STATE-OF-THE-ART REVIEW

The nexus between redox state and intermediary metabolism

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Reactive oxygen species (ROS) are not just a by-product of cellular metabolic processes but act as signalling molecules that regulate both physiological and pathophysiological processes. A close connection exists in cells between redox homeostasis and cellular metabolism. In this review, we describe how intracellular redox state and glycolytic intermediary metabolism are closely coupled. On the one hand, ROS signalling can control glycolytic intermediary metabolism by direct regulation of the activity of key metabolic enzymes and indirect regulation via redox-sensitive transcription factors. On the other hand, metabolic adaptation and reprogramming in response to physiological or pathological stimuli regulate intracellular redox balance, through mechanisms such as the generation of reducing equivalents. We also discuss the impact of these intermediary metabolism–redox circuits in physiological and disease settings across different tissues. A better understanding of the mechanisms regulating these intermediary metabolism–redox circuits will be crucial to the development of novel therapeutic strategies.

Introduction

Reactive oxygen species homeostasis

Reactive oxygen species (ROS) are highly reactive oxygen-containing chemical molecules that play a crucial role in physiological and pathological processes [1]. In cells, ROS include the superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($OH^-$),...
which have distinct biological properties including chemical reactivity, half-life and lipid solubility [2,3]. The main sources of cellular ROS are mitochondria and NADPH oxidases (or NOXs) although many other metabolic and enzymatic reactions may make smaller contributions. Mitochondria generate ROS during oxidative phosphorylation at the electron transport chain where electrons derived from NADH or FADH can directly react with oxygen or other electron acceptors and generate free radicals [4]. Other mitochondrial sources of ROS include monoamine oxidases [5]. NOXs are multiprotein complexes that catalyse the generation of ROS and protons as their primary function by transporting electrons across biological membranes, thereby leading to the reduction of oxygen to superoxide or hydrogen peroxide. There are seven mammalian members of the NOX family, with varying structure, activation mechanisms, tissue distribution and pathophysiological roles, as reviewed elsewhere [6–9].

Under normal physiological conditions, the balance between the rate and magnitude of ROS production and their elimination is finely tuned, not only at cellular but also subcellular level [10]. ROS elimination is facilitated by a complex array of nonenzymatic (e.g. glutathione [GSH]; vitamins A,C,E) and enzymatic antioxidant defence systems. Superoxide dismutases convert superoxide (O$_2^−$) to H$_2$O$_2$, while catalase reduces H$_2$O$_2$ to water and molecular oxygen. Glutathione peroxidases eliminate H$_2$O$_2$ using reducing power derived from GSH. Thioredoxins (TRXs) enable the reduction of oxidised proteins by cysteine thiol-disulphide exchange. The peroxiredoxins catalyse the reduction of H$_2$O$_2$, alkyl hydroperoxides and peroxynitrite to H$_2$O, the equivalent alcohol and nitrite, respectively. Glutathione synthetase also has a key role in ROS detoxification by synthesising the major cellular antioxidant GSH [11].

Historically, ROS were generally considered to be detrimental by-products of cellular metabolism and thought to cause toxic effects associated with several pathological conditions including inflammatory and immune system dysfunction, allergies, neurodegeneration, cardiovascular disease, diabetes, ageing and cancer [12–15]. Indeed, prolonged exposure to high ROS concentrations may lead to nonspecific damage to proteins, membranes and nucleic acids and ultimately induce cell death and disease. However, it is now well established that ROS also serve as signalling molecules to regulate physiological processes [1,2]. In fact, low concentrations of ROS have been shown to be fundamentally important for processes such as increased lifespan [16–18], physiological adaptation to exercise [19,20], cell differentiation [21], immune responses [22] and components of cardiac contractile function and vasodilatation [23]. Brief exposures to ROS trigger a prosurvival/adaptive mechanism termed hormesis [24–26]. Specific ROS-mediated signalling (redox signalling) depends crucially on the amount, type and duration of ROS generation as well as their spatial compartmentation (reviewed in [27,28]).

**Inter-relationship between redox state and metabolism**

A close connection exists between redox homeostasis and cellular metabolism under basal physiological conditions, during stress adaptation and in disease settings. On the one hand, cellular metabolism is profoundly affected by ROS signalling and/or oxidative stress while on the other, redox homeostasis is greatly influenced by metabolic alterations and metabolic reprogramming [15,29–32]. It is increasingly evident that this complex inter-relationship is an important driver of changes in cell phenotype and function and may provide novel therapeutic targets. Some of the most detailed information on how intermediary metabolism and redox homeostasis are intertwined comes from cancer studies. Metabolic alteration is a hallmark of cancer cells that was firstly described almost a century ago by Otto Warburg [33]. He observed that cancer cells increased their glucose utilisation even in the presence of oxygen (the so-called Warburg effect), as compared to normal cells which typically increase glycolysis under hypoxic conditions when oxidative phosphorylation declines as an energy source. This metabolic change was considered necessary to satisfy the increased demand for energy of rapidly proliferating cancer cells via glycolysis. It is now known, however, that the Warburg effect is considerably more complex and also involves a redirection of glycolytic intermediates into multiple anabolic branch pathways that support macromolecule synthesis, an increase in biomass and stress resistance [34–36]. Interestingly, such metabolic reprogramming towards anabolic reactions is not an exclusive feature of cancer cells and is observed in many other cell types, for example activated lymphocytes, macrophages, endothelial cells and embryonic stem cells [37–39]. Importantly, these metabolic changes are intricately linked to changes in redox homeostasis and redox signalling. However, the mechanistic underpinnings and directionalities of this relationship are incompletely understood and remain somewhat elusive. Mechanisms that may be involved include increased ROS production (both nonspecific, for example mitochondrial, and specific, for example NOXs), altered antioxidant balance [40–42], altered NADPH/NADP$^+$.
ratio (which regulates GSH regeneration), activation of transcriptional pathways that affect redox homeostasis [43,44] and direct or indirect ROS-mediated regulation of key metabolic enzymes [42,45]. We focus on some of these mechanisms in the subsequent sections of this review.

