nature reviews molecular cell biology

https://doi.org/10.1038/s41580-023-00649-0

Review article

Check for updates

FOXO transcription factors as mediators of stress adaptation

Maria J. Rodriguez-Colman[1], Tobias B. Dansen[1] & Boudewijn. M. T. Burgering[1,2]

Abstract

The forkhead box protein O (FOXO, consisting of FOXO1, FOXO3, FOXO4 and FOXO6) transcription factors are the mammalian orthologues of *Caenorhabditis elegans* DAF-16, which gained notoriety for its capability to double lifespan in the absence of *daf-2* (the gene encoding the worm insulin receptor homologue). Since then, research has provided many mechanistic details on FOXO regulation and FOXO activity. Furthermore, conditional knockout experiments have provided a wealth of data as to how FOXOs control development and homeostasis at the organ and organism levels. The lifespan-extending capabilities of DAF-16/FOXO are highly correlated with their ability to induce stress response pathways. Exogenous and endogenous stress, such as cellular redox stress, are considered the main drivers of the functional decline that characterizes ageing. Functional decline often manifests as disease, and decrease in FOXO activity indeed negatively impacts on major age-related diseases such as cancer and diabetes. In this context, the main function of FOXOs is considered to preserve cellular and organismal homeostasis, through regulation of stress response pathways. Paradoxically, the same FOXO-mediated responses can also aid the survival of dysfunctional cells once these eventually emerge. This general property to control stress responses may underlie the complex and less-evident roles of FOXOs in human lifespan as opposed to model organisms such as *C. elegans*.

Sections

[1]Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, Netherlands. [2]Oncode Institute, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht, Netherlands. e-mail: B.M.T.Burgering@umcutrecht.nl

Introduction

Life and reproduction are tightly coupled to environmental conditions. In *Caenorhabditis elegans,* for instance, worms that have an impaired *daf-2* gene not only have a very long lifespan and improved stress resistance but also develop slower and have a lower fecundity[1]. These long-lived worms are therefore rapidly outcompeted by wild-type worms when grown on the same plate under plentiful, unstressed conditions[2–4]. But under adverse conditions, long-lived worms easily outlive wild-type worms[5]. One could argue that for most cells in the metazoan body, evolutionary success does not depend on propagation of their own DNA, but on that of the DNA of the germ cells and that the ability of these cells to adapt to stress, not to proliferate, is what contributes to evolutionary fitness of the organism. In fact, aberrant proliferation of somatic cells is a hallmark of cancer. Hence, adaptation to handle stress is a key factor in evolutionary fitness, especially in metazoans such as humans.

At the end of the previous century, it became clear that the extended lifespan and stress resistance of the mutant *daf-2* worm depend on the *daf-16* gene[6]. This discovery had major implications for the research on ageing: it meant that lifespan is not fixed but can be modulated by genetic factors[5]. The prospect of potentially being able to extend healthy human lifespan and to ward off age-related disease spurred further research into the *daf-2*–*daf-16* network. *daf-2* encodes an insulin receptor (InsR) orthologue, the activity of which inactivates the DAF-16 transcription factor through nuclear exclusion that depends on a signalling pathway consisting of the lipid kinase AGE-1 (phosphoinositide 3-kinase (PI3K) in mammals) and the serine/threonine kinase AKT (protein kinase B, also known as AKT in mammals). The forkhead box protein O (FOXO) transcription factors consisting of FOXO1, FOXO3, FOXO4 and FOXO6 (Box 1) represents the mammalian orthologues of *C. elegans* DAF-16. Although the InsR–PI3K–AKT–FOXO cascade is entirely conserved in most eukaryotes, only in primitive eukaryotes such as *C. elegans* the changes in pathway activity clearly affect lifespan. In humans, a link between pathway activity and lifespan is, 30 years after the discovery that *daf-16* can double worm lifespan[6], at present less evident. Several genetic studies identified SNPs within the FOXO3 gene locus that were significantly enriched in centenarians[7]; the functional consequences of these SNPs on FOXO3 function are not yet resolved. A recent study showed that one of these FOXO3-associated SNPs results in the expression of a shorter FOXO3 allele that affects muscle glucose handling[8]. Although the association of this SNP with longevity could indeed be in line with better glucose tolerance, it is not clear whether and how lifespan is indeed causally affected by this allele.

Nevertheless, similar to *daf-16*, mammalian FOXOs are implicated in stress adaptation. A diverse range of stress conditions, for example, nutrient stress, redox stress and genotoxic stress, all activate FOXOs and start a transcriptional programme that counteracts these stresses to restore homeostasis[9]. When homeostasis cannot be restored, FOXOs prevent propagation of damage by inducing a permanent cell cycle arrest or apoptosis, preventing, for instance, the onset of cancer. FOXOs also ensure stem cell maintenance, allowing for efficient homeostatic tissue turnover[10]. Hence, FOXOs confer the ability to cope with various adverse conditions and thereby ensure that cells, organs and eventually an organism can be resilient and maintain homeostasis (Fig. 1a). Considering this suite of FOXO-dependent effects promoting resilience, it is puzzling that, for instance, multiple allele germline *Foxo* knockout mice show no overt shortening of lifespan[11]. Possibly, embryonic adaptation or the controlled environment of laboratory mouse husbandry precludes a role of FOXOs in lifespan to become apparent. Nevertheless, dietary restriction in certain mouse strains results in increased lifespan and this appeared to depend on FOXO3, whereas haploinsufficiency of FOXO1 diminishes the antineoplastic effect of dietary restriction in mice but not lifespan[12]. It is also clear that the FOXO function is involved in delaying the onset of age-related diseases, as loss of FOXO3 function leads to a premature decline of neural cells[13]. Also, FOXO3 deficiency leads to aberrant cortical astrocyte activation, and altered lipid metabolism, supporting a protective role of FOXOs against brain ageing[14].

