015).
102. Singh, C. K. et al. The role of sirtuins in antioxidant and redox signaling. *Antioxid. Redox Signal.* **28**, 643–661 (2018).
103. Cheng, X., Ku, C. H. & Siow, R. C. Regulation of the Nrf2 antioxidant pathway by microRNAs: new players in micromanaging redox homeostasis. *Free Radic. Biol. Med.* **64**, 4–11 (2013).
104. Karin, M. NF-kappaB as a critical link between inflammation and cancer. *Cold Spring Harb. Perspect. Biol.* **1**, a000141 (2009).
105. Pahl, H. L. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene.* **18**, 6853–6866 (1999).
106. Oliveira-Marques, V., Marinho, H. S., Cyrne, L. & Antunes, F. Role of hydrogen peroxide in NF-kappaB activation: from inducer to modulator. *Antioxid. Redox Signal.* **11**, 2223–2243 (2009).
107. Schreck, R., Rieber, P. & Baeuerle, P. A. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappaB transcription factor and HIV-1. *EMBO J.* **10**, 2247–2258 (1991). **Description of the role of H_2O_2 in NF-κB activation**.
108. Halvey, P. J. et al. Selective oxidative stress in cell nuclei by nuclear-targeted D-amino acid oxidase. *Antioxid. Redox Signal.* **9**, 807–816 (2007).
109. Kaelin, W. G. Jr. & Ratcliffe, P. J. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* **30**, 393–402 (2008).
110. Jiang, B. H., Rue, E., Wang, G. L., Roe, R. & Semenza, G. L. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. *J. Biol. Chem.* **271**, 17771–17778 (1996). **Seminal article on HIF**.

111. Hernansanz-Agustin, P. et al. Mitochondrial complex I deactivation is related to superoxide production in acute hypoxia. *Redox Biol.* **12**, 1040–1051 (2017).
112. Prabhakar, N. R., Kumar, G. K., Nanduri, J. & Semenza, G. L. ROS signaling in systemic and cellular responses to chronic intermittent hypoxia. *Antioxid. Redox Signal.* **9**, 1397–1403 (2007).
113. Waypa, G. B., Smith, K. A. & Schumacker, P. T. O_2 sensing, mitochondria and ROS signaling: The fog is lifting. *Mol. Asp. Med.* **47-48**, 76–89 (2016).
114. Chandel, N. S. et al. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing. *J. Biol. Chem.* **275**, 25130–25138 (2000).
115. Pouyssegur, J. & Mechta-Grigoriou, F. Redox regulation of the hypoxia-inducible factor. *Biol. Chem.* **387**, 1337–1346 (2006).
116. Acker, T., Fandrey, J. & Acker, H. The good, the bad and the ugly in oxygen-sensing: ROS, cytochromes and prolyl-hydroxylases. *Cardiovasc. Res.* **71**, 195–207 (2006).
117. Eijkelenboom, A. & Burgering, B. M. FOXOs: signalling integrators for homeostasis maintenance. *Nat. Rev. Mol. Cell Biol.* **14**, 83–97 (2013).
118. Klotz, L. O. & Steinbrenner, H. Cellular adaptation to xenobiotics: interplay between xenosensors, reactive oxygen species and FOXO transcription factors. *Redox Biol.* **13**, 646–654 (2017).
119. Liu, B., Chen, Y. & St Clair, D. K. ROS and p53: a versatile partnership. *Free Radic. Biol. Med.* **44**, 1529–1535 (2008).
120. Uehara, I. & Tanaka, N. Role of p53 in the regulation of the inflammatory tumor microenvironment and tumor suppression. *Cancers (Basel).* **10**, 219 (2018).
121. Herzig, S. & Shaw, R. J. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell Biol.* **19**, 121–135 (2018).
122. Hinchy, E. C. et al. Mitochondria-derived ROS activate AMP-activated protein kinase (AMPK) indirectly. *J. Biol. Chem.* **293**, 17208–17217 (2018).
123. Liu, G. Y. & Sabatini, D. M. mTOR at the nexus of nutrition, growth, ageing and disease. *Nat. Rev. Mol. Cell Biol.* 10-0199 (2020).
124. Schmeisser, K. & Parker, J. A. Pleiotropic effects of mTOR and autophagy during development and aging. *Front. Cell Dev. Biol.* **7**, 192 (2019).
125. Sirover, M. A. Pleiotropic effects of moonlighting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cancer progression, invasiveness, and metastases. *Cancer Metastasis Rev.* **37**, 665–676 (2018).
126. Peralta, D. et al. A proton relay enhances H_2O_2 sensitivity of GAPDH to facilitate metabolic adaptation. *Nat. Chem. Biol.* **11**, 156–163 (2015).
127. Lin, C. S. & Klingenberg, M. Isolation of the uncoupling protein from brown adipose tissue mitochondria. *FEBS Lett.* **113**, 299–303 (1980).
Discovery of uncoupling protein.