**Regulation of redox homeostasis by changes in glycolytic flux**

NADPH is critical for the maintenance of redox homeostasis and antioxidant defence through its role in GSH regeneration, as well as being required for reductive biosynthesis [46]. The predominant producer of NADPH in cells is the oxidative phase of the pentose phosphate pathway (ox-PPP), which branches off from glycolysis at the level of glucose-6-phosphate (G6P) [47]. G6P sits at the node between glycolysis, glycogen synthesis and the ox-PPP, and its fate depends on cell type and metabolic demand. In the ox-PPP, NADPH is generated when G6P is converted by glucose-6-phosphate dehydrogenase (G6PD) to 6-phosphogluconate, and this is then decarboxylated by 6-phosphogluconate dehydrogenase (6PGDH) to ribulose-5-phosphate (which feeds into nucleotide biosynthesis) [46,47] (Fig. 1). Several different mechanisms may promote an altered ox-PPP flux and modify redox homeostasis by changing NADPH levels.

G6PD is rate-limiting in the ox-PPP and its activity is regulated by the NADPH/NADP+ ratio so that, as the ratio decreases, activity increases to generate more NADPH [48–50]. G6PD activity is also regulated at transcriptional [51,52], post-translational [41,53] and intracellular localisation levels [54]. Its dimerisation is required for activity and it has been shown that the tumour suppressor p53 can directly bind to G6PD and reduce dimerisation, thereby decreasing NADPH production [41]. In tumours with p53 gene mutations [55], this inhibitory effect on G6PD is absent and enhanced PPP flux diverts glucose from bioenergetic to biosynthetic routes necessary for cancer cell growth and proliferation and facilitating increased NADPH production.

Altered regulation of the glycolytic pathway at the level of phosphofructokinase-1 (PFK1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or pyruvate kinase (PK) also impacts on flux through the PPP. PFK1 is the first committed step of glycolysis [56] and is tightly regulated: its activity is allosterically inhibited by ATP and citrate but can be activated by AMP and fructose-2,6-bisphosphate (F-2,6-BP). TIGAR (TP53-induced glycolysis and apoptosis regulator) decreases glycolytic flux downstream of PFK1 both by reducing F-2,6-BP levels and inhibiting PFK1 [40] (Fig. 1). This leads to accumulation of the G6P and F6P pools and increased flux into ox-PPP, driven also by upregulation of G6PD levels. This mechanism promotes cell survival not only in cancer models but also tissues such as brain and intestine [40,57–59]. GAPDH catalyses the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate (G3P) to 1,3-bisphosphoglycerate (1-BPG) and can be inhibited by oxidative stress, as described in section 4 [45]. This results in the accumulation of upstream metabolites and their diversion into the ox-PPP [60,61].

PK catalyses the final reaction of glycolysis, that is, conversion of phosphoenolpyruvate (PEP) to pyruvate. In cancer cells, it has been shown that a shift from the higher activity PK muscle isoform 1 (PKM1) to the lower activity PKM2 splice isoform leads to increased flux into the ox-PPP and other branch pathways [42,62]. Moreover, as with GAPDH, the activity of PKM2 is inhibited by oxidation (see section 4). Interestingly, it has also been described that the accumulation of phosphoenolpyruvate following PK inhibition leads to a direct inhibition of triosephosphate isomerase (TPI) by binding to its catalytic pocket, resulting in reduced GAPDH substrate availability and thus redirection of glycolytic intermediates into the PPP [63] (Fig. 1).

PK inhibition also promotes the accumulation of the glycolytic intermediate 3-phosphoglycerate (3-PG) which can be diverted into the de novo serine biosynthesis pathway (SBP) [64]. This intermediary pathway plays an important role in the antioxidant defence system as serine is a precursor for GSH synthesis and for NADPH production. The GSH tripeptide is generated from glutamate, cysteine and glycine; cysteine synthesis requires both serine and activity of one-carbon metabolism [65], while glycine is directly converted from serine by serine hydroxymethyltransferases (SHMTs) [66]. Serine, glycine and one-carbon metabolism are key for many biosynthetic pathways, including the one-carbon cycle that supports nucleotide synthesis (purine and thymidine), methylation reactions, epigenetic regulation and antioxidant defence [67,68]. In a recent study using quantitative flux analysis and mathematical modelling, Fan et al [69] found that in some cell types, a significant amount of NADPH is also produced from serine-driven one-carbon metabolism.

Methionine synthesis is fuelled by one-carbon units derived from the SHMT-catalysed conversion of serine to glycine. Notably, the cyclic oxidation and reduction of methionine sulfurs have been shown to function as a ROS scavenger mechanism. Provided there is sufficient NADPH, catalytic protection of specific proteins and adjacent macromolecules is mediated by an
enzymatic apparatus consisting of thioredoxin-dependent methionine sulfoxide reductases and thioredoxin reductases [70,71]. Furthermore, Campbell et al. [72] show that increased methionine levels promote an upregulation of PPP enzymes and its metabolites. Importantly, the authors show that the methionine-mediated antioxidant defence mechanism essentially depends on the induction of this metabolic reprogramming and subsequent PPP-derived NADPH generation. Recent quantitative flux studies have further defined the role of de novo serine biosynthesis and the key SBP enzyme, phosphoglycerate dehydrogenase (PHGDH). Upon pharmacological inhibition of PHGDH, Reid and colleagues describe a disruption of mass balance within central carbon metabolism and a downregulation of PPP activity [73].

Lactate dehydrogenase (LDH) is a key glycolytic enzyme that catalyses the interconversion of pyruvate to lactate and of NADH to NAD⁺. LDH is a tetrameric enzyme composed of varying combinations of LDHA and LDHB subunits. LDHA has a higher affinity for pyruvate and preferentially converts pyruvate to lactate (and NADH to NAD⁺), whereas LDHB possesses a higher affinity for lactate and...
preferentially converts lactate to pyruvate (and NAD+ to NADH) [74]. LDHA expression is increased in many human cancers thereby minimising pyruvate entry into the TCA cycle and supporting the ‘Warburg effect’, as well as increasing the NAD+/NADH ratio [75–77]. LDHA levels are also reported to increase in response to pressure overload stress in the heart and may contribute to cardiac hypertrophic growth [78]. Interestingly, LDH has recently been associated with antioxidant effects as genetic or pharmacological inhibition of LDH induces oxidative stress and inhibits tumour progression both in vitro and in vivo [79,80]. However, the mechanisms underlying these antioxidant effects still remain to be fully elucidated.

