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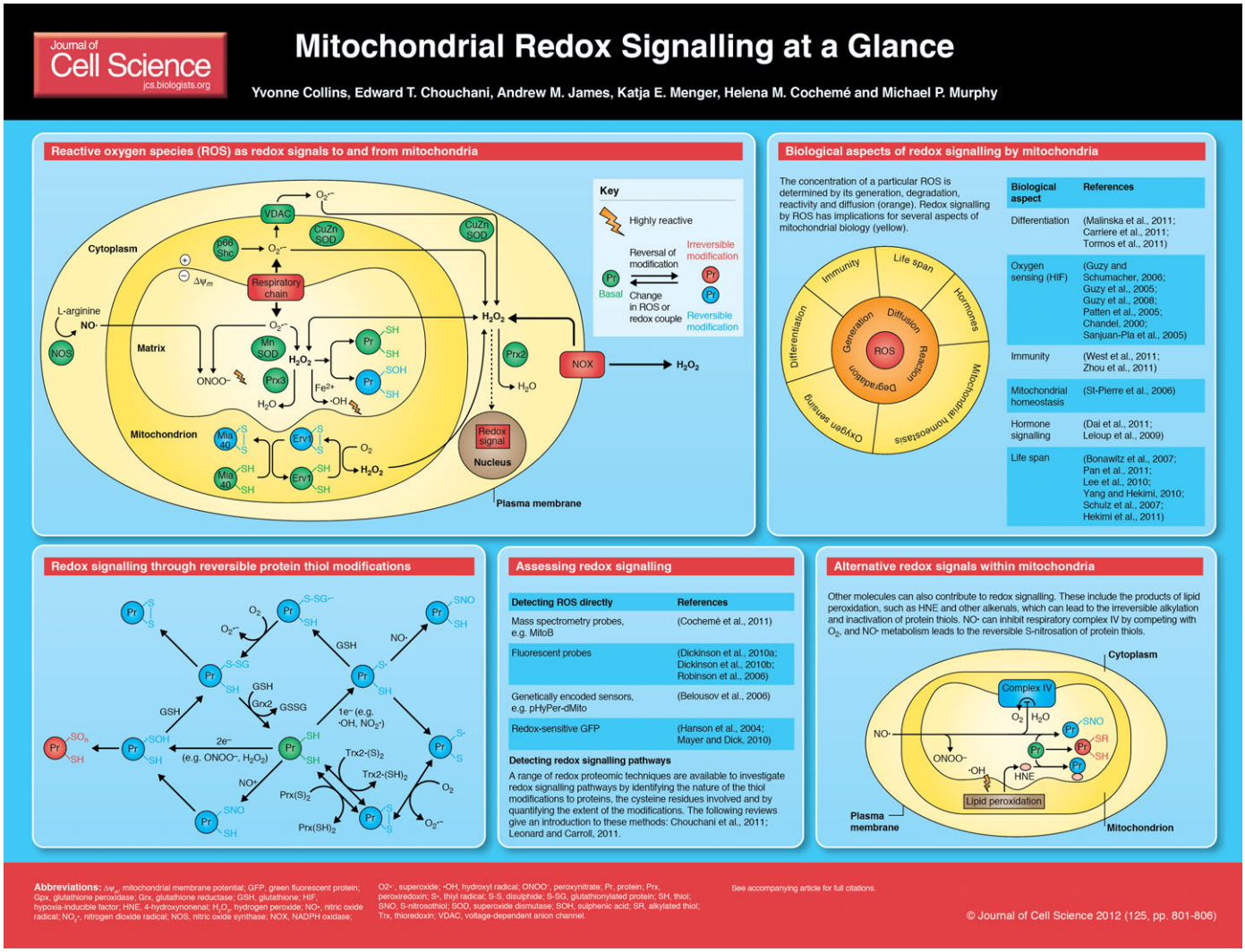
Redox signalling occurs when a biological system alters in response to a change in the level of a particular reactive oxygen species (ROS) or the shift in redox state of a responsive group such as a dithiol–disulphide couple (D'Autreaux and Toledano, 2007; Finkel, 2011; Fourquet et al., 2008; Janssen-Heininger et al., 2008; Rhee, 2006). Although ROS are best known as damaging agents in pathology, a more nuanced view has developed. It is now clear that some ROS, such as hydrogen peroxide (H_2O_2), can act as messengers both in the extracellular environment and within cells (D'Autreaux and Toledano, 2007; Fourquet et al., 2008; Janssen-Heininger et al., 2008; Rhee, 2006). Mitochondria seem to be an important redox signalling node, partly because of the flux of the ROS superoxide ($O_2^{\bullet-}$) generated by the respiratory chain and other core metabolic machineries within mitochondria (Balaban et al., 2005; Finkel, 2011; Murphy, 2009a). In addition, the mitochondrial matrix is central to metabolism, as oxidative phosphorylation, the citric acid cycle, fatty acid oxidation, the urea cycle and the biosynthesis of iron sulphur centres and haem take place there. Furthermore, mitochondria have key roles in apoptosis, calcium homeostasis and oxygen sensing (Duchen, 2004; Murphy, 2009a; Murphy, 2009b). Consequently, mitochondria are at the core of many biological processes, and redox signals to and from this organelle help to integrate mitochondrial function with that of the cell and organism. In this Cell Science at a Glance article we outline how mitochondrial redox signals are produced and modulated, the mechanisms by which redox signals can alter mitochondrial function and the experimental procedures available to assess this.

Production and modulation of redox signals to and from mitochondria

The initial ROS formed within mitochondria is $O_2^{\bullet-}$, which is generated by the respiratory chain and other enzymatic components within the mitochondrion (Finkel, 2011; Murphy, 2009a).