128. Echtay, K. S. et al. Uncoupling proteins: Martin Klingenberg's contributions for 40 years. *Arch. Biochem. Biophys.* **657**, 41–55 (2018).
129. Berry, B. J., Trewin, A. J., Amitrano, A. M., Kim, M. & Wojtovich, A. P. Use the protonmotive force: mitochondrial uncoupling and reactive oxygen species. *J. Mol. Biol.* **430**, 3873–3891 (2018).
130. Jezek, P., Holendova, B., Garlid, K. D. & Jaburek, M. Mitochondrial uncoupling proteins: subtle regulators of cellular redox signaling. *Antioxid. Redox Signal.* **29**, 667–714 (2018).
131. Echtay, K. S. et al. Superoxide activates mitochondrial uncoupling proteins. *Nature* **415**, 96–99 (2002).
132. Mailloux, R. J. & Harper, M. E. Uncoupling proteins and the control of mitochondrial reactive oxygen species production. *Free Radic. Biol. Med.* **51**, 1106–1115 (2011).
133. Dustin, C. M., Heppner, D. E., Lin, M. J. & van der Vliet, A. Redox regulation of tyrosine kinase signaling: more than meet the eye. *J, Biochem.* **167**, 151–163 (2020).
134. Truong, T. H. & Carroll, K. S. Redox regulation of protein kinases. *Crit. Rev. Biochem. Mol. Biol.* **48**, 332–356 (2013).
135. Londhe, A. D. et al. Regulation of PTP1B activation through disruption of redox-complex formation. *Nat. Chem Biol.* **16**, 122–125 (2020).
136. Truong, T. H. et al. Molecular basis for redox activation of epidermal growth factor receptor kinase. *Cell Chem. Biol.* **23**, 837–848 (2016).
137. Heppner, D. E. et al. Direct cysteine sulfenylation drives activation of the Src kinase. *Nat. Commun.* **9**, 4522–06790 (2018).
138. Dagnell, M. et al. Bicarbonate is essential for protein tyrosine phosphatase 1B (PTP1B) oxidation and cellular signaling through EGF-triggered phosphorylation cascades. *J. Biol. Chem.* **294**, 12330–12338 (2019).
139. Truzzi, D. R. et al. The bicarbonate/carbon dioxide pair increases hydrogen peroxide-mediated hyperoxidation of human peroxiredoxin 1. *J. Biol. Chem.* **294**, 14055–14067 (2019).
140. Löwe, O. et al. BIAM switch assay coupled to mass spectrometry identifies novel redox targets of NADPH oxidase 4. *Redox Biol.* **21**, 101125 (2019).
141. Bogeski, I. & Niemeyer, B. A. Redox regulation of ion channels. *Antioxid. Redox Signal.* **21**, 859–862 (2014).
142. Kourie, J. I. Interaction of reactive oxygen species with ion transport mechanisms. *Am. J. Physiol.* **275**, C1-C24 (1998).
143. Sahoo, N., Hoshi, T. & Heinemann, S. H. Oxidative modulation of voltage-gated potassium channels. *Antioxid. Redox Signal.* **21**, 933–952 (2014).
144. Ruppersberg, J. P. et al. Regulation of fast inactivation of cloned mammalian IK(A) channels by cysteine oxidation. *Nature* **352**, 711–714 (1991).
Description of redox regulation of K^+ channel.
145. Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q. & Griendling, K. K. Reactive oxygen species in metabolic and inflammatory signaling. *Circ. Res.* **122**, 877–902 (2018).
146. Chen, P. H., Chi, J. T. & Boyce, M. Functional crosstalk among oxidative stress and O-GlcNAc signaling pathways. *Glycobiology* **28**, 556–564 (2018).
147. Taniguchi, N. et al. Glyco-redox, a link between oxidative stress and changes of glycans: lessons from research on glutathione, reactive oxygen and nitrogen species to glycobiology. *Arch. Biochem. Biophys.* **595**, 72–80 (2016).
148. Nordzieke, D. E. & Medrano-Fernandez, I. The plasma membrane: a platform for intra- and intercellular redox signaling. *Antioxidants (Basel)* **7**, 168 (2018).
149. Patinen, T. et al. Regulation of stress signaling pathways by protein lipoxidation. *Redox Biol.* **23**, 101114 (2019).
150. Conrad, M. & Pratt, D. A. The chemical basis of ferroptosis. *Nat. Chem. Biol.* **15**, 1137–1147 (2019).
151. Ingold, I. et al. Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell* **172**, 409–422 (2018).
152. Somyajit, K. et al. Redox-sensitive alteration of replisome architecture safeguards genome integrity. *Science* **358**, 797–802 (2017).
153. Ahmed, W. & Lingner, J. PRDX1 and MTH1 cooperate to prevent ROS-mediated inhibition of telomerase. *Genes Dev.* **32**, 658–669 (2018).
154. Rhee, S. G., Woo, H. A. & Kang, D. The role of peroxiredoxins in the transduction of H_2O_2 signals. *Antioxid. Redox Signal.* **28**, 537–557 (2018).