Aldose reductase catalyses the first step in the polyol pathway of glucose metabolism reducing glucose to sorbitol while utilising NADPH [81]. At physiological glucose concentrations, a very small percentage of total glucose is converted to sorbitol. However, in a hyperglycaemic environment, excessive amounts of glucose are shunted to the polyol pathway, with a concomitant decrease in NADPH and consequent reduction of cellular antioxidant defence [82]. Thus, increased flux to the polyol pathway has been linked to hyperglycaemia-induced oxidative stress and is thought to contribute to the pathogenesis of diabetic complications such as retinopathy, nephropathy and atherothrombotic cardiovascular disease [83].

Therefore, altered flux into the ox-PPP, the SBP and serine–glycine–methionine one-carbon metabolism as well as LDH isof orm shifts and flux to the polyol pathway impact significantly on redox state and redox homeostasis.

**Direct redox regulation of glycolytic and intermediary metabolism enzymes**

A large body of evidence indicates that the activity of key glycolytic enzymes may be modulated by direct redox modifications [84] (Fig. 2). This is typically mediated by the reversible oxidation of critical reactive thiol groups that profoundly impact on enzymatic functionality [85]. Such direct, structural enzyme modifications have wider impact through changes in metabolite levels, including altered flux into other pathways as discussed in the previous section.

A well-described mechanism of ROS-mediated regulation is the inhibition of GAPDH via the oxidation of catalytic site cysteine 152. GAPDH inhibition reroutes flux into the oxidative branch of the ox-PPP to promote NADPH regeneration and antioxidant defence. PK can be directly oxidised by ROS at the level of Cys residues. PK inhibition result in accumulation of the PK substrate PEP, which in turn inhibits TPI and divert glycolytic flux into the ox-PPP. PEP-dependent phosphorylation of PGAM increases PGAM enzymatic activity, decreases 3-PG levels and also increases flux into the ox-PPP. Inhibition of PK results in an accumulation 2-PG, activation of phosphoglycerate dehydrogenase (PHGDH) and flux diversion into serine, glycine and one-carbon metabolism. Abbreviations: glucose-6-phosphate (G6P); glucose-6-phosphate dehydrogenase (G6PD); fructose-1,6-bisphosphate (F-1,6-BP); glyceraldehyde-3-phosphate (GAP); dihydroxyacetone phosphate (DHAP); 1,3-Bisphosphoglycerate (1,3-BPG).

Perelta and colleagues [60] recently described that the exceptional susceptibility of GAPDH to changes in cellular redox balance relates to a highly conserved and previously unrecognised proton shuttling mechanism. Metabolomic approaches unravelled that the inhibition of GAPDH by oxidant species serves as an evolutionarily conserved, adaptive mechanism that dynamically reroutes glycolytic flux into the PPP to restore...
NADPH levels [60,90,91]. This metabolic switch is further supported by increased expression levels of different PPP enzymes in settings of increased oxidative stress (see section 5) [92].

PKM2, which in the cancer field has been shown to contribute to metabolic rewiring during the Warburg effect [42,62], is strongly influenced by oxidative stress [42]. PKM2 is the predominant PK isoform in tissues with high anabolic requirements and exists in an equilibrium of monomers, dimers and tetramers. The assembly of tetramers is required for optimal enzymatic activity [93]. The oxidation of Cys358 [42,94], Cys424 [94] and Cys326 [95] have independently been shown to inhibit PKM2 via blockage of intersubunit interaction and thus a reduced formation of active tetramers. PK inhibition results in the accumulation of its substrate, phosphoenolpyruvate, which not only inhibits TPI (see section 3) but also serves as a phosphate donor to phosphoglycerate mutase (PGAM). phosphoenolpyruvate-dependent phosphorylation of PGAM1 on the one hand increases PGAM1 enzymatic activity and on the other uncouples pyruvate production from ATP generation [96]. Van der Heiden and colleagues [96] suggested that this might be relevant in preventing the inhibition of glycolytic enzymes by high ATP/ADP ratios, thus sustaining the provision of glycolytic intermediates to support antioxidant defence (via NADPH and serine) and anabolic growth. PGAM activity is also influenced by acetylation at the highly conserved K100 residue. Importantly, oxidative stress may induce a SIRT2-mediated deacetylation of PGAM, leading to increased enzymatic activity and improved antioxidant defence [97]. Hitosugi et al [98] reported that PGAM activity also has profound effects on glycolytic intermediary metabolism through the allosteric effects of the PGAM substrate, 3-PG, and its product, 2-PG. For example, 3-PG competes with 6-PG for binding to G6PD; thus, PGAM phosphorylation and increase in activity lead to a decrease in 3-PG levels and increased ox-PPP flux. Moreover, 2-PG activates PHGDH, a key enzyme in the SBP.

While the above studies indicate direct oxidative inhibition of PKM2 as mediating an upregulation of anabolic and antioxidative glycolytic branch pathways, a recent study proposes a different indirect mechanism that contributes to cancer drug resistance [99]. These authors reported that PKM2 stability is enhanced secondary to ROS-dependent inhibition of its acetylation by P300/CREB-associated factor (PCAF) – which normally leads to lysosomal degradation of PKM2 [100]. In this study, it was suggested that these ROS derive from NOX4 and are triggered by mitochondrial dysfunction [99]. Additional work is needed, however, to determine the importance of this novel mechanism either in cancer or other cell types.

**Redox-regulated transcription factors that modulate glycolytic and intermediary metabolism**

Accumulating evidence indicates that several redox-sensitive transcription factors are involved in regulating intermediary metabolism. In this section, we discuss selected transcription factors as examples of this type of regulation.