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Mitochondrial $O_2^{\bullet -}$ generation provides an indication of functional status because its production is altered by many cellular factors. These include the membrane potential, the reduction state of electron carriers and post-translational modification or damage to the respiratory chain (Murphy, 2009a). However, $O_2^{\bullet -}$ itself is not the main ROS signal within mitochondria because it is mostly converted to H_2O_2 by manganese superoxide dismutase (MnSOD), which reacts very rapidly with $O_2^{\bullet -}$ and is present at a high concentration within the matrix (Balaban et al., 2005; Chance et al., 1979; Finkel, 2005; Murphy, 2009a). As H_2O_2 can pass easily through mitochondrial membranes, it can act as a redox signal from mitochondria to the rest of the cell and vice versa (Balaban et al., 2005; D'Autreaux and Toledano, 2007; Droge, 2002; Fourquet et al., 2008; Janssen-Heininger et al., 2008; Murphy, 2009a).

Respiratory complex III can also release $O_2^{\bullet -}$ into the intermembrane space (St-Pierre et al., 2002; Muller et al., 2004; Han et al., 2001). The intermembrane space enzyme p66Shc (the 66 kDa isoform of the growth factor adapter Shc) can also generate $O_2^{\bullet -}$, which can regulate apoptotic cell death (Giorgio et al., 2005). The $O_2^{\bullet -}$ can diffuse from the intermembrane space to the cytosol or be converted to H_2O_2 by an intermembrane space Cu,Zn-SOD (Okado-Matsumoto and Fridovich, 2001). The Mia40p and Erv1p system of the intermembrane space, which inserts disulphide bonds into intermembrane space proteins during import, also generates H_2O_2 (Koehler et al., 2006), but the potential of this for redox signalling is unclear.

Matrix H_2O_2 concentration is further regulated by degradation through peroxiredoxin 3 and 5 (Prx3 and Prx5, respectively) and glutathione peroxidase 1 (Gpx1), with Prx3 being the most significant because of its relative abundance and reactivity (Cox et al., 2010). Prx proteins degrade H_2O_2 using the mitochondrial thioredoxin 2 (Trx2) system as a reducing source, whereas Gpx1 uses the mitochondrial glutathione (GSH) pool (Cox et al., 2010). During its reaction cycle, dimeric Prx3 forms an inter-subunit disulphide that is reduced back to the dithiol form by Trx2 (Rhee, 2006; Rhee et al., 2001). Exposure to H_2O_2 can lead to a significant fraction of Prx3 being in the disulphide form at any given time, thereby affecting H_2O_2 release from mitochondria (Cox et al., 2009; Cox et al., 2008). The activity of Prx3 might also be affected by post-translational modification or by the extent of its oligomerisation (Rhee et al., 2001; Rhee et al., 2005b; Cox et al., 2010). The extent of this H_2O_2 signal can be modulated both by its production, which is highly responsive to mitochondrial status (Murphy, 2009a), and by the rate of its degradation by matrix peroxidases – predominantly Prx3 – and diffusion into and out of the organelle.

The H_2O_2 that is produced by one mitochondrion can diffuse to another, coordinating or relaying signals between the organelles (Murphy, 2009a). Additionally, H_2O_2 can diffuse to mitochondria from the cell surface through the activation of NADPH oxidase (NOX) enzymes by growth factors (Janssen-Heininger et al., 2008; Rhee et al., 2005a; Rhee et al., 2005b).

The main ROS involved in redox signalling to and from mitochondria seems to be H_2O_2; however, other forms of ROS can also contribute. Nitric oxide (NO) is generated by NO synthases, and can diffuse into mitochondria and modulate mitochondrial function by competing with O_2 at respiratory complex IV – thereby slowing respiration – and by the S-nitrosation of mitochondrial thiol groups (Moncada and Erusalimsky, 2002). Iron sulphur centres in proteins such as aconitase can react rapidly with $O_2^{\bullet -}$ (D'Autreaux and Toledano, 2007), thereby modifying activity independently of H_2O_2. In addition, $O_2^{\bullet -}$ can diffuse from the intermembrane space through the outer membrane voltage-dependent anion channel to the cytosol, where it can act as a redox signal (Zhou et al., 2010). However, as $O_2^{\bullet -}$ is shorter lived and less diffusible than H_2O_2, its signalling roles are thought to be more limited. A number of other redox signals might also be produced within mitochondria, including peroxynitrite ($ONOO^-$) and the products of mitochondrial lipid peroxidation, such as prostaglandin-like molecules and 4-hydroxynonenal (HNE) (Levonen et al., 2004). These compounds can modify mitochondrial protein thiols and, thereby, affect their activity; however, the metabolic significance of these interactions is unclear.

Post-translational protein modification by H_2O_2 and NO

To act as effective biological messengers, molecules such as H_2O_2 and NO have to bring about a reversible change in the activity of a protein. Generally, this involves modification of a thiol group on a cysteine residue that mediates redox signalling (Eaton, 2006; Gilbert, 1990; Gilbert, 1995; Schafer and Buettner, 2001). For example, when H_2O_2 acts as a redox signal it oxidises the thiol group on the target protein to a disulphide group, thereby changing the function of the protein; once the level of H_2O_2 has returned to basal levels the alteration is reversed and the activity of the protein reverts to its initial level (Beltran et al., 2000; D'Autreaux and Toledano, 2007; Hess et al., 2001; Jacob et al., 2003; Janssen-Heininger et al., 2008; Ziegler, 1985). If the modification is to an active-site thiol, for example oxidation of the crucial thiol in tyrosine phosphatases (Boivin et al., 2010), then the impact on the protein is a clear loss of function. However, thiol oxidation can alter proteins and, thereby, mediate the redox signal in other ways, such as by changing binding affinity to another protein, altering its action as a transcription factor, or by modifying the activity of a transporter or channel (Balaban et al., 2005; D'Autreaux and Toledano, 2007; Droge, 2002; Fourquet et al., 2008; Murphy, 2009a; Rhee, 2006; Rhee et al., 2000).