155. Sarsour, E. H., Kumar, M. G., Chaudhuri, L., Kalen, A. L. & Goswami, P. C. Redox control of the cell cycle in health and disease. *Antioxid. Redox Signal.* **11**, 2985–3011 (2009).
156. Srinivas, U. S., Tan, B. W. Q., Vellayappan, B. A. & Jeyasekharan, A. D. ROS and the DNA damage response in cancer. *Redox Biol.* **25**, 101084 (2019).
157. Mailloux, R. J. Teaching the fundamentals of electron transfer reactions in mitochondria and the production and detection of reactive oxygen species. *Redox Biol.* **4**, 381–398 (2015).
158. Matilainen, O., Quiros, P. M. & Auwerx, J. Mitochondria and epigenetics - crosstalk in homeostasis and stress. *Trends Cell Biol.* **27**, 453–463 (2017).
159. Castro, L., Tortora, V., Mansilla, S. & Radi, R. Aconitases: non-redox iron-sulfur proteins sensitive to reactive species. *Acc. Chem. Res.* **52**, 2609–2619 (2019).
160. Braymer, J. J., Stumpfig, M., Thelen, S., Muhlenhoff, U. & Lill, R. Depletion of thiol reducing capacity impairs cytosolic but not mitochondrial iron-sulfur protein assembly machineries. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 240–251 (2019).
161. Bulthuis, E. P., Adjobo-Hermans, M. J. W., Willems, P. H. G. M. & Koopman, W. J. H. Mitochondrial morphofunction in mammalian cells. *Antioxid. Redox Signal.* **30**, 2066–2109 (2019).
162. Kondadi, A. K., Anand, R. & Reichert, A. S. Functional interplay between cristae biogenesis, mitochondrial dynamics and mitochondrial DNA integrity. *Int. J. Mol. Sci.* **20**, ijms20174311 (2019).
163. Murley, A. & Nunnari, J. The emerging network of mitochondria-organelle contacts. *Mol. Cell* **61**, 648–653 (2016).
164. Frank, M. et al. Mitophagy is triggered by mild oxidative stress in a mitochondrial fission dependent manner. *Biochim. Biophys. Acta* **1823**, 2297–2310 (2012).
165. Zorov, D. B., Juhaszova, M. & Sollott, S. J. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* **94**, 909–950 (2014).
166. Sies, H. Biochemistry of the peroxisome in the liver cell. *Angew. Chem. Int. Ed. Engl.* **13**, 706–718 (1974).
167. Gebicka, L. & Krych-Madej, J. The role of catalases in the prevention/promotion of oxidative stress. *J. Inorg. Biochem.* **197**, 110699 (2019).
168. Böhm, B., Heinzelmann, S., Motz, M. & Bauer, G. Extracellular localization of catalase is associated with the transformed state of malignant cells. *Biol. Chem.* **396**, 1339–1356 (2015).
169. Wang, L., Zhang, L., Niu, Y., Sitia, R. & Wang, C. C. Glutathione peroxidase 7 utilizes hydrogen peroxide generated by Ero1alpha to promote oxidative protein folding. *Antioxid. Redox Signal.* **20**, 545–556 (2014).
170. Cenci, S. & Sitia, R. Managing and exploiting stress in the antibody factory. *FEBS Lett.* **581**, 3652–3657 (2007).
171. Laporte, A., Lortz, S., Schaal, C., Lenzen, S. & Elsner, M. Hydrogen peroxide permeability of cellular membranes in insulin-producing cells. *Biochim. Biophys. Acta Biomembr.* **1862**, 183096 (2019).
172. Cao, S. S. & Kaufman, R. J. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxid. Redox Signal.* **21**, 396–413 (2014).
173. Eletto, D., Chevet, E., Argon, Y. & Appenzeller-Herzog, C. Redox controls UPR to control redox. *J. Cell Sci.* **127**, 3649–3658 (2014).
174. Amodio, G., Moltedo, O., Faraonio, R. & Remondelli, P. Targeting the endoplasmic reticulum unfolded protein response to counteract the oxidative stress-induced endothelial dysfunction. *Oxid. Med. Cell Longev.* **2018**, 4946289 (2018).
175. Görlach, A., Bertram, K., Hudecova, S. & Krizanova, O. Calcium and ROS: a mutual interplay. *Redox Biol.* **6**, 260–271 (2015).
176. Hempel, N. & Trebak, M. Crosstalk between calcium and reactive oxygen species signaling in cancer. *Cell Calcium.* **63**, 70–96 (2017).
177. Feno, S., Butera, G., Vecellio, R. D., Rizzuto, R. & Raffaello, A. Crosstalk between calcium and ROS in pathophysiological conditions. *Oxid. Med. Cell Longev.* **2019**, 9324018 (2019).
178. Joseph, S. K. et al. Redox regulation of type-I inositol trisphosphate receptors in intact mammalian cells. *J. Biol. Chem.* **293**, 17464–17476 (2018).