**Nuclear factor erythroid-related factor 2 (NFE2L2 or NRF2)**

NRF2 is perhaps the best example of a transcription factor that mediates crosstalk between cellular redox homeostasis and regulation of glucose intermediary metabolism [44] (Fig. 3). In fact, NRF2 activity is on the one hand tightly regulated by cellular oxidative status, while on the other, NRF2 regulates the expression of an extensive network of antioxidant and detoxification enzymes, genes regulating intermediary metabolism and NADPH-generating enzymes [101]. Thus, oxidative stress promotes activation of NRF2 which in turn activates cytoprotective and metabolic pathways necessary to restore redox homeostasis and promote stress adaptation and cell survival [44]. The mechanisms underlying redox activation of NRF2 have been well defined and are reviewed elsewhere [102,103]. In addition to promoting the transcription of detoxification enzymes such as glutathione S-transferases (GSTs) and NAD(P)H:quinone oxidoreductase-1 (NQO1) [104], NRF2 induces many components of endogenous antioxidant systems, including the system Xc– cysteine membrane transporter and the glutamate–cysteine ligase catalytic (GCLC) and modifier (GCLM) subunits which catalyse the rate-limiting step in GSH biosynthesis [105,106]. NRF2 also promotes transcription of glutathione peroxidase (GPX) [107] and glutathione reductase (GSR) [108] which are essential for the reduction of H2O2 and of oxidised GSH (GSSH) and of enzymes important for reducing oxidised protein thiols such as thioredoxin-1 (TRX1) [107,109], thioredoxin reductase-1 (TRXR1) and sulfiredoxin-1 [107,108]. Thus, NRF2-mediated transcriptional activation of these enzymes enhances cellular resistance and protection against oxidative stress.

NRF2 also has an important role in enhancing cellular stress adaptation via modulation of intermediary metabolism, especially through enzymes that are involved in NADPH generation. In lung cancer cells,
NRF2 controls the transcription of key PPP enzymes including the NADPH-generating G6PD and PGD; enzymes of the nonoxidative arm of PPP, transaldolase (Tald01) and transketolase (TKT); and the NADPH-regenerating TCA cycle enzymes, malic enzyme 1 and isocitrate dehydrogenase 1 [110]. Interestingly, Singh et al. [111] have suggested that NRF2 may indirectly regulate the expression of PPP enzymes via modulation of miR-1 and miR-206 levels. These authors showed that in cancer cells, activation of NRF2 decreased miR-1 and miR-206 expression and increased expression of G6PD, PGD and TKT, while overexpression of miR-1 and miR-206 reduced expression of the PPP enzymes, decreased NADPH production and ribose synthesis, and significantly inhibited in vivo tumour growth in mice [111]. Therefore, NRF2 promotes diversion of glucose flux into the PPP, an increase in NADPH regeneration and purine biosynthesis [110]. Interestingly, PK expression levels are negatively regulated by NRF2 [112,113]. As a decrease in PK activity can induce a build-up of glycolytic intermediates, this could be an additional mechanism by
which NRF2 diverts glucose flux into the PPP and SBP. Regarding the link between NRF2 and purine biosynthesis, a recent study showed that knockdown of NRF2 in lung cancer cells decreased levels of phosphoribosyl pyrophosphate amidotransferase (PPAT) which catalyses the rate-limiting step in the de novo purine biosynthetic pathway and of methylentetrahydrofolate dehydrogenase 2 (MTHFD2) which provides one-carbon units for purine biosynthesis [110]. These authors also found that carbon flux from glutamine is directed towards GSH biosynthesis and the TCA cycle upon NRF2 activation [110]. The authors concluded that this is largely due to NRF2-mediated increase of GCLC and GLCM and of malic enzyme 1, which may increase glutaminolysis [110,114]. Overall, these observations suggest that NRF2 induces metabolic reprogramming during stress conditions. It should be noted that the role of NRF2 in cancer is still controversial as, depending on the context, NRF2 can act as a tumour suppressor or an oncogene [115–117]. For example, studies have shown that NRF2-deficient mice are more sensitive to carcinogenesis [118,119] and NRF2 loss has been linked to increased metastasis [120,121]. However, in certain types of tumours, constitutive activation of NRF2 or mutations in the Keap1 gene that lead to NRF2 activation have been linked to increased cancer cell survival, chemoresistance, aggressive proliferation and overall poor prognosis [122–124]. An explanation for these seemingly diverse findings may be that NRF2 promotes survival and stress adaptation not only in cancer cells but also normal cells; thus, NRF2 hyperactivation in malignant cells may contribute to aggressive evolution of the disease [110,123].

NRF2-dependent regulation of PPP enzymes and NADPH production is evident not only in cancer cells but in numerous (perhaps all) tissues, for example the liver [125], motor neurons [126], inflammatory macrophages [127] and stem cells [128]. While the activation of NRF2 by oxidative stress is well recognised, intriguingly, recent studies indicate that in certain settings the physiological or adaptive activation of NRF2 is specifically dependent upon endogenous ROS generation by NOX4. In the heart, NRF2 has been shown to play a beneficial role by mediating adaptation and protection against injury and contractile dysfunction in response to both physiological and pathological stresses [19,129,130]. In cultured cardiomyocytes, NRF2 protects cells from oxidative stress-induced injury and cell death [131], and NRF2 was reported to exert protective cardiac effects during pressure overload in vivo [129,130]. Our laboratory found that, both in the heart in vivo and in cardiac myocytes, NRF2 activation requires ROS derived from NOX4. As such, the activation of NRF2 in the heart in response to haemodynamic overload was blunted in NOX4 knockout mice, while in cardiomyocytes, NRF2 activation by neurohumoral agonists was blocked by the knockdown of NOX4 but not NOX2 [130]. Similarly, we have recently described that NOX4-ROS are an obligatory activator of cardiac NRF2 in response to physiological exercise. NOX4-mediated activation of NRF2 during physiological exercise enhances heart function and exercise performance by triggering an adaptive response that preserves redox balance and mitochondrial function [19]. Whether these beneficial effects of NRF2 activation also involve changes in intermediary metabolism requires further study but it is of interest that an increase in NOX4 expression is associated with extensive changes in metabolic proteins [132].