Generally, in response to H_2O_2, protein thiol groups will initially form a sulphenic acid (–SOH) (Brennan et al., 2004; Charles et al., 2007; Cotgreave and Gerdes, 1998; Fratelli et al., 2004; Leonard et al., 2009; Seres et al., 1996; Ziegler, 1985; Dalle-Donne et al., 2008; Dalle-Donne et al., 2009), which can occur by direct reaction of H_2O_2 with the thiolate ($–S^-$). This reaction is dependent on the local environment of the thiol and also its pK_a, which can lead to certain thiols being particularly sensitive to oxidation. Once formed, the sulphenic acid can itself be a relevant post-translational modification, or it can form other post-translational modifications by reacting with a GSH to form a glutathionylated protein, with an adjacent thiol to form a disulphide (Brennan et al., 2004; Charles et al., 2007; Dalle-Donne et al., 2009; Delaunay et al., 2002; Hurd et al., 2008), or with amides within the protein to form a sulphenyl amide (Sivaramakrishnan et al., 2010). An alternative route to thiol oxidation during redox signalling is the single-electron oxidation of a thiol to a thiyl radical ($–S^{\bullet}$), which can then react to form disulphide bonds with GSH or with another protein thiol (Wardman and Von Sonntag, 1995; Winterbourn, 1993).

NO metabolism can also modify a protein thiol group into an S-nitrosothiol group (SNO) in a process known as S-nitrosation or S-nitrosylation (Beltran et al., 2000; Hess et al., 2001; Hogg, 2002; Stamler, 1994; Stamler and Hausladen, 1998). The mechanism of SNO formation in vivo is obscure (Hogg, 2002) but, once generated, the SNO can be passed between thiols by transnitrosation, with the formation and stability of the SNO determined by protein sequence motifs that surround the modified cysteine residue (Benhar et al., 2009; Doulias et al., 2010; Hou et al., 1996; Marino and Gladyshev, 2010; Nikitovic and Holmgren, 1996). In addition, an initial SNO on a protein can be modified into other thiol-based groups, such as disulphide, sulphenic acid or into a glutathionylated protein (Nikitovic and Holmgren, 1996; Stamler et al., 1992).

All of these post-translational modifications can potentially act as 'redox switches' (Cabiscol and Levine, 1996; Mallis et al., 2000; Schafer and Buettner, 2001; Zheng et al., 1998), altering the function of a protein and, thereby, enabling it to respond sensitively to the reduction potential of a particular redox couple or to the production of a particular ROS. Although structural alterations brought about by these modifications can potentially have a major effect on protein function, in only a few cases have detailed structural analyses shown clearly how this occurs. To be effective signals, these thiol alterations must be readily reversible. This is achieved by the glutathione-reductase–GSH–glutaredoxin (Grx2) system or by the thioredoxin reductase (TrxR2)–Trx2 system that is present in the mitochondrial matrix (Dalle-Donne et al., 2009; Hurd et al., 2005a; Hurd et al., 2005b; Schafer and Buettner, 2001).

Protein thiols can be modified by a direct reaction with H_2O_2, independently of bulk changes to the redox state of thiol pools. Alternatively, protein thiol modifications can occur through reactions with another thiol–disulphide redox couple. An example of this is the change in the extent of glutathionylation of particular protein thiols in response to changes in the ratio of glutathione to glutathione disulphide (GSH:GSSG), mediated by Grx2 (Costa et al., 2003; Schafer and Buettner, 2001; Beer et al., 2004). However, as this process requires the GSH pool to be significantly oxidised, this situation probably does not occur under most physiological conditions. Alterations to the redox state of Trx2 might also lead to further modifications to protein thiols, provided that a sufficiently oxidised reduction potential can be achieved by the Trx2 pool. More generally, other dithiol proteins – such as peroxidases with appropriate reduction potentials relative to both oxidants and target proteins – can affect the activity of target proteins by introducing internal disulphides (Delaunay et al., 2002).

There are other potential modes of redox signalling in addition to the reversible modification of protein thiols. Proteins can be modified irreversibly by the alkylation of thiols. This is exemplified in the cytosolic pathway of nuclear factor erythroid 2-related factor 2 (NRF2) and Kelch-like ECH-associated protein 1 (KEAP1) (NRF2–KEAP1 pathway), in which one of the KEAP1 thiol groups can react irreversibly with electrophiles to release the NRF2 transcription factor. NRF2 then translocates to the nucleus where it induces transcription of genes under the control of promoters that contain the antioxidant response element (ARE) (Hayes et al., 2010; Kobayashi and Yamamoto, 2006). Alternatively, other interactions are possible, such as the competition of NO with O_2 in binding to respiratory complex IV and, thus, altering mitochondrial respiration and the redox state of the respiratory chain (Brown, 1995; Moncada and Erusalimsky, 2002). These and other modes of redox signalling might complement or extend the central role of reversible thiol oxidation.

Biologically important mitochondrial redox signals

The concept of redox signalling in biology initially emerged from studies on ROS production from NOXs and on the interactions of NO with biological systems (reviewed by, Finkel, 2011; Rhee, 2006; Janssen-Heininger et al., 2008). Since then, mitochondria have emerged as an important node of redox signalling in numerous biologically important areas. Among the most intriguing is the role of mitochondrial ROS in O_2 sensing, especially during hypoxia (Guzy and Schumacker, 2006; Guzy et al., 2008; Patten et al., 2010; Brunelle et al., 2005). In this process, it seems that the production of $O_2^{\cdot-}$ by the respiratory chain increases under conditions of low O_2 levels (Chandel et al., 1998; Chandel et al., 2000; Guzy et al., 2005). The site of the $O_2^{\cdot-}$ production is thought to be respiratory complex III, but the mechanism is unclear (Chandel et al., 2000; Guzy et al., 2005). The elevated mitochondrial $O_2^{\cdot-}$ is converted to H_2O_2 in the mitochondrial matrix, followed by diffusion into the cytosol where it stabilises hypoxia-inducible factor-1α (HIF-1α), thus leading to the transcription of genes that enable the cell to respond to hypoxia (Sanjuán-Pla et al., 2005). Redox signalling by mitochondrial ROS is now implicated in a disparate range of biologically important areas, including as a determinant of chronological lifespan (the time cells in a stationary phase culture remain viable) in yeast (Bonawitz et al., 2007; Pan et al., 2011; Bell et al., 2007), a factor controlling lifespan in *Caenorhabditis elegans* (Lee et al., 2010; Yang and Hekimi, 2010; Schulz et al., 2007; Hekimi et al., 2011), in the regulation of the immune system (West et al., 2011; Zhou et al., 2011; Wang et al., 2010), in angiotensin II signalling (Dai et al., 2011), in insulin secretion (Leloup et al., 2009) and mitochondrial homeostasis (St-Pierre et al., 2006).