179. Booth, D. M., Enyedi, B., Geiszt, M., Varnai, P. & Hajnoczky, G. Redox nanodomains are induced by and control calcium signaling at the ER-mitochondrial interface. *Mol. Cell* **63**, 240–248 (2016).
Description of H_2O_2 redox nanodomains.
180. Csordas, G., Weaver, D. & Hajnoczky, G. Endoplasmic reticulum-mitochondrial contactology: structure and signaling functions. *Trends Cell Biol.* **28**, 523–540 (2018).
181. Egea, J. et al. European contribution to the study of ROS: a summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). *Redox Biol.* **13**, 94–162 (2017).
182. Go, Y. M. & Jones, D. P. Redox theory of aging: implications for health and disease. *Clin. Sci.* **131**, 1669–1688 (2017).
183. Valko, M. et al. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **39**, 44–84 (2007).
184. Milkovic, L., Cipak, G. A., Cindric, M., Mouthuy, P. A. & Zarkovic, N. Short overview of ROS as cell function regulators and their implications in therapy concepts. *Cells* **8**, 793 (2019).
185. Timme-Laragy, A. R., Hahn, M. E., Hansen, J. M., Rastogi, A. & Roy, M. A. Redox stress and signaling during vertebrate embryonic development: regulation and responses. *Semin. Cell Dev. Biol.* **80**, 17–28 (2018).
186. Rampon, C., Volovitch, M., Joliot, A., Vriz, S. Hydrogen peroxide and redox regulation of developments. *Antioxidants (Basel)* **7**, 159 (2018).
187. Oswald, M. C. W., Garnham, N., Sweeney, S. T. & Landgraf, M. Regulation of neuronal development and function by ROS. *FEBS Lett.* **592**, 679–691 (2018).
188. Wilson, C., Munoz-Palma, E. & Gonzalez-Billault, C. From birth to death: a role for reactive oxygen species in neuronal development. *Semin. Cell Dev. Biol.* **80**, 43–49 (2018).
189. Wilson, C. & Gonzalez-Billault, C. Regulation of cytoskeletal dynamics by redox signaling and oxidative stress: implications for neuronal development and trafficking. *Front. Cell Neurosci.* **9**, 381 (2015).

190. Tan, D. Q. & Suda, T. Reactive oxygen species and mitochondrial homeostasis as regulators of stem cell fate and function. *Antioxid. Redox Signal.* **29**, 149–168 (2018).
191. Rhee, S. G. & Kil, I. S. Mitochondrial H_2O_2 signaling is controlled by the concerted action of peroxiredoxin III and sulfiredoxin: linking mitochondrial function to circadian rhythm. *Free Radic. Biol. Med.* **100**, 73–80 (2016).
An account of the role of peroxiredoxins in circadian rhythms.
192. Nagy, A. D. & Reddy, A. B. Redox clocks: time to rethink redox interventions. *Free Radic. Biol. Med.* **119**, 3–7 (2018).
193. Reinke, H. & Asher, G. Crosstalk between metabolism and circadian clocks. *Nat. Rev. Mol. Cell Biol.* **20**, 227–241 (2019).
194. Pei, J. F. et al. Diurnal oscillations of endogenous H_2O_2 sustained by $p66^{Shc}$ regulate circadian clocks. *Nat. Cell Biol.* **21**, 1553–1564 (2019).
195. Kempf, A., Song, S. M., Talbot, C. B. & Miesenbock, G. A potassium channel beta-subunit couples mitochondrial electron transport to sleep. *Nature* **568**, 230–234 (2019).
196. Patke, A., Young, M. W. & Axelrod, S. Molecular mechanisms and physiological importance of circadian rhythms. *Nat. Rev. Mol. Cell Biol.* **21**, 67–84 (2020).
197. Nayernia, Z., Jaquet, V. & Krause, K. H. New insights on NOX enzymes in the central nervous system. *Antioxid. Redox Signal.* **20**, 2815–2837 (2014).
198. Cobley, J. N., Fiorello, M. L. & Bailey, D. M. 13 reasons why the brain is susceptible to oxidative stress. *Redox Biol.* **15**, 490–503 (2018).
199. Tarafdar, A. & Pula, G. The role of NADPH oxidases and oxidative stress in neurodegenerative disorders. *Int. J. Mol. Sci.* **19**, ijms19123824 (2018).
200. Sbodio, J. I., Snyder, S. H. & Paul, B. D. Redox mechanisms in neurodegeneration: from disease outcomes to therapeutic opportunities. *Antioxid. Redox Signal.* **30**, 1450–1499 (2019).
201. Steinbrenner, H. & Sies, H. Selenium homeostasis and antioxidant selenoproteins in brain: implications for disorders in the central nervous system. *Arch. Biochem. Biophys.* **536**, 152–157 (2013).
202. Lepka, K. et al. Iron-sulfur glutaredoxin 2 protects oligodendrocytes against damage induced by nitric oxide release from activated microglia. *Glia* **65**, 1521–1534 (2017).