**Hypoxia-inducible factor 1α (HIF-1α)**

Another transcription factor that links redox homeostasis and the reprogramming of glucose metabolism is HIF-1α, which is activated by hypoxia and induces genes involved in angiogenesis, cell proliferation, differentiation and metabolism [133,134] (Fig. 3). HIF-1α is also activated by ROS, including ROS that are endogenously generated by NOX4 [135]. In cancer cells, the activation of HIF-1α promotes the reprogramming of glucose metabolism from oxidative phosphorylation to anaerobic glycolysis by increasing the expression of glucose transporters (i.e. GLUT1 and GLUT3) and glycolytic enzymes such as aldolase A, enolase 1, phosphoglycerate kinase, GAPDH and TPI [136–138]. At the same time, HIF-1α mediates suppression of mitochondrial oxidative phosphorylation by increasing the expression of LDHA and pyruvate dehydrogenase kinase 1 (PDK1, which inhibits pyruvate dehydrogenase and thereby pyruvate entry into the TCA cycle) [139,140]. Recently, HIF1α has been shown to be required for hypoxic induction of the SBP and one-carbon metabolism enzymes [141]. Samanta and colleagues showed that hypoxia-induced expression of the PHGDH, phosphoserine aminotransferase 1 (PSAT1), phosphoserine phosphatase (PSPH), SHMT2, MTHFD2 and methylentetrahydrofolate dehydrogenase 1 like genes (which belong to the serine biosynthesis and one-carbon metabolism pathways) was impaired in breast cancer cell lines where HIF1α was either silenced or pharmacologically inhibited [141]. Moreover, the authors demonstrated that activation of serine–glycine one-carbon metabolism is required for NADPH generation and maintenance of redox homeostasis under hypoxic conditions [141,142].
Interestingly, it has been described that NRF2 and HIF-1α may cooperate to promote the metabolic switch to glycolytic energy production that occurs during induced pluripotent stem cell (iPSC) reprogramming [128]. Mechanistically, during early stages of iPSC reprogramming, increased proliferation of cells induces an initial increase in mitochondrial respiration and increase in ROS production which activates NRF2. NRF2 activation channels glucose to the PPP to support increased nucleotide synthesis and the maintenance of redox homeostasis. Therefore, peaks in NRF2 activity correlate with a peak in cell proliferation, oxidative phosphorylation and PPP activation. This is then followed by an NRF2-dependent increase in HIF-1α activity and switch to glycolysis for energy production [128]. The mechanism by which NRF2 activates HIF-1α in iPSC is not yet clear although the authors suggest it may be driven by increase in TRX1 which was shown to mediate activation of HIF-1α in lung adenocarcinoma A549 cells [143]. Overall, more studies are required to dissect the inter-relationship between these transcription factors in terms of the regulation of intermediary metabolism.

**MYC proto-oncogene**

MYC is a family of proto-oncogene consisting of three members, CMYC, MYCN and MYCL, which encode for the transcription factors c-Myc, N-Myc and L-Myc, respectively. MYC is considered a master regulator of cellular metabolism and has been extensively studied in cancer cells, where is often upregulated [144]. In this setting, MYC drives the ‘Warburg effect’ by directly and indirectly upregulating the expression of glycolytic enzymes (glucose transporter protein type 1 (GLUT1), hexokinase 2, enolase, PFK, GAPDH, TPI and LDHA) [145,146] and increasing flux into anabolic pathways (Fig. 3). MYC can also induce the expression of splicing factors which favour the splicing of PKM2 over PKM1 [43], thus leading to diversion of glycolytic intermediates to glycolytic branch pathways such as the ox-PPP (see section 3) [42,62]. Another branch pathway of glycolysis regulated by MYC is the SBP, with upregulation of PHGDH, PSAT1, PSPH, SHMT1 and SHMT2 [147], enhancement of antioxidant defence and nucleotide biosynthesis, and ultimately tumour growth. MYC is involved in the regulation of cellular redox balance and response to oxidative stress by transcriptionally regulating γ-GCS, the first rate-limiting step in GSH biosynthesis [148]. Benassi et al [148] described that in human melanoma cell lines, exposure to H2O2 induces ERK-mediated phosphorylation of MYC at Ser-62 and its stabilisation, thereby enhancing its recruitment to γ-GCS regulatory regions and promoting antioxidant defence. Thus, oxidative stress can activate MYC which in turn transcriptionally regulates enzymes involved in antioxidant defence mechanisms. Many tumour cells rely on glutamine metabolism to fuel their growth and proliferation. MYC has been shown to enhance glutamine metabolism by promoting glutamine uptake through a higher expression of glutamine transporters SLClA5 and SLC7A5/SLC3A2 [149]. MYC also promotes glutamine synthesis by upregulating glutamine synthetase, which catalyses the de novo synthesis of glutamine from glutamate and ammonia [150]. Glutaminolysis is regulated by MYC through increasing the expression of the glutaminase (GLS) via transcriptional suppression of the GLS repressor miR-23a/b [151]. Interestingly, Zeng et al [152] have demonstrated that, in non-small cell lung cancer cells, NOX4 promotes glycolysis, ox-PPP for production of NADPH, and glutaminolysis for GSH synthesis, thus contributing to adaptation to oxidative stress. Mechanistically, NOX4 stimulates glycolysis and glutaminolysis via H2O2-dependent activation of the PI3K/Akt pathway and MYC stabilisation [152]. Besides cancer cells, MYC plays key biological roles in other tissues. For example, in the heart, MYC expression is rapidly induced in response to hypertrophic stimuli [153]. In a mouse model, inducible activation of MYC in adult heart induced myocyte hypertrophy, increased protein synthesis and activated a fetal gene programme. MYC activation also triggered DNA synthesis, leading to increased nuclei number per cardiomyocyte and ploidy [153]. Accordingly, Zhong and colleagues showed that cardiac hypertrophy is attenuated in cardiomyocyte-specific MYC-deficient mice after haemodynamic stress [154]. MYC has been shown to act as an important regulator of energy metabolism in the heart. Indeed, Ahuja et al [155] reported that MYC activation in adult mouse heart increases glucose uptake and utilisation, downregulates fatty acid oxidation and induces mitochondrial biogenesis. Moreover, inactivation of MYC in the adult myocardium decreased the expression of glycolytic and mitochondrial biogenesis genes and attenuated hypertrophic growth in response to haemodynamic load. Similarly, Olson et al [156] showed that in inducible, cardiomyocyte-specific MYC transgenic mice, increased expression of MYC induced cardiac hypertrophy with preserved or better function. However, in contrast with data from Ahuja and coworkers, the authors here reported an increase in fatty acid oxidation and a concomitant decrease in glucose utilisation upon MYC activation. Interestingly, they also reported...
changes in intermediary metabolism as myocardial O-GlcNAcylation was increased in MYC overexpressing mice. Although these studies overall indicate that MYC activation affects myocardial substrate utilisation and potentially can influence glycolytic intermediary metabolism, further work is necessary to clarify these discrepancies, establish the relationship between changes in redox state and MYC activation and elucidate how the MYC-mediated shifts in substrate utilisation are related to hypertrophic response in the stressed heart.