How to investigate redox signalling pathways

Although there is considerable evidence indicating the importance of mitochondrial redox signalling, changes in ROS concentration or a thiol modification also occur during pathologies. Consequently, it is imperative not to assume that such events are necessarily evidence of a redox signal, and to show that changes in the levels of a particular ROS and the subsequent modification of target proteins correlate with and are sufficient to explain the biological modification. However, assessing changes in ROS and protein redox modifications in biological systems is technically demanding and requires an understanding of the underlying chemistry (Murphy et al., 2011). Despite this, considerable evidence demonstrates the presence of protein thiols within mitochondria that can be modified by H_2O_2 and S-nitrosating agents (Chouchani et al., 2010; Hurd et al., 2005a; Hurd et al., 2005b; Hurd et al., 2007; Prime et al., 2009; Sun et al., 2007). There are now a variety of methods that can be used to assess the levels of particular ROS within mitochondria, and these include mitochondria-targeted small-molecule fluorescence probes (Dickinson et al., 2010a; Dickinson et al., 2010b; Robinson et al., 2006), the use of mitochondria-targeted proteins derived from green fluorescent protein – whose fluorescence is redox sensitive (Meyer and Dick, 2010), and mitochondria-targeted mass spectrometry probes that enable mitochondrial ROS levels to be estimated in vivo (Cochemé et al., 2011). The proteins modified and the nature of the thiol modification can also be determined by using a number of redox proteomic techniques (Chouchani et al., 2010; Dahm et al., 2006; Danielson et al., 2011; Taylor et al., 2003; Held et al., 2010; Hurd et al., 2007).

Once the involved cysteine residues have been determined it is vital to quantify the extent of the modification to ensure that it correlates with a change in protein activity that is sufficient to account for the phenotypic change (Murphy et al., 2011). Mass spectrometric techniques to assess this are now available (Danielson et al., 2011; Held et al., 2010). Proteomic approaches have also been extended to in-vivo models and a range of mitochondrial proteins have been identified that have reversible modifications (Burwell et al., 2006; Doulias et al., 2010; Charles et al., 2007; Fratelli et al., 2003; Murray et al., 2011; Schroder and Eaton, 2008; Sun and Murphy, 2010; Nadtochiy et al., 2007). Without such measurements it might be that the changes in the level of the putative signalling ROS and in the protein redox modification merely correlate with the change in activity, rather than cause it.

Perspectives

There is increased recognition that protein modifications that are induced by certain ROS, such as H_2O_2 and NO, are not solely damaging events in biological systems, but might also be important components of feedback and signalling pathways. Mitochondria are at the heart of metabolism and cell death and are,

therefore, important for many physiological pathways. It is also clear that ROS and redox modifications of proteins enable mitochondria to respond to and modulate function(s) of cells and whole organisms. However, despite the development of a more nuanced view of the role of ROS and redox modification in biology, caution is still warranted. This is owing to the technical difficulties in measuring and quantifying ROS and protein redox modifications in biological systems. Consequently, it is important to make sure that any changes measured are responsible for the biological changes and are not merely correlates with no signalling function – such as a response to damage or a repair process.

Often redox signalling is compared, explicitly or tacitly, with signalling by reversible protein phosphorylation. However, it is important to bear in mind that with phosphorylation there is a large thermodynamic driving force for the modification of serine, threonine or tyrosine residues that is channelled and kinetically controlled by tightly regulated kinases. The introduction of a bulky, charged phosphate group has a significant effect on the target protein, resulting in a change in its function or location. The reversal of the modification is also tightly regulated by specific phosphatases. Few redox signalling pathways are as well-defined as established phosphorylation signalling pathways, with most only matching a few aspects. Often, the processes that lead to the redox modifications are less specific as there is no kinase equivalent that can selectively modify proteins, with thiol sensitivity usually owing to the influence of local sequence and structural motifs on the pK_a and reactivity of the thiol. Consequently, many thiols are susceptible to redox modification, but only a few are important in genuine signalling pathways. This can lead to all redox changes being interpreted as signalling events through a kind of 'phosphorylation envy' that has to be guarded against, so the true significance of redox signalling and modifications in mitochondrial biology can emerge.

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References

Balaban, R. S., Nemoto, S. and Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell* **120**, 483-495.

Beer, S. M., Taylor, E. R., Brown, S. E., Dahm, C. C., Costa, N. J., Runswick, M. J. and Murphy, M. P. (2004). Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: Implications for mitochondrial Redox regulation and antioxidant defense. *J. Biol. Chem.* **279**, 47939-47951.

Bell, E., Klimova, T. A., Eisenbart, J., Schumacker, P. T. and Chandel, N. S. (2007). Mitochondrial ROS trigger HIF-dependent extension of replicative lifespan during hypoxia. *Mol. Cell. Biol.* **27**, 5737-5745.

Belousov, V. V., Fradkov, A. F., Lukyanov, K. A., Staroverov, D. B., Shakhbazov, K. S., Terskikh, A. V. and Lukyanov, S. (2006). Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nat. Methods* **3**, 281-286.

Beltran, B., Orsi, A., Clementi, E. and Moncada, S. (2000). Oxidative stress and S-nitrosylation of proteins in cells. *Br. J. Pharmacol.* **129**, 953-960.

Benhar, M., Forrester, M. T. and Stamler, J. S. (2009). Protein denitrosylation: enzymatic mechanisms and cellular functions. *Nat. Rev. Mol. Cell Biol.* **10**, 721-732.

Boivin, B., Yang, M. and Tonks, N. K. (2010). Targeting the reversibly oxidized protein tyrosine phosphatase superfamily. *Sci. Signal.* **3**, pl2.

Bonawitz, N. D., Chatenay-Lapointe, M., Pan, Y. and Shadel, G. S. (2007). Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. *Cell. Metab.* **5**, 265-277.

Brennan, J. P., Wait, R., Begum, S., Bell, J. R., Dunn, M. J. and Eaton, P. (2004). Detection and mapping of widespread intermolecular protein disulfide formation during cardiac oxidative stress using proteomics with diagonal electrophoresis. *J. Biol. Chem.* **279**, 41352-41360.