203. Casas, A. I. et al. NOX4-dependent neuronal autotoxicity and BBB breakdown explain the superior sensitivity of the brain to ischemic damage. *Proc. Natl Acad. Sci. USA* **114**, 12315–12320 (2017).
204. Meda, F. et al. Nerves, H_2O_2 and Shh: three players in the game of regeneration. *Semin. Cell Dev. Biol.* **80**, 65–73 (2018).
205. Hervera, A. et al. Reactive oxygen species regulate axonal regeneration through the release of exosomal NADPH oxidase 2 complexes into injured axons. *Nat. Cell Biol.* **20**, 307–319 (2018).
206. Vicente-Gutierrez, C. et al. Astrocytic mitochondrial ROS modulate brain metabolism and mouse behaviour. *Nat. Metab.* **1**, 201–211 (2019).
207. Bierhaus, A. et al. A mechanism converting psychosocial stress into mononuclear cell activation. *Proc. Natl Acad. Sci. USA* **100**, 1920–1925 (2003).
A report describing how psychosocial stress is translated into a cell response pattern.
208. Aschbacher, K. et al. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology* **38**, 1698–1708 (2013).
209. Aschbacher, K. & Mason, A. E. Eustress, distress and oxidative stress: promising pathways for mind-body medicine. In *Oxidative Stress: Eustress and Distress* (ed. Sies, H.) 583–617 (Academic, 2020).
210. Golbidi, S., Li, H. & Laher, I. Oxidative stress: a unifying mechanism for cell damage induced by noise, (water-pipe) smoking, and emotional stress-therapeutic strategies targeting redox imbalance. *Antioxid. Redox Signal.* **28**, 741–759 (2018).
211. Münzel, T. et al. Effects of noise on vascular function, oxidative stress, and inflammation: mechanistic insight from studies in mice. *Eur. Heart J.* **38**, 2838–2849 (2017).
212. Rousset, F., Carnesecchi, S., Senn, P. & Krause, K. H. NOX3-targeted therapies for inner ear pathologies. *Curr. Pharm. Des.* **21**, 5977–5987 (2015).
213. Lugrin, J., Rosenblatt-Velin, N., Parapanov, R. & Liaudet, L. The role of oxidative stress during inflammatory processes. *Biol. Chem.* **395**, 203–230 (2014).
214. Pei, L. & Wallace, D. C. Mitochondrial etiology of neuropsychiatric disorders. *Biol. Psychiatry* **83**, 722–730 (2018).
215. Nathan, C. & Cunningham-Bussel, A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nat. Rev. Immunol.* **13**, 349–361 (2013).
A review bridging immunology and redox biology.
216. Brinkmann, V. et al. Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
217. Kenny, E. F. et al. Diverse stimuli engage different neutrophil extracellular trap pathways. *eLife*. **6**, e24437 (2017).
218. Anelli, T., Sannino, S. & Sitia, R. Proteostasis and "redoxtasis" in the secretory pathway: tales of tails from ERp44 and immunoglobulins. *Free Radic. Biol. Med.* **83**, 323–330 (2015).
219. Garaude, J. Reprogramming of mitochondrial metabolism by innate immunity. *Curr. Opin. Immunol.* **56**, 17–23 (2018).
220. Abais, J. M., Xia, M., Zhang, Y., Boini, K. M. & Li, P. L. Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxid. Redox Signal.* **22**, 1111–1129 (2015).
221. Jones, R. M. & Neish, A. S. Redox signaling mediated by the gut microbiota. *Free Radic. Biol. Med.* **105**, 41–47 (2017).
222. Aviello, G. & Knaus, U. G. NADPH oxidases and ROS signaling in the gastrointestinal tract. *Mucosal Immunol.* **11**, 1011–1023 (2018).
223. Cano, S. M., Lancel, S., Boulanger, E. & Neviere, R. Targeting oxidative stress and mitochondrial dysfunction in the treatment of impaired wound healing: a systematic review. *Antioxidants (Basel)* **7**, 98 (2018).
224. Niethammer, P. Wound redox gradients revisited. *Semin. Cell Dev. Biol.* **80**, 13–16 (2018).
225. Love, N. R. & Chen, Y. Amputation-induced reactive oxygen species are required for successful Xenopus tadpole tail regeneration. *Nat. Cell Biol.* **15**, 222–228 (2013).
226. Levigne, D., Modarressi, A., Krause, K. H. & Pittet-Cuenod, B. NADPH oxidase 4 deficiency leads to impaired wound repair and reduced dityrosine-crosslinking, but does not affect myofibroblast formation. *Free Radic. Biol. Med.* **96**, 374–384 (2016).
227. Kunkemoeller, B. & Kyriakides, T. R. Redox signaling in diabetic wound healing regulates extracellular matrix deposition. *Antioxid. Redox Signal.* **27**, 823–838 (2017).
228. Handy, D. E. & Loscalzo, J. Responses to reductive stress in the cardiovascular system. *Free Radic. Biol. Med.* **109**, 114–124 (2017).