Activating transcription factor 4 (ATF4)

The integrated stress response (ISR) is an evolutionarily conserved adaptive pathway driven by ATF4, which is activated in response to numerous stress conditions such as oxidative stress, hypoxia, glucose or amino acid starvation, endoplasmic reticulum (ER) stress and infection [157]. During the initiation of the ISR, the eukaryotic translation initiation factor 2 alpha (eIF2α) is phosphorylated by eIF2α kinases to mediate a decrease in global protein synthesis but enhanced translation of a subset of genes that include ATF4 [157,158]. This transcription factor induces a wide network of metabolic genes, notably many that are involved in amino acid metabolism and antioxidative stress responses [159]. Among the genes regulated by ATF4 are many involved in protein translation, amino acid import, the SBP and the one-carbon methylation cycle [159–161]. ATF4 also activates the transcription factor ATF5 [162], which is important in the mitochondrial unfolded protein response – an adaptive response that serves to preserve mitochondrial function [163]. In some cancer types, ATF4 expression is deregulated and correlates with more aggressive phenotype and poor prognosis [164]. Conversely, in certain conditions of chronic stress, ATF4 activation can induce apoptosis, which may be related to its induction of the transcription factor CHOP [165]. NRF2 has been reported to regulate ATF4 both by direct transcriptional activation [166,167] and by heterodimerisation to induce gene expression [168]. DeNicola et al [169] showed that in non-small cell lung cancer cell lines, NRF2 regulates serine biosynthesis via ATF4-mediated transcription of PHGDH, PSAT1 and SHMT2 to support antioxidant defence mechanisms and nucleotide biosynthesis (Fig. 3). Although the mechanism by which NRF2 indirectly regulates ATF4 transcriptional activity remains unknown, the authors suggest that the newly described indirect activation of serine biosynthesis via ATF4 combined with NRF2-mediated activation of the PPP (which collectively promote NADPH regeneration and nucleotide production) correlates with poor prognosis in non-small cell lung cancer patients [169].

Recently, our laboratory has shown that ATF4 activation during ER stress or during heart ischaemia–reperfusion is enhanced by NOX4 through an intricate and spatially confined redox signalling mechanism [170]. During ER stress conditions, NOX4 located at the ER forms a complex with the protein, GADD34, which binds to and targets a subfraction of cellular serine–threonine protein phosphatase-1 (PP1) to this location. The GADD34-PP1 complex normally acts to dephosphorylate eIF2α and limit ATF4 translation; however, NOX4 mediates a redox inhibition of PP1 at the ER and therefore enables sustained eIF2α phosphorylation and ATF4 activation. The atomic mechanism underpinning NOX4 inhibition of PP1 is the oxidation of its metal centre, in contrast to the cysteine oxidation that underlies redox inhibition of tyrosine phosphatases. In the heart, the activation of this NOX4-eIF2α-ATF4 pathway robustly enhances cell survival and mediates cardiac protection during ischaemia–reperfusion injury, while in the kidney, it enhances organ function in a model of acute tubular necrosis [170]. Interestingly, we also found that NOX4 itself is a transcriptional target of ATF4 so that there is a positive feedback loop between the two proteins [170]. This finding may also explain why NOX4 is induced in diverse stress settings. A study from a different group also reported a NOX4-dependent activation of ATF4 in the heart during starvation or energy deprivation [171]. Whether the actions of ATF4 on intermediary metabolism are involved in the above effects was not investigated in these studies. However, we have recently reported that a NOX4-mediated activation of ATF4 in the heart or in cultured cardiomyocytes induces an increase in the level of glutamine fructose-6-phosphate aminotransferase 1 (GFAT1), the rate-limiting enzyme of the hexosamine biosynthesis pathway (HBP), a branch pathway of glycolysis [172]. NOX4-ATF4-mediated modulation of GFAT1 is associated with an increase in the levels of O-GlcNAcylated proteins in the heart as a marker of increased HBP activity. This study showed that O-GlcNAcylation of the fatty acid transporter CD36 was linked to an increase in fatty acid oxidation in these hearts [172]. However, since O-GlcNAcylation of numerous proteins was altered, there are likely to be many other effects on metabolism.

The above studies indicate that redox-regulated transcription factors have a major impact on intermediary metabolism both in cancer and in noncancerous tissues during various physiological and pathological conditions.
stress conditions. An intriguing finding from recent studies is that the ROS-generating protein, NOX4, appears to be specifically linked to the activation of each of the four transcription factors discussed above. Our work suggests that in several cases, this represents a specific signalling and regulatory role of NOX4 rather than nonspecific effects related to any source that generates ROS. This hypothesis is supported by recent findings that NOX4 is localised predominantly to ER–mitochondrial contact sites (MAM) [173], which are hotspots for metabolic regulation, as well as by the complex mechanism involved in NOX4-mediated activation of ATF4 (suggestive of a regulatory role) [170].

**Bidirectional control of intermediary metabolism and redox homeostasis: Relevance to physiology and disease**

In this final section, we consider several examples of cell types or disease settings where the interplay between redox homeostasis and intermediary metabolism has been studied and is revealing important insights that may extend to more general concepts.