Brown, G. C. (1995). Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrome oxidase. *FEBS Lett.* **369**, 136-139.

Brunelle, J. K., Bell, E. L., Quesada, N. M., Vercauteren, K., Tiranti, V., Zeviani, M., Scarpulla, R. C. and Chandel, N. S. (2005). Oxygen Sensing requires ROS but not oxidative phosphorylation. *Cell Metab.* **1**, 409-414.

Burwell, L. S., Nadtochiy, S. M., Tompkins, A. J., Young, S. and Brookes, P. S. (2006). Direct evidence for S-nitrosation of mitochondrial complex I. *Biochem. J.* **394**, 627-634.

Cabiscol, E. and Levine, R. L. (1996). The phosphatase activity of carbonic anhydrase III is reversibly regulated by glutathiolation. *Proc. Natl. Acad. Sci. USA* **93**, 4170-4174.

Carriere, A., Carmona, M. C., Fernandez, Y., Rigoulet, M., Wenger, R. H., Penicaud, L. and Casteilla, L. (2004). Mitochondrial reactive oxygen species control the transcription factor CHOP-10/GADD153 and adipocyte differentiation: a mechanism for hypoxia-dependent effect. *J. Biol. Chem.* **279**, 40462-40469.

Chance, B., Sies, H. and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**, 527-605.

Chandel, N. S., Maltepe, E., Goldwasser, E., Mathieu, C. E., Simon, M. C. and Schumacker, P. T. (1998). Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc. Natl. Acad. Sci. USA* **95**, 11715-11720.

Chandel, N. S., McClintock, D. S., Feliciano, C. E., Wood, T. M., Melendez, J. A., Rodriguez, A. M. and Schumacker, P. T. (2000). Reactive oxygen species generated at complex III during hypoxia stablize HIF-1a: A mechanism of oxygen sensing. *J. Biol. Chem.* **275**, 25130-25138.

Charles, R. L., Schroder, E., May, G., Free, P., Gaffney, P. R., Wait, R., Begum, S., Heads, R. J. and Eaton, P. (2007). Protein sulfenation as a redox sensor: proteomics studies using a novel biotinylated dimedone analogue. *Mol. Cell. Proteomics* **6**, 1473-1484.

Chouchani, E. T., Hurd, T. R., Nadtochiy, S. M., Brookes, P. S., Fearnley, I. M., Lilley, K. S., Smith, R. A. and Murphy, M. P. (2010). Identification of S-nitrosated mitochondrial proteins by S-nitrosothiol difference in gel electrophoresis (SNO-DIGE): implications for the regulation of mitochondrial function by reversible S-nitrosation. *Biochem. J.* **430**, 49-59.

Chouchani, E. T., James, A. M., Fearnley, I. M., Lilley, K. S. and Murphy, M. P. (2011). Proteomic approaches to the characterization of protein thiol modification. *Curr. Opin. Chem. Biol.* **15**, 120-128.

Cocheme, H. M., Quin, C., McQuaker, S. J., Cabreiro, F., Logan, A., Prime, T. A., Abakumova, I., Patel, J. V., Fearnley, I. M., James, A. M. et al. (2011). Measurement of H_2O_2 within living Drosophila during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. *Cell Metab.* **13**, 340-350.

Costa, N. J., Dahm, C. C., Hurrell, F., Taylor, E. R. and Murphy, M. P. (2003). Interactions of mitochondrial thiols with nitric oxide. *Antioxid. Redox Signal.* **5**, 291-305.

Cotgreave, I. A. and Gerdes, R. G. (1998). Recent trends in glutathione biochemistry–glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? *Biochem. Biophys. Res. Commun.* **242**, 1-9.

Cox, A. G., Pullar, J. M., Hughes, G., Ledgerwood, E. C. and Hampton, M. B. (2008). Oxidation of mitochondrial peroxiredoxin 3 during the initiation of receptor-mediated apoptosis. *Free Radic. Biol. Med.* **44**, 1001-1009.

Cox, A. G., Peskin, A. V., Paton, L. N., Winterbourn, C. C. and Hampton, M. B. (2009). Redox potential and peroxide reactivity of human peroxiredoxin 3. *Biochemistry* **48**, 6495-6501.

Cox, A. G., Winterbourn, C. C. and Hampton, M. B. (2010). Mitochondrial peroxiredoxin involvement in antioxidant defence and redox signalling. *Biochem. J.* **425**, 313-325.

D'Autreaux, B. and Toledano, M. B. (2007). ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat. Rev Mol. Cell Biol.* **8**, 813-824.

Dahm, C. C., Moore, K. and Murphy, M. P. (2006). Persistent S-nitrosation of complex I and other mitochondrial membrane proteins by S-nitrosothiols but not nitric oxide or peroxynitrite: Implications for the interaction of nitric oxide with mitochondria. *J. Biol. Chem.* **281**, 10056-10065.

Dai, D. F., Johnson, S. C., Villarin, J. J., Chin, M. T., Nieves-Cintrón, M., Chen, T., Marcinek, D. J., Dorn, G. W., Kang, Y. J., Prolla, T. A. et al. (2011). Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Gαq overexpression-induced heart failure. *Circ. Res.* **108**, 837-846.

Dalle-Donne, I., Milzani, A., Gagliano, N., Colombo, R., Giustarini, D. and Rossi, R. (2008). Molecular mechanisms and potential clinical significance of S-glutathionylation. *Antioxid. Redox Signal.* **10**, 445-473.

Dalle-Donne, I., Rossi, R., Colombo, G., Giustarini, D. and Milzani, A. (2009). Protein S-glutathionylation: a regulatory device from bacteria to humans. *Trends Biochem. Sci.* **34**, 85-96.

Danielson, S. R., Held, J. M., Oo, M., Riley, R., Gibson, B. W. and Andersen, J. K. (2011). Quantitative mapping of reversible mitochondrial complex I cysteine oxidation in a Parkinson disease mouse model. *J. Biol. Chem.* **286**, 7601-7608.

Delaunay, A., Pflieger, D., Barrault, M. B., Vinh, J. and Toledano, M. B. (2002). A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. *Cell* **111**, 471-481.