In this Review, we first briefly discuss mechanism (mechanisms) that controls FOXO activity and their transcriptional output. We then focus on how FOXOs maintain cellular, organ and organismal homeostasis through their roles in cell cycle, metabolism, redox signalling and stem cell function.

Control of FOXO activity

The control and downstream output of FOXOs have been described extensively in several excellent reviews[9,15–17]. A common theme in the FOXO-dependent regulation of adaptation to stress is that the activity of FOXOs is regulated by several upstream signalling cascades that are switched on or off, dependent on the stress that is encountered. Loss of insulin signalling is a signal for limited glucose availability and nutrient stress and relieves AKT-dependent inhibition of FOXOs. Stress-activated protein kinases such as JNK and p38 mitogen-activated protein kinase (MAPK) are activated by oxidative stress through redox signalling (Supplementary Box 1) and activate FOXOs by direct phosphorylation. Of note, redox signalling also has JNK or p38 MAPK-independent regulatory effects on FOXOs, as discussed further subsequently. FOXOs are also activated in response to heat and genotoxic stress (Fig. 1a). Active FOXOs subsequently induce transcription programmes that help to resolve or adapt to the encountered stress. Some of these programmes, such as cell cycle arrest, are universal to FOXO-dependent stress adaption, whereas others are more stress-type or context-specific. Specific combinations of post-translational modifications (PTMs), which depend in turn on the type of stress encountered, may aid in the transcription of specific target genes[18].

FOXO activity can be controlled at various levels (Fig. 2): first, the level of FOXO protein expression. Second, PTM-regulated nucleo-cytoplasmic shuttling determines whether FOXOs have access to DNA. Third, open or closed chromatin determines the ability of FOXOs to access target genes. Finally, transcription co-regulators determine FOXO transcriptional activity.

The level of FOXO expression is balanced by production and degradation at the level of both mRNA and protein. FOXO mRNA expression is controlled by several transcription factors, including FOXO itself, as FOXO3 can regulate the expression of FOXO1 and FOXO4 (refs. 12,19). Several microRNAs have been described that regulate the mRNA expression of FOXO (reviewed elsewhere[20]).

E3 ubiquitin-protein ligase MDM2 activates FOXOs by monoubiquitylation, but MDM2 and SKP2 (a subunit of the SCFSKP2 ubiquitin ligase) (reviewed recently[21]) can also polyubiquitylate FOXOs and thus target them for proteasomal degradation[22], which occurs upon AKT-mediated phosphorylation of FOXOs (reviewed elsewhere[23]). The E3 ligase activities of MDMX[24] and p300 (ref. 25) have been shown to have a role in regulating the switch from MDM2-dependent monoubiquitylation to polyubiquitylation of p53, and a similar regulatory mechanism could be in place for FOXOs. AKT can also phosphorylate MDM2, resulting in the nuclear translocation of MDM2. This may result in MDM2-mediated ubiquitylation and subsequent

Box 1

FOXO isoforms and their roles

The freshwater polyp *Hydra vulgaris* expresses one forkhead box (FOXO) allele[139], whereas mammals express four FOXO isoforms and, in *Caenorhabditis elegans*, multiple DAF-16 isoforms are expressed, albeit from a single genetic locus[207]. This raises the question whether different FOXO isoforms mediate the same function, yet their role is determined by context, or that FOXO function has diverged between these isoforms. In mammals, the FOXO family consists of FOXO1, FOXO3, FOXO4 and FOXO6. Although regulation of FOXO1, FOXO3 and FOXO4 shows a high degree of similarity, FOXO6 appears to differ, most importantly FOXO6 does not appear to shuttle between nucleus and cytosol. Many studies acclaim a FOXO isoform-specific function, but studying possible isoform-specific function is not straightforward and unfortunately confounded by several experimental issues. First, FOXO3 has been shown to regulate FOXO1 expression[19], indicating that manipulation of one isoform may also affect the other isoforms. Second, FOXOs induce complex feedback signalling. FOXO transcriptionally regulates various phosphoinositide 3-kinase (PI3K) signalling intermediates, including the insulin receptor, PI3K and mTORC2 (ref. 208) and especially the latter appears in many cell types to mediate FOXO-dependent PI3K and AKT activation and consequent inhibition of FOXO activity. Third, the FOXO function is in part determined by the expression level. In *C. elegans*, the DAF-16 protein level impacts on the cellular location whereby low DAF-16a expression results in mostly nuclear localization and high DAF-16a expression results mostly in cytosolic localization[207]. This expression-dependent cellular location will affect regulation by upstream signalling and thus may underlie apparent isoform-specific regulation by upstream signalling. Also, in mice, there may be a dosage-dependent effect in FOXO function. In contrast to *Foxo1* null mice, *Foxo3* and *Foxo4* null mice did not show a haemangioma phenotype; deletion of both *Foxo3* and *Foxo4* in a *Foxo1* null background did, however, increase severity of the haemangioma phenotype, demonstrating that FOXOs have overlapping roles as a tumour suppressor[103]. As all FOXO isoforms bind to the same canonical DNA sequence, differential gene regulation by specific FOXO isoforms probably requires some additional form of regulation such as the recruitment of isoform-specific interaction partners[35].