**Cancer**

The inter-relationship between intermediary metabolism and redox homeostasis is nowhere better illustrated than in cancer biology. The hyperproliferative cancer phenotype is supported and sustained by the bidirectional effects of altered redox and intermediary metabolism. Cancer cells generally feature a more oxidative environment (related to aberrant signalling pathways and oncogene-driven rewired metabolic pathways) which may compromise cell viability. In some cancer cells, mitochondrial respiration may be elevated and associated with impaired coupling efficiency and resultant ROS production due to increased electron leak [34,174]. However, the upregulation of multiple antioxidant pathways (e.g. enhanced NADPH generation via the PPP and other pathways, increased NRF2, increased GSH synthesis) provides a survival advantage [169,175–177]. At the same time, many of the changes in intermediary metabolism support the proliferative state through increased generation of cellular ‘building blocks’ for biomass generation in the form of nucleotides, amino acids and cell membrane constituents. The studies discussed in earlier sections indicate that some of these changes in intermediary metabolism are themselves driven by altered redox signalling. An involvement of NOX4 in such signalling in many settings is supported by studies showing that an increase in NOX4 levels is associated with (and in some cases causatively linked to) increased survival of cancer cells such as melanoma [178], non-small cell lung cancer [152], prostate cancer [179], colorectal cancer [180], gastric cancer [181] and glioblastoma [182]. Understanding the link between ROS homeostasis and metabolism in the adaptation towards a proliferative phenotype has led to a renewed concept in cancer therapeutics whereby targeting metabolic pathways to affect ROS metabolism rather than directly targeting ROS generation itself may help to reduce cancer cell survival and improve patient prognosis [30].

**Stem cells**

The nexus between redox and intermediary metabolism is increasingly recognised to play a key role in stem cell biology including proliferation, pluripotency and differentiation. Recent studies indicate that metabolic control of redox signalling can determine the stemness of embryonic stem cells (ESC). ESC are generally characterised by reduced ATP levels and a higher reliance on glycolysis for energetic requirements [183]. This is associated with increased activation of glycolytic branch pathways such as the PPP, with consequent increase in antioxidant status and anabolic metabolism. This may serve to sustain proliferative potential, protect against DNA damage and maintain genomic stability and stemness characteristics. Less reliance on mitochondrial respiration also produces less ROS. A similar phenomenon is observed in iPSC—whereby ESC which exhibit a relative reduction in oxidative metabolism and a shift towards glycolysis [184]. The effects of ROS have also been studied in adult stem cells, particularly in haematopoietic stem cells (HSC) which differentiate into myeloid and lymphoid progenitors. Ito and colleagues [185] showed that in ataxia telangiectasia mutated (ATM) knockout mice, ROS levels were increased in HSC and the adult HSC pool was defective, thus demonstrating that self-renewal capacity of HSC depends on ATM-mediated control of oxidative stress. Mechanistically, in Atm<sup>−/−</sup> mice, an increase in ROS levels induced HSC-specific phosphorylation and activation of p38MAPK, which impaired the self-renewal capacity of HSC and resulted in defects in stem cell function in vivo [186]. Interestingly, Juntilla et al [187] demonstrated that low basal levels of ROS are required for normal HSC functions. HSC from Akt1/2 double-knockout mice exhibit decreased intracellular ROS levels and defects in long-term haematopoietic reconstitution after transplantation. Importantly, pharmacological increase of ROS levels rescued the differentiation defect in Akt1/2 double-
deficient mice, thus demonstrating the importance of maintaining low basal ROS levels for HSC proliferation and haematopoietic differentiation. Therefore, a fine balance of ROS levels is crucial to maintain normal functions of HSC, as changes in intracellular ROS levels can determine HSC fate either by promoting proliferation and self-renewal or haematopoietic differentiation.

Immune cells

Redox homeostasis and intermediary metabolism are closely intertwined in processes that regulate immune cells, for example T cells [31]. T cells are central regulators of antigen-specific adaptive immune responses and rapidly switch from a naïve to an active state upon stimulation of antigen receptors, followed by rapid proliferative expansion. It has become evident that T cells undergo profound metabolic reprogramming to fulfil bioenergetic and biosynthetic demands during activation, the precise pattern of which differs depending upon the phenotypic and functional subsets [188–190]. For example, activated effector T cells (Teffs) switch from oxidative phosphorylation to aerobic glycolysis, activation of the PPP and glutaminolysis to sustain their biological functions, whereas regulatory T cells (Tregs) rely predominantly on fatty acid oxidation and oxidative phosphorylation [191]. Intracellular ROS levels also play a key role in regulating T-cell biological functions [192,193]. Initial studies showed that antioxidant treatment inhibited the proliferation of T cells [194,195], and pharmacological or genetic disruption of mitochondrial ROS production reduced T-cell activation in vitro and in vivo [193,196]. Besides mitochondria, ROS generated by NOX2 have also been implicated in maintaining T-cell activation [197,198]. Furthermore, the transient generation of physiologically relevant levels of ROS has been shown to be necessary for T-cell activation and function [196,199,200] via the modulation of a wide spectrum of redox-sensitive transcription factors (e.g. NF-κB, AP-1, and GATA-binding protein 3) [199,201–203]. However, it remains to be definitively established whether the requirement for ROS involves effects on intermediary metabolism.

Endothelial cells

The relationship between redox homeostasis and glycolytic intermediary metabolism is also evident in endothelial cells (EC). Under normal physiological conditions, EC primarily rely on glycolysis as their ATP source, and glycolytic pathways are essential for EC proliferation and angiogenesis. In fact, the Carmeliet laboratory demonstrated that PFKFB3 regulates EC sprouting — involving proliferation, migration and branching — indicating that glycolysis has a central role in the formation of new blood vessels [204]. In diabetes, the inter-relationship between redox homeostasis and intermediary metabolism is disrupted and may contribute to disease pathophysiology, for example EC dysfunction, vasoconstriction, pro-inflammatory and prothrombotic effects [205,206]. Persistent hyperglycaemia is accompanied by an increase in intracellular ROS levels through multiple mechanisms, including increased protein kinase C-dependent NOX activation [207,208] and increased mitochondrial ROS production [209]. Interestingly, this may be associated with altered flux into ancillary pathways of glycolysis, for example a downregulation of G6PD levels and reduced PPP activity — which leads to lowering of NADPH levels and contributes to a further oxidised state [210,211]. On the contrary, flux through the HBP is increased in diabetic EC and leads ultimately to excessive protein O-GlcNAc modification and impairment of angiogenesis [212].