229. Incalza, M. A. et al. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vascul. Pharmacol.* **100**, 1–19 (2018).
230. Münzel, T. et al. Impact of oxidative stress on the heart and vasculature: part 2 of a 3-part series. *J. Am. Coll. Cardiol.* **70**, 212–229 (2017).
231. Schröder, K. Redox control of angiogenesis. *Antioxid. Redox Signal.* **30**, 960–971 (2019).
232. Förstermann, U., Xia, N. & Li, H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circ. Res.* **120**, 713–735 (2017).
233. Siu, K. L. et al. NOX isoforms in the development of abdominal aortic aneurysm. *Redox Biol.* **11**, 118–125 (2017).
234. Oikonomou, E. K. & Antoniades, C. Immunometabolic regulation of vascular redox state: the role of adipose tissue. *Antioxid. Redox Signal.* **29**, 313–336 (2018).
235. Stocker, R. & Keaney, J. F. Jr. Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* **84**, 1381–1478 (2004).
236. Trinity, J. D., Broxterman, R. M. & Richardson, R. S. Regulation of exercise blood flow: role of free radicals. *Free Radic. Biol. Med.* **98**, 90–102 (2016).
237. Jackson, M. J. Redox regulation of muscle adaptations to contractile activity and aging. *J. Appl. Physiol.* **119**, 163–171 (2015).
238. Le Moal, E. et al. Redox control of skeletal muscle regeneration. *Antioxid. Redox Signal.* **27**, 276–310 (2017).
239. El Assar, M., Angulo, J. & Rodriguez-Manas, L. Frailty as a phenotypic manifestation of underlying oxidative stress. *Free Radic. Biol. Med.*10 (2019).
240. McArdle, A., Pollock, N., Staunton, C. A. & Jackson, M. J. Aberrant redox signalling and stress response in age-related muscle decline: role in inter- and intra-cellular signalling. *Free Radic. Biol. Med.* **132**, 50–57 (2019).
241. Cobley, J. N., Close, G. L., Bailey, D. M. & Davison, G. W. Exercise redox biochemistry: conceptual, methodological and technical recommendations. *Redox Biol.* **12**, 540–548 (2017).
242. Hancock, M. et al. Myocardial NADPH oxidase-4 regulates the physiological response to acute exercise. *eLife* **7**, 41044 (2018).
243. Jackson, M. J. Mechanistic models to guide redox investigations and interventions in musculoskeletal ageing. *Free Radic. Biol. Med.* **149**, 2–7 (2020).
244. Watson, J. D. Type 2 diabetes as a redox disease. *Lancet* **383**, 841–843 (2014).
245. Haeusler, R. A., McGraw, T. E. & Accili, D. Biochemical and cellular properties of insulin receptor signalling. *Nat. Rev. Mol. Cell Biol.* **19**, 31–44 (2018).
246. Petersen, M. C. & Shulman, G. I. Mechanisms of insulin action and insulin resistance. *Physiol. Rev.* **98**, 2133–2223 (2018).
247. Onyango, A. N. Cellular stresses and stress responses in the pathogenesis of insulin resistance. *Oxid. Med. Cell Longev.* **2018**, 4321714 (2018).
248. Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300 (1956).
Seminal article on the free radical theory of ageing.
249. Golubev, A., Hanson, A. D. & Gladyshev, V. N. Non-enzymatic molecular damage as a prototypic driver of aging. *J. Biol. Chem.* **292**, 6029–6038 (2017).
250. Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The hallmarks of aging. *Cell* **153**, 1194–1217 (2013).
Comprehensive overview of the hallmarks of ageing.
251. Pomatto, L. C. D. & Davies, K. J. A. The role of declining adaptive homeostasis in ageing. *J. Physiol.* **595**, 7275–7309 (2017).
252. Schmidlin, C. J., Dodson, M. B., Madhavan, L. & Zhang, D. D. Redox regulation by NRF2 in aging and disease. *Free Radic. Biol. Med.* **134**, 702–707 (2019).
253. Taetzsch, T., Benusa, S., Levesque, S., Mumaw, C. L. & Block, M. L. Loss of NF-kappaB p50 function synergistically augments microglial priming in the middle-aged brain. *J. Neuroinflammation* **16**, 60–1446 (2019).
254. Salminen, A., Kauppinen, A. & Kaarniranta, K. AMPK activation inhibits the functions of myeloid-derived suppressor cells (MDSC): impact on cancer and aging. *J. Mol. Med.* **97**, 1049–1064 (2019).
255. Rose, G., Crocco, P., De, R. F., Montesanto, A. & Passarino, G. Further support to the uncoupling-to-survive theory: the genetic variation of human UCP genes is associated with longevity. *PLoS One* **6**, e29650 (2011).
256. Kirstein, J. et al. Proteotoxic stress and ageing triggers the loss of redox homeostasis across cellular compartments. *EMBO J.* **34**, 2334–2349 (2015).
257. Höhn, A. et al. Happily (n)ever after: aging in the context of oxidative stress, proteostasis loss and cellular senescence. *Redox Biol.* **11**, 482–501 (2017).