Taken together, the possibility of dosage effects outlined earlier warrants caution in interpreting mammalian data using overexpression and/or incomplete short hairpin RNA-mediated knockdown to indicate isoform-specific function. Irrespective, in many cases, the possibility cannot be excluded that functions are similar yet tissue-specific expression of isoforms, or differential timely expression, or even relative level of isoforms within one cell underlies these acclaimed isoform-specific phenotypes. In this Review, we therefore use the generic term FOXO to indicate any or all the FOXO isoforms. When a specific FOXO isoform is mentioned, this is because a cited study focused on that isoform. We do not exclude that similar regulation or effects can be attributed to the other FOXO isoforms as well.

proteasomal degradation of FOXOs, similar to what has been described for p53 (refs. 26,27). So far, USP7 is the only deubiquitylating enzyme (DUB) that has been described to deubiquitylate FOXOs[28], and this is conserved in *C. elegans*[29].

Nucleo-cytoplasmic shuttling likely represents the most critical step in the control of FOXO activity. Nuclear export of FOXOs requires AKT-mediated phosphorylation and consequent binding of 14-3-3 scaffold protein to FOXOs[30,31] and involves the exportin CRM1 (ref. 32). The importin responsible for nuclear entry following inhibition of insulin signalling is unknown, but FOXOs harbour an nuclear localization sequence, and nuclear accumulation may be the mere result of loss of AKT–14-3-3-dependent export. Following cellular redox stress, nuclear import of FOXOs correlates with monoubiquitylation[28], phosphorylation mediated by JNK and p38 MAPK[33] and disulfide-dependent binding to the importins TNPO1 (ref. 34) (for FOXO4), IPO7 and IPO8 (for FOXO3)[35].

Several other PTMs that modify the activity of FOXOs have been described, and most converge on the regulation of nucleo-cytoplasmic shuttling. These PTMs include phosphorylation by a suit of other kinases besides the aforementioned AKT, JNK and p38 MAPK, as well as acetylation, ubiquitylation and methylation. Many of the enzymes catalysing addition and removal of these PTMs have been identified. Several reviews have summarized the role of PTMs in the regulation of FOXO, and we refer to these for more detail[16,17] (Supplementary Box 2 and Supplementary Tables).

Following nuclear entry, the ability to regulate gene expression requires binding to DNA. A FOXO-binding sequence can be present in chromatin-condensed or chromatin-open genomic regions. FOXO1, similar to some other forkhead transcription factors, can act as the so-called pioneering factor and directly bind to open condensed chromatin to affect transcription in an ATP-independent process[36,37]. This is a common trait of FOXO factors, which contain a winged helix DNA-binding domain that mimics the 3D structure of the histone H5 (ref. 37).

Access to DNA in open chromatin regions is limited by the ability of FOXOs to adopt an auto-inhibited conformation, in which the CR3 domain of FOXOs folds back onto the DNA-binding domain thereby inhibiting DNA binding[38,39]. β-Catenin, the canonical transcriptional co-activator downstream of WNT signalling, can bind to the CR3 and part of the CR2 domain and this releases the auto-inhibition, allowing for DNA binding[39]. The forkhead domain has a defined 3D structure (reviewed elsewhere[40]) and mediates DNA binding to the consensus FOXO DNA-binding site. FOXOs bind to DNA mostly as monomers, but recently FOXO dimer binding to specific palindrome sequences has also been suggested[41]. The structure of the DNA-binding domain has been determined for all FOXO isoforms and reveals only subtle isoform-specific differences, the relevance of which still needs to be determined[42].

Similar to many other transcription factors, FOXOs are largely intrinsically disordered proteins (apart from the DNA-binding domain).

This provides structural flexibility that allows FOXOs to interact with many different proteins to integrate various signalling inputs into specific outcomes. Interactions involving unstructured regions gain high affinity by using multiple low-affinity small interfaces[43] that interact in a process termed coupled folding and binding[44]. For FOXOs, this mode of binding through coupled folding has been resolved for p300/CBP, p53, TNPO1 and β-catenin. Under oxidizing conditions, the non-covalent, medium-affinity interaction between FOXOs and p300 (ref. 45), TNPO1 (ref. 34), IPO7 and IPO8 (ref. 35) and many others becomes locked by a covalent but reversible cysteine disulfide bridge (resulting from cysteine oxidation)[46] (Supplementary Box 1 and the section 'Reciprocal control of FOXO, ROS and redox signalling').