Dickinson, B. C., Huynh, C. and Chang, C. J. (2010a). A palette of fluorescent probes with varying emission colors for imaging hydrogen peroxide signaling in living cells. *J. Am. Chem. Soc.* **132**, 5906-5915.

Dickinson, B. C., Srikun, D. and Chang, C. J. (2010b). Mitochondrial-targeted fluorescent probes for reactive oxygen species. *Curr. Opin. Chem. Biol.* **14**, 50-56.

Doulias, P. T., Greene, J. L., Greco, T. M., Tenopoulou, M., Seeholzer, S. H., Dunbrack, R. L. and Ischiropoulos, H. (2010). Structural profiling of endogenous S-nitrosocysteine residues reveals unique features that accommodate diverse mechanisms for protein S-nitrosylation. *Proc. Natl. Acad. Sci. USA* **107**, 16958-16963.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.* **82**, 47-95.

Duchen, M. R. (2004). Mitochondria in health and disease: Perspectives on a new mitochondrial biology. *Mol. Aspects Med.* **25**, 365-451.

Eaton, P. (2006). Protein thiol oxidation in health and disease: techniques for measuring disulfides and related modifications in complex protein mixtures. *Free Radic. Biol. Med.* **40**, 1889-1899.

Finkel, T. (2005). Opinion: Radical medicine: treating ageing to cure disease. *Nat. Rev. Mol. Cell Biol.* **6**, 971-976.