Heart

An inter-relationship between oxidative metabolism, ROS production and a shift from cardiomyocyte proliferation to cell cycle arrest has been identified in the postnatal heart. Cardiomyocytes proliferate for a limited period after birth (approximately 7–10 days in the mouse heart) but then undergo cell cycle arrest, with subsequent growth occurring by the enlargement of individual cells. Puente et al. [213] demonstrated in a landmark study that the postnatal cell cycle arrest of cardiomyocytes in the mouse heart is driven by the shift from glycolytic to oxidative metabolism consequent upon an increase in oxygen delivery to the heart after birth. These authors found that the switch to oxidative metabolism was accompanied by an increase in mitochondrial ROS production and the activation of the DNA damage response, resulting in cell cycle arrest [213]. The postnatal cardiomyocyte proliferative window could be prolonged by maintaining perinatal hypoxia (which was accompanied by lower ROS levels) or by the scavenging of mitochondrial ROS. This effect appears analogous to the effect of hypoxia on the proliferative potential of adult stem cells [183]. Cardiomyocyte differentiation (with loss of proliferative capacity) is also reported to be favoured by a reduction in activity of glycolytic intermediary branch pathways that support NADPH production, resulting in a more oxidised redox state [214]. The induction of cell cycle arrest by elevated ROS at first sight appears
to contrast to studies that have found a ROS-dependent increase in cardiomyocyte cell cycling [215]; however, this difference is likely to be related to the source and/or location of ROS generation [27,28,216].

Beyond the setting of the developing heart, bidirectional crosstalk between redox homeostasis and intermediary metabolism may also have important effects in the adult heart. The heart is characterised by its high-energy requirements, related to its continuous contractile activity. The vast majority of the ATP for contractile function is derived through mitochondrial oxidative phosphorylation, a process that may generate some ROS through electron leak in the respiratory chain. Normal mitochondrial function therefore requires efficient scavenging of ROS, based on intricate antioxidant machinery [217]. Interestingly, we recently found that a NOX4-ROS-dependent activation of NRF2 is required to maintain the mitochondrial antioxidant machinery during physiological exercise and thereby maximise exercise performance [19]. NADPH derived from the PPP function is thought to be important in maintaining redox homeostasis particularly during pathological settings such as ischaemia–reperfusion [218]. Significant alterations in intermediary metabolism as well as redox state are reported in the failing heart although the contributions to disease pathophysiology remain to be fully elucidated. It has been shown that inhibition of the PPP increases oxidative stress and worsens heart failure [219], whereas driving intermediary metabolism towards the PPP can ameliorate oxidative stress [220]. It was also reported that ATF4-dependent metabolic changes may augment cardioprotective antioxidant mechanisms during haemodynamic overload [221] or cardiac ischaemia [222]. More recently, we found that NOX4-dependent activation of ATF4 contributed to alterations in intermediary metabolism that were associated with cardioprotective effects during chronic haemodynamic overload [172]. In the latter study, ATF4 activation led to an increase in flux through the HBP and mediated an augmentation of fatty acid oxidation secondary to increased O-GlcNAcylation of the fatty acid transporter, CD36. Increased activity of glycolytic branch pathways may also contribute to the process of cardiac remodelling whereby the heart’s structure and function change in response to chronic alterations in workload [223,224]. Examples of such effects include the process of myocardial recovery from failure, induced by mechanical unloading of the heart.

It is important to note, when using mouse models, that the widely used inbred C57BL/6J strain is characterised by a loss-of-function deletion of the nicotinamide nucleotide transhydrogenase (Nnt) gene [225]. Nnt is an inner mitochondrial membrane protein that couples the protonmotive force across the inner membrane to hydride transfer from NADH to NADP⁺, thereby forming NADPH (which has a key role in maintaining mitochondrial antioxidative capacity). In

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Fig. 4. Intermediary metabolism–redox circuits are critical for maintenance of cellular physiological functions. Intermediary metabolism and redox signalling are intimately coupled to form dynamic intermediary metabolism–redox circuits in cells. ROS-mediated oxidative post-translational modifications (oxPTMs) and transcriptional regulation of glycolytic and intermediary metabolism contributes to rewiring of metabolic pathways to activate antioxidant systems and regulate many physiological processes across different cell types and tissues.
response to stresses such as pressure overload of the heart, Nnt can act in reverse mode to maintain NADH levels but deplete NADPH pools and antioxidant capacity [226]. C57BL/6J mice have been shown to exhibit protection against oxidative stress and heart failure because this reverse mode Nnt activity is absent in these animals [225,227]. Given that Nnt is expressed in many tissues, its physiological functions will be unclear in studies that solely utilise C57BL/6J mice.

Conclusion

A growing body of evidence supports the concept that intermediary metabolism and ROS signalling are inextricably intertwined and represent critical determinants of cellular physiology. Beyond a detrimental role when produced in excess, ROS are essential signalling molecules that regulate many critical physiological processes [216]. Metabolic ‘rewiring’ is a key feature of many physiological processes (e.g. stem cell proliferation, immune cell activation), stress adaptation and pathophysiological conditions (e.g. cancer). Alterations in glycolytic intermediary metabolism are especially important in regulating redox state and generating building blocks for cell growth and proliferation. In this review, we have discussed the multilevel regulation between intermediary metabolism, redox state and redox signalling. Fine control and maintenance of these intermediary metabolism–redox circuits is crucial for normal physiological functions, with disruption contributing to the development of disease (Fig. 4). Interestingly, it is increasingly apparent that there is significant conservation of these circuits and mechanisms across many different tissues, hinting at their fundamental biological importance. It is also highly likely that perturbations of these intermediary metabolism–redox circuits have important roles in physiological and pathological stress settings. Unravelling these changes and their specific contributions to pathophysiology in the types of cells and disease settings discussed in this review holds significant promise in revealing novel therapeutic targets. This task will benefit from the application of state-of-the-art approaches, such as the assessment of spatially localised changes in ROS levels, redox state and redox signalling and of metabolic fluxes. While the technical challenges in this complex field are significant, the prize is also likely to be large.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

AZ and AMS conceived the manuscript. AZ, AAN, RRO and CXCS searched the literature and wrote the first draft. AZ and AMS edited and revised the manuscript.

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