Transcription regulation by FOXOs

FOXOs regulate gene transcription by binding transcription regulators, such as histone acetyl transferases including p300/CBP[47], PCAF[48] and KAT5/tip60 (ref. 49) and several deacetylases such as SIRT1 (refs. 47,50), SIRT2 (ref. 51), SIRT3 (ref. 52) as well as HDAC3 (ref. 53). The interaction between FOXOs and these acetyltransferases and deacetylases, on the one hand, enable FOXOs to drive gene transcription through regulation of histone acetylation and subsequent chromatin remodelling. But, on the other hand, these enzymes also regulate acetylation of lysines within the FOXO DNA-binding region, which is reported to alter the affinity of FOXOs for DNA and has a role in transcriptional target selection[54].

In *C. elegans,* DAF-16 interacts with the switch/sucrose non-fermentable (SWI/SNF) chromatin remodelling complex[55], which likely is also the case for mammalian FOXOs (discussed in ref. 56). Transcription elongation is controlled by the p-TEFb complex, and a screen for FOXO regulators in flies identified cyclin-dependent kinase 9 (CDK9), a component of p-TEFb complex to regulate FOXO activity[57]. The interaction between FOXO and BRD4, another p-TEFb complex member, has been described to regulate human breast cancer cell resistance to AKT inhibitors[58].

Meta-analysis of chromatin immunoprecipitation (ChIP) followed by sequencing experiments combined with gene expression of FOXO-regulated genes in mammalian cells, *C. elegans* and *Drosophila* revealed an overview of conserved and 'canonical' FOXO transcriptional targets[59]. However, besides these archetypical FOXO gene targets, there appears to be limited overlap in the many genes regulated downstream of active FOXO comparing different conditions (cell line, treatment, organism and so on). In part, this may be due to the role of FOXOs in the stress response: distinct cell types may deal with a certain stress in a different way. As described in the previous section, FOXO-mediated gene expression can be regulated by multiple cues and thereby FOXOs can respond in a context-dependent manner to the type and amplitude of stress. Here we discuss emerging concepts in transcription regulation that we consider relevant to further our understanding as to how FOXOs mediate cell resilience and restoration of homeostasis in the event of stress.