Finkel, T. (2011). Signal transduction by reactive oxygen species. *J. Cell Biol.* **194**, 7-15.
Fourquet, S., Huang, M. E., D'Autreaux, B. and Toledano, M. B. (2008). The dual functions of thiol-based peroxidases in H_2O_2 scavenging and signaling. *Antioxid. Redox Signal.* **10**, 1565-1575.
Fratelli, M., Demol, H., Puype, M., Casagrande, S., Villa, P., Eberini, I., Vandekerckhove, J., Gianazza, E. and Ghezzi, P. (2003). Identification of proteins undergoing glutathionylation in oxidatively stressed hepatocytes and hepatoma cells. *Proteomics* **3**, 1154-1161.
Fratelli, M., Gianazza, E. and Ghezzi, P. (2004). Redox proteomics: identification and functional role of glutathionylated proteins. *Expert Rev. Proteomics* **1**, 365-376.
Gilbert, H. F. (1990). Molecular and cellular aspects of thiol-disulfide exchange. *Adv. Enzymol. Relat. Areas Mol. Biol.* **63**, 69-172.
Gilbert, H. F. (1995). Thiol/disulfide exchange equilibria and disulfide bond stability. *Methods Enzymol.* **251**, 8-28.
Giorgio, M., Migliaccio, E., Orsini, F., Paolucci, D., Moroni, M., Contursi, C., Pelliccia, G., Luzi, L., Minucci, S., Marcaccio, M., et al. (2005). Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **122**, 221-233.
Guzy, R. D. and Schumacker, P. T. (2006). Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp. Physiol.* **91**, 807-819.
Guzy, R. D., Hoyos, B., Robin, E., Chen, H., Liu, L., Mansfield, K. D., Simon, M. C., Hammerling, U. and Schumacker, P. T. (2005). Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab.* **1**, 401-408.
Guzy, R. D., Sharma, B., Bell, E., Chandel, N. S. and Schumacker, P. T. (2008). Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol. Cell. Biol.* **28**, 718-731.
Han, D., Williams, E. and Cadenas, E. (2001). Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. *Biochem. J.* **353**, 411-416.
Hanson, G. T., Aggeler, R., Oglesbee, D., Cannon, M., Capaldi, R. A., Tsien, R. Y. and Remington, S. J. (2004). Investigating mitochondrial redox potential with redox-sensitive green fluorescent protein indicators. *J. Biol. Chem.* **279**, 13044-13053.
Hayes, J. D., McMahon, M., Chowdhry, S. and Dinkova-Kostova, A. T. (2010). Cancer chemoprevention mechanisms mediated through the keap1-Nrf2 pathway. *Antioxid. Redox Signal.* **13**, 1713-1748.
Hekimi, S., Lapointe, J. and Wen, Y. (2011). Taking a "good" look at free radicals in the aging process. *Trends Cell. Biol.* 569-575.
Held, J. M., Danielson, S. R., Behring, J. B., Atsriku, C., Britton, D. J., Puckett, R. L., Schilling, B., Campisi, J., Benz, C. C. and Gibson, B. W. (2010). Targeted quantitation of site-specific cysteine oxidation in endogenous proteins using a differential alkylation and multiple reaction monitoring mass spectrometry approach. *Mol. Cell. Proteomics* **9**, 1400-1410.
Hess, D. T., Matsumoto, A., Nudelman, R. and Stamler, J. S. (2001). S-nitrosylation: spectrum and specificity. *Nat. Cell. Biol.* **3**, E46-E49.
Hogg, N. (2002). The biochemistry and physiology of S-nitrosothiols. *Annu. Rev. Pharmacol. Toxicol.* **42**, 585-600.
Hou, Y., Guo, Z., Li, J. and Wang, P. G. (1996). Seleno compounds and glutathione peroxidase catalyzed decomposition of S-nitrosothiols. *Biochem. Biophys. Res. Commun.* **228**, 88-93.
Hurd, T. R., Costa, N. J., Dahm, C. C., Beer, S. M., Brown, S. E., Filipovska, A. and Murphy, M. P. (2005a). Glutathionylation of mitochondrial proteins. *Antioxid. Redox Signal.* **7**, 999-1010.
Hurd, T. R., Filipovska, A., Costa, N. J., Dahm, C. C. and Murphy, M. P. (2005b). Disulphide formation on mitochondrial protein thiols. *Biochem. Soc. Trans.* **33**, 1390-1393.
Hurd, T. R., Prime, T. A., Harbour, M. E., Lilley, K. S. and Murphy, M. P. (2007). Detection of Reactive Oxygen Species-sensitive Thiol Proteins by Redox Difference Gel Electrophoresis: Implications for mitochondrial Redox signaling. *J. Biol. Chem.* **282**, 22040-22051.
Hurd, T. R., Requejo, R., Filipovska, A., Brown, S., Prime, T. A., Robinson, A. J., Fearnley, I. M. and Murphy, M. P. (2008). Complex I within oxidatively stressed bovine heart mitochondria is glutathionylated on Cys-531 and Cys-704 of the 75-kDa subunit: potential role of CYS residues in decreasing oxidative damage. *J. Biol. Chem.* **283**, 24801-24815.
Jacob, C., Giles, G. I., Giles, N. M. and Sies, H. (2003). Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew. Chem. Int. Ed. Engl.* **42**, 4742-4758.
Janssen-Heininger, Y. M., Mossman, B. T., Heintz, N. H., Forman, H. J., Kalyanaraman, B., Finkel, T., Stamler, J. S., Rhee, S. G. and van der Vliet, A. (2008). Redox-based regulation of signal transduction: principles, pitfalls, and promises. *Free Radic. Biol. Med.* **45**, 1-17.
Kobayashi, M. and Yamamoto, M. (2006). Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. *Adv. Enzyme Regul.* **46**, 113-140.
Koehler, C. M., Beverly, K. N. and Leverich, E. P. (2006). Redox pathways of the mitochondrion. *Antioxid. Redox Signal.* **8**, 813-822.
Lee, S. J., Hwang, A. B. and Kenyon, C. (2010). Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr. Biol.* **20**, 2131-2136.
Leloup, C., Tourrel-Cuzin, C., Magnan, C., Karaca, M., Castel, J., Carneiro, L., Colombani, A. L., Ktorza, A., Casteilla, L. and Penicaud, L. (2009). Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. *Diabetes* **58**, 673-681.
Leonard, S. E. and Carroll, K. S. (2011). Chemical 'omics' approaches for understanding protein cysteine oxidation in biology. *Curr. Opin. Chem. Biol.* **15**, 88-102.
Leonard, S. E., Reddie, K. G. and Carroll, K. S. (2009). Mining the thiol proteome for sulfenic acid modifications reveals new targets for oxidation in cells. *ACS Chem. Biol.* **4**, 783-799.
Levonen, A. L., Landar, A., Ramachandran, A., Ceaser, E. K., Dickinson, D. A., Zanoni, G., Morrow, J. D. and Darley-Usmar, V. M. (2004). Cellular mechanisms of redox cell signalling: role of cysteine modification in controlling antioxidant defences in response to electrophilic lipid oxidation products. *Biochem. J.* **378**, 373-382.
Malinska, D., Kudin, A. P., Bejtka, M. and Kunz, W. S. (2012). Changes in mitochondrial reactive oxygen species synthesis during differentiation of skeletal muscle cells. *Mitochondrion* **12**, 144-148.
Mallis, R. J., Poland, B. W., Chatterjee, T. K., Fisher, R. A., Darmawan, S., Honzatko, R. B. and Thomas, J. A. (2000). Crystal structure of S-glutathiolated carbonic anhydrase III. *FEBS Lett.* **482**, 237-241.
Marino, S. M. and Gladyshev, V. N. (2010). Structural analysis of cysteine S-nitrosylation: a modified acid-based motif and the emerging role of trans-nitrosylation. *J. Mol. Biol.* **395**, 844-859.
Meyer, A. J. and Dick, T. P. (2010). Fluorescent protein-based redox probes. *Antioxid. Redox Signal.* **13**, 621-650.
Moncada, S. and Erusalimsky, J. D. (2002). Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nat. Rev. Mol. Cell Biol.* **3**, 214-220.
Muller, F. L., Liu, Y. and Van Remmen, H. (2004). Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J. Biol. Chem.* **279**, 49064-49073.
Murphy, M. P. (2009a). How mitochondria produce reactive oxygen species. *Biochem. J.* **417**, 1-13.
Murphy, M. P. (2009b). Mitochondria–a neglected drug target. *Curr. Opin. Investig. Drugs* **10**, 1022-1024.
Murphy, M. P., Holmgren, A., Larsson, N. G., Halliwell, B., Chang, C. J., Kalyanaraman, B., Rhee, S. G., Thornalley, P. J., Partridge, L., Gems, D. et al. (2011). Unraveling the biological roles of reactive oxygen species. *Cell Metab.* **13**, 361-366.
Murray, C. I., Kane, L. A., Uhrigshardt, H., Wang, S. B. and Van Eyk, J. E. (2011). Site-mapping of in vitro S-nitrosation in cardiac mitochondria: Implications for cardioprotection. *Mol. Cell. Proteomics* **10**, M110.004721.
Nadtochiy, S. M., Burwell, L. S. and Brookes, P. S. (2007). Cardioprotection and mitochondrial S-nitrosation: effects of S-nitroso-2-mercaptopropionyl glycine (SNO-MPG) in cardiac ischemia-reperfusion injury. *J. Mol. Cell. Cardiol.* **42**, 812-825.
Nikitovic, D. and Holmgren, A. (1996). S-nitrosoglutathione is cleaved by the thioredoxin system with liberation of glutathione and redox regulating nitric oxide. *J. Biol. Chem.* **271**, 19180-19185.
Okado-Matsumoto, A. and Fridovich, I. (2001). Subcellular distribution of superoxide dismutases (SOD) in rat liver. Cu,Zn-SOD in mitochondria. *J. Biol. Chem.* **276**, 38388-38393.
Pan, Y., Schroeder, E. A., Ocampo, A., Barrientos, A. and Shadel, G. S. (2011). Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. *Cell Metab.* **13**, 668-678.
Patten, D. A., Lafleur, V. N., Robitaille, G. A., Chan, D. A., Giaccia, A. J. and Richard, D. E. (2010). Hypoxia-inducible factor-1 activation in nonhypoxic conditions: the essential role of mitochondrial-derived reactive oxygen species. *Mol. Biol. Cell* **21**, 3247-3257.
Prime, T. A., Blaikie, F. H., Evans, C., Nadtochiy, S. M., James, A. M., Dahm, C. C., Vitturi, D. A., Patel, R. P., Hiley, C. R., Abakumova, I. et al. (2009). A mitochondria-targeted S-nitrosothiol modulates respiration, nitrosates thiols, and protects against ischemia-reperfusion injury. *Proc. Natl. Acad. Sci. USA* **106**, 10764-10769.
Rhee, S. G. (2006). Cell signaling. H_2O_2, a necessary evil for cell signaling. *Science* **312**, 1882-1883.
Rhee, S. G., Bae, Y. S., Lee, S. R. and Kwon, J. (2000). Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. *Sci. STKE* **2000**, pe1.
Rhee, S. G., Kang, S. W., Chang, T. S., Jeong, W. and Kim, K. (2001). Peroxiredoxin, a novel family of peroxidases. *IUBMB Life* **52**, 35-41.
Rhee, S. G., Kang, S. W., Jeong, W., Chang, T. S., Yang, K. S. and Woo, H. A. (2005a). Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr. Opin. Cell Biol.* **17**, 183-189.
Rhee, S. G., Yang, K. S., Kang, S. W., Woo, H. A. and Chang, T. S. (2005b). Controlled elimination of intracellular H_2O_2: regulation of peroxiredoxin, catalase, and glutathione peroxidase via post-translational modification. *Antioxid. Redox Signal.* **7**, 619-626.
Robinson, K. M., Janes, M. S., Pehar, M., Monette, J. S., Ross, M. F., Hagen, T. M., Murphy, M. P. and Beckman, J. S. (2006). Selective fluorescent imaging of superoxide in vivo using ethidium-based probes. *Proc. Natl. Acad. Sci. USA* **103**, 15038-15043.
Sanjuán-Pla, A., Cervera, A. M., Apostolova, N., Garcia-Bou, R., Víctor, V. M., Murphy, M. P. and McCreath, K. J. (2005). A targeted antioxidant reveals the importance of mitochondrial reactive oxygen species in the hypoxic signaling of HIF-1alpha. *FEBS Lett.* **579**, 2669-2674.
Schafer, F. Q. and Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* **30**, 1191-1212.
Schroder, E. and Eaton, P. (2008). Hydrogen peroxide as an endogenous mediator and exogenous tool in cardiovascular research: issues and considerations. *Curr. Opin. Pharmacol.* **8**, 153-159.
Schulz, T. J., Zarse, K., Voigt, A., Urban, N., Birringer, M. and Ristow, M. (2007). Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* **6**, 280-293.
Seres, T., Ravichandran, V., Moriguchi, T., Rokutan, K., Thomas, J. A. and Johnston, R. B., Jr (1996). Protein S-thiolation and dethiolation during the respiratory burst in human monocytes. A reversible post-translational modification with potential for buffering the effects of oxidant stress. *J. Immunol.* **156**, 1973-1980.
Sivaramakrishnan, S., Cummings, A. H. and Gates, K. S. (2010). Protection of a single-cysteine redox switch from oxidative destruction: On the functional role of sulfenyl amide formation in the redox-regulated enzyme PTP1B. *Bioorg. Med. Chem. Lett.* **20**, 444-447.
St-Pierre, J., Buckingham, J. A., Roebuck, S. J. and Brand, M. D. (2002). Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J. Biol. Chem.* **277**, 44784-44790.