258. Hipp, M. S., Kasturi, P. & Hartl, F. U. The proteostasis network and its decline in ageing. *Nat. Rev. Mol. Cell Biol.* **20**, 421–435 (2019).
259. Akbari, M., Kirkwood, T. B. L. & Bohr, V. A. Mitochondria in the signaling pathways that control longevity and health span. *Ageing Res. Rev.* **54**, 100940 (2019).
260. Campisi, J. et al. From discoveries in ageing research to therapeutics for healthy ageing. *Nature* **571**, 183–192 (2019).
261. Labunskyy, V. M. & Gladyshev, V. N. Role of reactive oxygen species-mediated signaling in aging. *Antioxid. Redox Signal.* **19**, 1362–1372 (2013).
262. Palmeira, C. M. et al. Mitohormesis and metabolic health: the interplay between ROS, cAMP and sirtuins. *Free Radic. Biol. Med.* **141**, 483–491 (2019).
263. Bazopoulou, D. et al. Developmental ROS individualizes organismal stress resistance and lifespan. *Nature* **576**, 301–306 (2019).
264. Golubev, A., Hanson, A. D. & Gladyshev, V. N. A tale of two concepts: harmonizing the free radical and antagonistic pleiotropy theories of aging. *Antioxid. Redox Signal.* **29**, 1003–1017 (2018).
265. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
Comprehensive overview of the hallmarks of cancer.
266. Hornsveld, M. & Dansen, T. B. The hallmarks of cancer from a redox perspective. *Antioxid. Redox Signal.* **25**, 300–325 (2016).
267. Moloney, J. N. & Cotter, T. G. ROS signalling in the biology of cancer. *Semin. Cell Dev. Biol.* **80**, 50–64 (2018).
268. Kalyanaraman, B. et al. Teaching the basics of reactive oxygen species and their relevance to cancer biology:

Mitochondrial reactive oxygen species detection, redox signaling, and targeted therapies. *Redox Biol.* **15**, 347–362 (2018).
269. DeBerardinis, R. J. & Chandel, N. S. Fundamentals of cancer metabolism. *Sci. Adv.* **2**, e1600200 (2016).
270. Kim, J., Kim, J. & Bae, J. S. ROS homeostasis and metabolism: a critical liaison for cancer therapy. *Exp. Mol. Med.* **48**, e269 (2016).
271. Steinbrenner, H., Speckmann, B. & Sies, H. Toward understanding success and failures in the use of selenium for cancer prevention. *Antioxid. Redox Signal.* **19**, 181–191 (2013).
272. Yang, H. et al. The role of cellular reactive oxygen species in cancer chemotherapy. *J. Exp. Clin. Cancer Res.* **37**, 266–0909 (2018).
273. Chaiswing, L., St Clair, W. H. & St Clair, D. K. Redox paradox: a novel approach to therapeutics-resistant cancer. *Antioxid. Redox Signal.* **29**, 1237–1272 (2018).
274. Panieri, E. & Santoro, M. M. ROS homeostasis and metabolism: a dangerous liason in cancer cells. *Cell Death. Dis.* **7**, e2253 (2016).
275. Allen, B. G. et al. First-in-human phase I clinical trial of pharmacologic ascorbate combined with radiation and temozolomide for newly diagnosed glioblastoma. *Clin. Cancer Res.* **25**, 6590–6597 (2019).
276. Schoenfeld, J. D. et al. Pharmacological ascorbate as a means of sensitizing cancer cells to radio-chemotherapy while protecting normal tissue. *Semin. Radiat. Oncol.* **29**, 25–32 (2019).
277. Elbatreek, M. H., Pachado, M. P., Cuadrado, A., Jandeleit-Dahm, K. & Schmidt, H. H. H. W. Reactive oxygen comes of age: mechanism-based therapy of diabetic end-organ damage. *Trends Endocrinol. Metab.* **30**, 312–327 (2019).
278. Keleku-Lukwete, N., Suzuki, M. & Yamamoto, M. An overview of the advantages of KEAP1-NRF2 system activation during inflammatory disease treatment. *Antioxid. Redox Signal.* **29**, 1746–1755 (2018).
279. Ames, B. N. Prolonging healthy aging: longevity vitamins and proteins. *Proc. Natl Acad. Sci. USA* **115**, 10836–10844 (2018).
An overview of healthy ageing and the role of micronutrients.
280. Banba, A., Tsuji, A., Kimura, H., Murai, M. & Miyoshi, H. Defining the mechanism of action of S1QELs, specific suppressors of superoxide production in the quinone-reaction site in mitochondrial complex I. *J. Biol. Chem.* **294**, 6550–6561 (2019).
281. Brand, M. D. et al. Suppressors of superoxide-H_2O_2 production at site IQ of mitochondrial complex I protect against stem cell hyperplasia and ischemia-reperfusion injury. *Cell Metab.* **24**, 582–592 (2016).