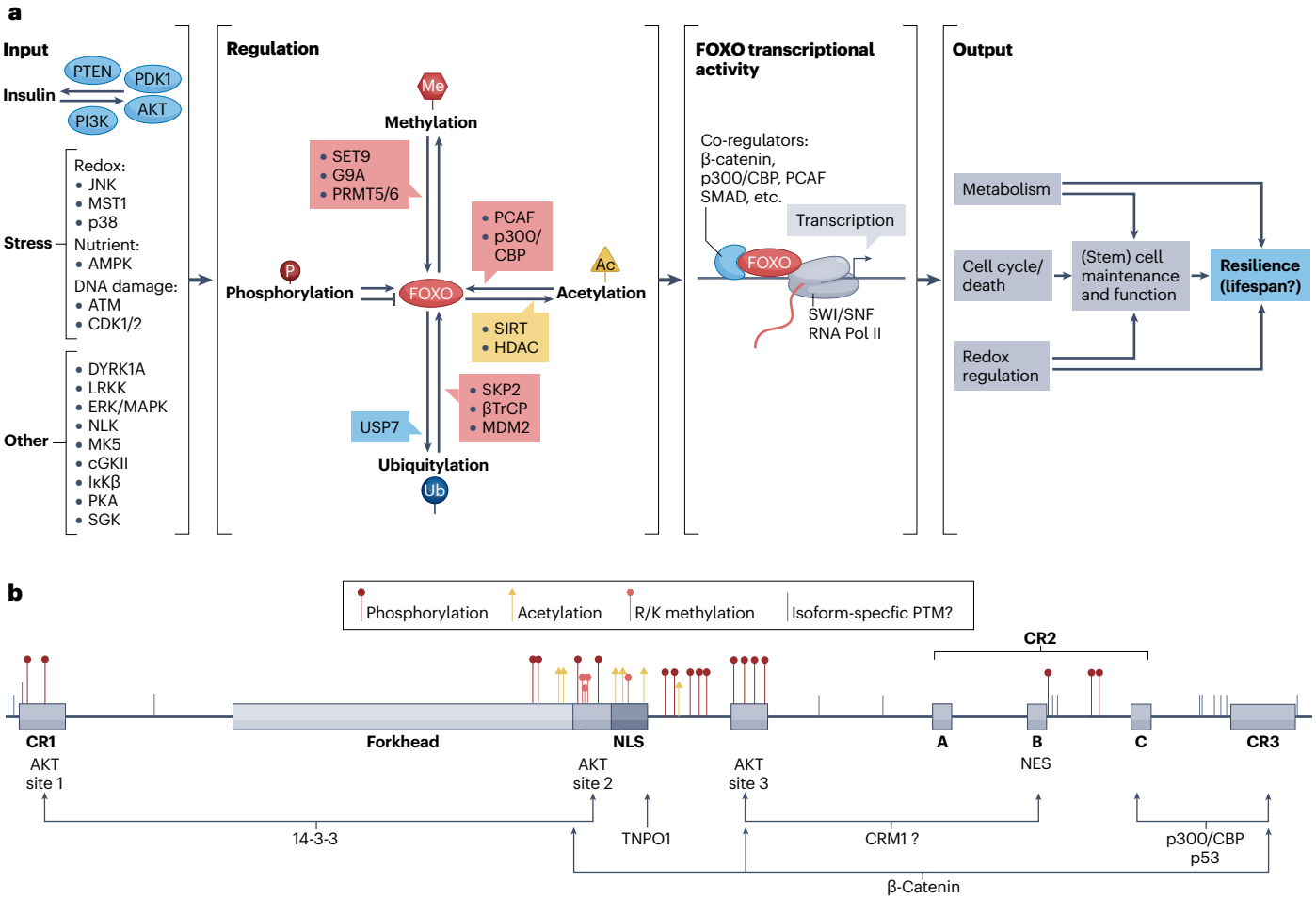
Gene control

Transcription regulation has long been regarded in a binary fashion: either 'on' or 'off'. But more recently the relevance of dynamics in transcription factor activity has become evident. For the yeast transcription factor Msn2, which similar to FOXOs is also involved in the stress response, it was shown that nucleo-cytoplasmic shuttling oscillates and that these oscillations differ in duration, amplitude and frequency[60]. A higher frequency of nucleo-cytoplasmic oscillations has a greater impact on transcriptional target gene expression when compared with the total length of time that Msn2 localizes to the nucleus[60]. High-frequency nucleo-cytoplasmic oscillations of DAF-16 were recently also shown to yield higher levels of target gene expression than continuous nuclear localization[61]. These observations suggest that the mere translocation of a transcription factor to the nucleus is not the only parameter that regulates the level of target gene transcription. For FOXOs, nucleo-cytoplasmic shuttling dynamics also differ in response to redox stress compared with in response to growth factor deprivation, resulting in different phenotypic outcomes. Treatment of cultured cells with a single high dose of H_2O_2 resulted in a mixed all-or-none response, in which some cells showed no nuclear FOXO accumulation, whereas other cells showed strong nuclear FOXO signal. The timing of nuclear residence of FOXOs appeared to be H_2O_2 dose-dependent with increased dose also linking with induction of FOXO-dependent cell death. By contrast, serum starvation causes low amplitude pulses of nuclear FOXOs and predominantly results in cell cycle arrest. Hence, different nucleo-cytoplasmic shuttling dynamics of FOXOs may result in the establishment of different cell fates[62].

FOXOs are also dynamically interacting with regions of open or condensed chromatin. ChIP is commonly used to determine the binding of a transcription factor to its target gene recognition sites. However, ChIP only provides an averaged-out picture of transcription factor binding at a given time point and gives little information on the dynamics and variation between cells. As mentioned, FOXOs may act as pioneering factors and FOXO1 can bind in vitro to its cognate sites on a nucleosome. This binding stably perturbs core histone–DNA contacts and results in chromatin opening by FOXO1 (ref. 63). Of note, although acetylation of FOXO1 reduces its DNA-binding affinity, it does not destabilize the binding of FOXO1 to nucleosomal DNA and has no effect on stable nucleosome remodelling[64]. Although there is ample evidence for FOXO1 as pioneer factor, it remains to be established whether all FOXOs can act as pioneer transcription factors[65].

Transcriptional noise and gene expression variability

The differential dynamics that control gene expression may also contribute to cell-to-cell variability in gene expression timing and amplitude. In unicellular organisms, it has been observed that genes involved in stress responses display high variability in expression[66], reminiscent of transcriptional noise. This may seem counterintuitive, as it would come at the cost of reduced fitness of the individual organism, but could support increased fitness of the population in stressful conditions: a large variation in transcriptional profiles after all increases the chance that some cells express a combination of genes that synergize to survive the insult and repopulate the culture afterwards. Likewise, *C. elegans* produce more male offspring under stressed conditions and this ensures more genetic variation compared with hermaphrodite reproduction. Variation in the gene pool also in this case would increase the chance of survival of some of the offspring. Genomic instability, a hallmark of cancer cells, may provide a similar solution to increase survival of a subset of cancer stem cells under stressful conditions, and this may even contribute to therapy resistance. In agreement, it has been shown that DNA damage[67] and consequent DNA repair regulate transcriptional noise[68]. Gene expression variability can occur at multiple levels (reviewed elsewhere[69,70]), and a role for some transcription factors is observed (nuclear factor-κB[71] and AP-1 (ref. 72)), but as far as we know has not been studied in the context of FOXOs. It could be speculated that because FOXOs have a role in stress resistance, they specifically increase gene expression variability at least for genes involved in stress resistance. Interestingly, ageing is correlated with an increase in cell-to-cell variability in gene expression[73]. This is also



observed in *C. elegans*[74], but here DAF-16 is suggested to suppress gene variability[75]. Irrespective, it will be of interest to address a possible link between FOXOs and gene expression variability more directly and more specifically, for instance, through single-cell RNA sequencing following stress challenges combined with manipulation of FOXO activity.

Enhancer-mediated gene control for adaptive stress regulation

Transcription control by FOXOs can proceed in a classical manner by FOXO binding to its consensus DNA sequence within promoter regions. In addition, we and others have shown that FOXO3 also contributed extensively to gene regulation by binding to and activation of enhancer regions. Importantly, gene regulation through enhancers provides a mechanism whereby FOXO3 can regulate the stress response and homeostasis not only in a cell-specific manner but also in a gene-specific manner (Box 2). This mode of regulation also suggests that whenever the epigenetic landscape changes, for example, as observed during stem cell differentiation, cancer development (discussed subsequently) and ageing[76] (reviewed elsewhere[77,78]), this may result in a changing landscape of enhancer activity and thus may have a profound impact on the set of genes that can be transcriptionally controlled by FOXOs. Consequently, this impacts on the ability to counteract cellular stress and thus also has an impact on the ability of FOXOs to maintain homeostasis in health and disease.