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jager, S., Handschin, C., Zheng, K., Lin, J., Yang, W. et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* **127**, 397-408.

Stamler, J. S. (1994). Redox signalling: nitrosylation and related target interactions of nitric oxide. *Cell* **78**, 931-936.

Stamler, J. S. and Hausladen, A. (1998). Oxidative modifications in nitrosative stress. *Nat. Struct. Biol.* **5**, 247-249.

Stamler, J. S., Singel, D. J. and Loscalzo, J. (1992). Biochemistry of nitric oxide and its redox activated forms. *Science* **258**, 1898-1902.

Sun, J. and Murphy, E. (2010). Protein S-nitrosylation and cardioprotection. *Circ. Res.* **106**, 285-296.

Sun, J., Morgan, M., Shen, R. F., Steenbergen, C. and Murphy, E. (2007). Preconditioning results in S-nitrosylation of proteins involved in regulation of mitochondrial energetics and calcium transport. *Circ. Res.* **101**, 1155-1163.

Taylor, E. R., Hurrell, F., Shannon, R. J., Lin, T. K., Hirst, J. and Murphy, M. P. (2003). Reversible glutathionylation of complex I increases mitochondrial superoxide formation. *J. Biol. Chem.* **278**, 19603-19610.

Tormos, K. V., Anso, E., Hamanaka, R. B., Eisenbart, J., Joseph, J., Kalyanaraman, B. and Chandel, N. S. (2011). Mitochondrial Complex III ROS Regulate Adipocyte Differentiation. *Cell Metab.* **14**, 537-544.

Wang, D., Malo, D. and Hekimi, S. (2010). Elevated mitochondrial reactive oxygen species generation affects the immune response via hypoxia-inducible factor-1alpha in long-lived Mclk1+/– mouse mutants. *J. Immunol.* **184**, 582-590.

Wardman, P. and Von Sonntag, C. (1995). Kinetic factors that control the fate of thiyl radicals in cells. *Methods Enzymol.* **251**, 31-45.

West, A. P., Brodsky, I. E., Rahner, C., Woo, D. K., Erdjument-Bromage, H., Tempst, P., Walsh, M. C., Choi, Y., Shadel, G. S. and Ghosh, S. (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* **472**, 476-480.

Winterbourn, C. C. (1993). Superoxide as an intracellular radical sink. *Free Radic. Biol. Med.* **14**, 85-90.

Yang, W. and Hekimi, S. (2010). A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol*. **8**, e1000556.

Zheng, M., Aslund, F. and Storz, G. (1998). Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**, 1718-1721.

Zhou, L., Aon, M. A., Almas, T., Cortassa, S., Winslow, R. L. and O'Rourke, B. (2010). A reaction-diffusion model of ROS-induced ROS release in a mitochondrial network. *PLoS Comput. Biol.* **6**, e1000657.

Zhou, R., Yazdi, A. S., Menu, P. and Tschopp, J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**, 221-225.

Ziegler, D. M. (1985). Role of reversible oxidation-reduction of enzyme thiols-disulfides in metabolic regulation. *Annu. Rev. Biochem.* **54**, 305-329.