282. Galaris, D., Barbouti, A. & Pantopoulos, K. Iron homeostasis and oxidative stress: an intimate relationship. *Biochim. Biophys. Acta Mol. Cell Res.* **1866**, 118535 (2019).
283. Koppenol, W. H. & Hider, R. H. Iron and redox cycling. Do's and don'ts. *Free Radic. Biol. Med.* **133**, 3–10 (2019).
284. Pugh, C. W. & Ratcliffe, P. J. New horizons in hypoxia signaling pathways. *Exp. Cell Res.* **356**, 116–121 (2017).
285. Jain, I. H. et al. Hypoxia as a therapy for mitochondrial disease. *Science* **352**, 54–61 (2016).
286. Gorrini, C., Harris, I. S. & Mak, T. W. Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug. Discov.* **12**, 931–947 (2013).
287. von Woedtke, T., Schmidt, A., Bekeschus, S., Wende, K. & Weltmann, K. D. Plasma medicine: a field of applied redox biology. *Vivo* **33**, 1011–1026 (2019).
288. Chacko, B. K., Zhi, D., Darley-Usmar, V. M. & Mitchell, T. The bioenergetic health index is a sensitive measure of oxidative stress in human monocytes. *Redox Biol.* **8**, 43–50 (2016).
289. Hill, B. G., Shiva, S., Ballinger, S., Zhang, J. & Darley-Usmar, V. M. Bioenergetics and translational metabolism: implications for genetics, physiology and precision medicine. *Biol. Chem.* **401**, 3–29 (2019).
290. Trachootham, D., Alexandre, J. & Huang, P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug. Discov.* **8**, 579–591 (2009).
291. Kirkpatrick, D. L. & Powis, G. Clinically evaluated cancer drugs inhibiting redox signaling. *Antioxid. Redox Signal.* **26**, 262–273 (2017).
292. Adhikari, A., Mondal, S., Darbar, S. & Kumar, P. S. Role of nanomedicine in redox mediated healing at molecular level. *Biomol. Concepts* **10**, 160–174 (2019).
293. Yang, B., Chen, Y. & Shi, J. Reactive oxygen species (ROS)-based nanomedicine. *Chem. Rev.* **119**, 4881–4985 (2019).
294. Barabasi, A. L., Gulbahce, N. & Loscalzo, J. Network medicine: a network-based approach to human disease. *Nat. Rev. Genet.* **12**, 56–68 (2011).
295. Go, Y. M., Fernandes, J., Hu, X., Uppal, K. & Jones, D. P. Mitochondrial network responses in oxidative physiology and disease. *Free Radic. Biol. Med.* **116**, 31–40 (2018).
296. Di Mascio, P. et al. Singlet molecular oxygen reactions with nucleic acids, lipids, and proteins. *Chem. Rev.* **119**, 2043–2086 (2019).
297. Mano, C. M. et al. Excited singlet molecular $O_2(^1\Delta_g)$ is generated enzymatically from excited carbonyls in the dark. *Sci. Rep.* **4**, 5938 (2014).
298. Brash, D. E., Goncalves, L. C. P. & Bechara, E. J. H. Chemiexcitation and its implications for disease. *Trends Mol. Med.* **24**, 527–541 (2018).
299. Poole, L. B. The basics of thiols and cysteines in redox biology and chemistry. *Free Radic. Biol. Med.* **80**, 148–157 (2015).
300. Leisegang, M. S., Schröder, K. & Brandes, R. P. Redox regulation and noncoding RNAs. *Antioxid. Redox Signal.* **29**, 793–812 (2018).
301. Kalinina, E. V., Ivanova-Radkevich, V. I. & Chernov, N. N. Role of microRNAs in the regulation of redox-dependent processes. *Biochemistry (Mosc,)* **84**, 1233–1246 (2019).
302. Bartesaghi, S. & Radi, R. Fundamentals on the biochemistry of peroxynitrite and protein tyrosine nitration. *Redox Biol.* **14**, 618–625 (2018).
303. MacMillan-Crow, L. A., Crow, J. P., Kerby, J. D., Beckman, J. S. & Thompson, J. A. Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 11853–11858 (1996).
304. Filipovic, M. R., Zivanovic, J., Alvarez, B. & Banerjee, R. Chemical biology of H_2S signaling through persulfidation. *Chem. Rev.* **118**, 1253–1337 (2018).
Chemistry of H_2S signaling.
305. Paul, B. D. & Snyder, S. H. H_2S signalling through protein sulfhydration and beyond. *Nat. Rev. Mol. Cell Biol.* **13**, 499–507 (2012).
306. Biteau, B., Labarre, J. & Toledano, M. B. ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin. *Nature* **425**, 980–984 (2003).
307. Akter, S. et al. Chemical proteomics reveals new targets of cysteine sulfinic acid reductase. *Nat. Chem. Biol.* **14**, 995–1004 (2018).
308. Watson, W. H. et al. Redox potential of human thioredoxin 1 and identification of a second dithiol/disulfide motif. *J. Biol. Chem.* **278**, 33408–33415 (2003).

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