Transcriptional programmes downstream of FOXOs

FOXO activation in response to several stresses elicits cellular responses to counteract stress. Some of the responses are general and irrespective of whether, for instance, metabolic, genotoxic or redox stress is encountered, but other responses may be specific for certain types of stress. In *C. elegans*, formation of Dauer (a highly stress-resistant, developmentally arrested larval stage that can survive for several months) is *daf-16*-dependent and triggered at larval stage 2 by various stresses including lack of nutrients, high temperature and overcrowding[79]. Dauer formation represents a reversible arrest and worms develop to fertile adults once unfavourable conditions have ceased. In analogy, in mammalian cells experiencing stress, FOXOs typically initially trigger a reversible cell cycle arrest, which provides time to resolve and adapt to stress and to prevent propagation of damage down the lineage. Here, we discuss cell cycle regulation, metabolism and maintenance of redox homeostasis as important hallmarks of the FOXO-mediated stress response.

Regulation of cell cycle progression

Regulation of cell cycle progression is key to organismal maintenance. For instance, it not only ensures balance between nutrient availability and cell number but also provides tumour suppression and tissue repair in metazoans. Cell cycle progression is stimulated by various growth

Fig. 1 | Upstream regulation and output of FOXO transcription factors in stress adaptation. a, Forkhead box (FOXO) transcription factors are activated by a plethora of inputs that in turn respond to numerous external and internal stress cues including disturbances in nutrient availability, redox balance and genomic stability. These inputs regulate FOXOs largely by mediating post-translational modifications (PTMs) (part **b**). Little is known as to whether all these inputs act individually or combined. Regulation downstream of these cues involves combinations of, for example, p38 mitogen-activated protein kinase (MAPK) phosphorylation, JUN N-terminal kinase (JNK) phosphorylation and E3 ubiquitin-protein ligase MDM2 ubiquitylation, but the details of this combined regulation and how this translates to strength and or specificity of gene regulation remain largely elusive. Once activated, FOXOs will transcriptionally control gene expression (by associating with co-regulatory proteins such as β-catenin, p300/CBP-associated factor (PCAF), SMAD (homologous to *Caenorhabditis elegans* SMA ('small' worm phenotype) and the mothers against decapentaplegic family from *Drosophila*) and others), regulating pathways widely associated with stress adaptation. These pathways include regulation of cell cycle progression, metabolism and cellular redox homeostasis, in which cell cycle regulation provides a time window to repair and resolve stress and metabolism and enzymes involved in redox regulation allow cells to reduce cellular redox. These mechanisms are particularly important in stem cell maintenance, which is an evolutionary conserved output of these FOXO controlled processes that ultimately affects organismal resilience, and consequently lifespan. Although stem cells are of importance with respect to lifespan, the FOXO-regulated processes will also impact on resilience and lifespan through other cell types, for instance, by protection of the soma from loss of function or oncogenic transformation. **b**, Schematic representation of PTMs for FOXO1, FOXO3 and FOXO4 (human) on the basis of PhosphoSitePlus (Supplementary Tables). FOXO1, FOXO3 and FOXO4 isoforms consist of a structured forkhead domain responsible for binding to a consensus DNA sequence (5′-TTGTTTAC-3′)[184]. The N-terminal and C-terminal parts of all FOXOs are intrinsically disordered regions that harbour small stretches that may have a propensity to adopt an α-helical fold. These represent semi-conserved regions (CR1, CR2A, CR2B, CR2C and CR3). Besides, there is a conserved region flanking the C terminus of the DNA-binding domain that harbours the AKT (also known as PKB) phosphorylation site 2 followed by a nuclear localization sequence (NLS). The nuclear export sequence (NES)[185] overlaps with the previously identified CR2B region[186]. The binding interfaces for CREB-binding protein (CBP) and p300 (refs. 186,187), p53 (ref. 38), transportin 1 (TNPO1)[38] and β-catenin[39] have been determined by the structural analysis and are indicated. AMPK, 5′ AMP-activated protein kinase; ATM, ataxia telangiectasia mutated; CDK, cyclin-dependent kinase; cGKII, cyclic GMP kinase II; CRM1, chromosomal region maintenance 1; DYRK1A, dual-specificity tyrosine phosphorylation-regulated kinase 1; ERK/MAPK, extracellular signal-regulated kinase/mitogen-activated protein kinase; G9A, histone-lysine *N*-methyltransferase G9A; HDAC, histone deacetylase; IκKβ, inhibitor of nuclear factor-κB kinase subunit-β; LRKK, leucine-rich repeat kinase; MK5, MAP kinase-activated protein kinase 5; MST1, mammalian STE20-like kinase 1; PDK1, phosphoinositide-dependent kinase-1; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PRMT, protein arginine *N*-methyltransferase; PTEN, phosphatase and tensin homologue; RNA Pol II, RNA polymerase II; SET9, histone-lysine *N*-methyltransferase SET9; SGK, serum and glucocorticoid-regulated kinase; SIRT, sirtuin; SKP2, S phase kinase-associated protein 2; USP7, ubiquitin-specific processing protease 7; SWI/SNF, switch/sucrose non-fermentable; βTrCP, β-transducing repeat containing protein.

factors such as insulin, EGF and WNT. FOXOs trigger a cell cycle arrest when growth factor signalling is switched off, for instance, owing to low glucose (nutrient stress). But activation of FOXOs by other stresses such as DNA damage or redox stress can also impose a cell cycle arrest by counteracting active proliferative signalling. FOXO activation, through loss of insulin–PI3K–AKT signalling or expression of a constitutively active FOXO allele, results in a G1 cell cycle arrest[80]. By contrast, activation of FOXO by redox stress mostly results in cell cycle arrest at G2, followed by direct progression to G0/G1 without undergoing mitosis (mitotic bypass)[81,82]. FOXO transcriptionally regulates several cell cycle regulators, such as $p27^{KIP1}$ (also known as p27), $p21^{CIP1}$ (also known as p21), EMI1 and cyclin D. We focus here on the regulation of p27.

The CDK inhibitor p27 not only is a gatekeeper of the G1-to-S transition but also regulates G2-to-M progression and cytokinesis completion. p27 can therefore be considered a pivot in cell cycle control by FOXOs. p27 expression is transcriptionally controlled by FOXOs[80], and similar to FOXOs, p27 is phosphorylated by AKT (at Thr157 and Thr198). AKT-mediated p27 phosphorylation results in the binding of the 14-3-3 protein[83], relocation from nucleus to cytosol and a reduction in the p27 protein level (reviewed elsewhere[84]). At the G2-to-M phase transition, p27 ubiquitylation and degradation are regulated by the SKP2 ubiquitin ligase. Interestingly, AKT also positively regulates SKP2 function by direct phosphorylation and this leads to cytoplasmic translocation and stabilization of SKP2 by preventing the interaction between SKP2 and the APC/C^{CDH1} (activator protein CDH1 of the anaphase-promoting complex (also known as the cyclosome) ubiquitin ligase complex — an important regulator of cell cycle that is active during late mitosis and early G1 phase to control exit from mitosis and further rounds of proliferation[85]. Recently, we have shown that FOXOs repress the expression of the APC/C^{CDH1} inhibitor EMI1 by binding to the E2F1 transcription factor and that the lowered levels of EMI1 lead to a premature activation of APC/C^{CDH1} and subsequent mitotic bypass from G2 under conditions of replication stress[82]. As mentioned earlier, SKP2 can also ubiquitylate and degrade FOXOs[86], which means that AKT-dependent SKP2 activation controls both G1-to-S and G2-to-M transitions[87] through the negative regulation of FOXOs and their downstream targets p27 and relieve of EMI1 repression (Fig. 3). In addition to inhibiting the cell cycle by binding to cyclin–CDK complexes, p27 has also been shown to be involved in the activation of CDK4 (CDK required for transition into S phase) under certain conditions, but it is not clear whether FOXO-dependent regulation of p27 also has a role in activation rather than inhibition of CDKs[38,88,89].

Long-term cell cycle arrest

The duration of a FOXO-induced cell cycle arrest can differ, and FOXOs have also been shown to contribute to both quiescence (dormancy) and senescence, forms of prolonged cell cycle arrest. Quiescence, often referred to as G0, differs from a G1 arrest, although the discriminating features are not fully clear[90].

The p105-RB protein, an essential component of the G1-to-S checkpoint, is stably expressed during the cell cycle and is regulated by CDK-dependent phosphorylation. The other RB family members, p107 and p130 (also known as pRB2), are regulated at the protein expression level as well as by phosphorylation. p107 protein levels are low during quiescence and early G1 but high during the other stages of the cell cycle. p130 protein levels are low in cycling cells but increase once cells exit the cell cycle. This is accompanied by a change in the phosphorylation of p130 from a hyperphosphorylated form to the hypophosphorylated form in G0 cells (reviewed elsewhere[91]). Hypophosphorylated p130 in G0 cells binds to the E2F4 transcription factor, which is thought to repress genes required for re-entry into early G1 phase, thereby maintaining the quiescent state[92]. FOXO activation increases expression of

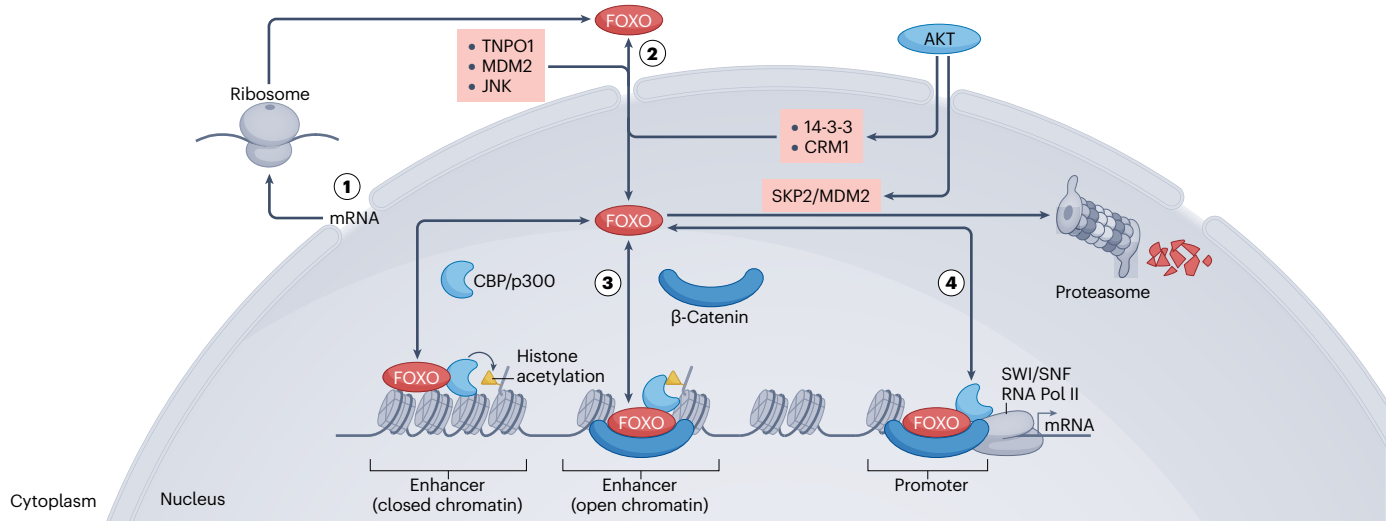


Fig. 2 | Levels of FOXO regulation. Activity of forkhead box (FOXO) can be controlled at different levels. These are: protein expression (level 1); nucleo-cytoplasmic shuttling (level 2); binding to condensed chromatin (level 3) and binding to open chromatin (level 4), including promoter and enhancer regions and. The first level is a balance among FOXO production through regulation of mRNA expression, microRNA-mediated regulation, protein translation and FOXO protein breakdown through proteasomal degradation regulated by ubiquitylation. The other levels of control all represent an equilibrium in which post-translational modifications on FOXOs and FOXO interaction (interactions) with regulating proteins determine the balance of the equilibrium. For example, AKT (also known as PKB)-mediated phosphorylation, and consequent binding to 14-3-3 facilitate nuclear export and inhibit nuclear re-entry of FOXOs and thereby shift the nucleo-cytoplasmic equilibrium towards the cytosol. Binding of FOXOs to DNA is also regulated. It is known that, for example, β-catenin binding to FOXOs relieves auto-inhibition[29] and therefore will shift the balance towards DNA binding. Binding of the acetyltransferase p300 or its homologue CREB-binding protein (CBP) to FOXO can, on the one hand, facilitate histone tail acetylation and opening of the chromatin and allow for DNA binding of FOXO. But, on the other hand, acetylation of FOXO itself by p300/CBP has been described to lower its affinity for DNA[47] and thus will shift the equilibrium towards FOXO being unbound to DNA. CRM1, chromosomal region maintenance 1; JNK, JUN N-terminal kinase; MDM2, E3 ubiquitin-protein ligase MDM2; SKP2, S-phase kinase-associated protein 2; SWI/SNF, switch/sucrose non-fermentable; RNA Pol II, RNA polymerase II.

the hypophosphorylated p130 and promotes complex formation with E2F4 (ref. 93), indicating that FOXOs induce a p27-induced G1 arrest that can proceed into a G0 arrest.

Senescence, in contrast to quiescence and a G1 arrest, is considered an irreversible cell cycle arrest. Senescence is induced by severe stress conditions such as oncogene expression and severe DNA damage. Under these conditions, senescence provides a protective mechanism towards the emergence of cancerous cells because it is accompanied by a cessation of proliferation. Senescent cells, when not removed by the immune system, remain resident and start to secrete a plethora of cytokines and other growth modulatory factors. This is termed the senescence-associated secretory phenotype and it is causally implicated in ageing and disease such as cancer progression (reviewed elsewhere[94]). Senescence is therefore seen both as tumour-suppressive and tumour-promoting. Initially, a role for FOXOs in senescence was inferred from studies showing that loss of AKT in primary mouse embryonic fibroblasts caused resistance towards induction of senescence and that this in part was due to a loss of FOXO inhibition[95]. FOXO3 activation was shown to mediate resistance towards senescence by inducing increased resistance towards oxidative stress (reviewed elsewhere[95]). Cancer cells in culture typically display high basal levels of oxidative stress, but have been shown to bypass replicative senescence by upregulating antioxidant enzymes. However, melanoma cells with an oncogenic mutation in *BRAF* can still enter senescence through JNK-mediated activation of FOXO4 and consequent induction of p21 (ref. 96). Establishment of senescence in cultured cells usually requires several days, and it was shown that FOXO4 expression is gradually induced during senescence establishment[97] and that this is required for tethering of p53 to promyelocytic leukaemia bodies and subsequent survival of senescent cells. Disruption of the FOXO4–p53 interaction resulted in relocation of p53 from nucleus to cytosol and p53-dependent apoptosis of senescent cells[97].

Initially, senescence was mostly studied in the context of severe stress in cultured cell lines. However, tissue remodelling during normal embryonic development also involves senescence induction and consequent removal of senescent cells. Interestingly, senescence during embryonic development is strictly dependent on p21, but independent of DNA damage, p53, or other cell cycle inhibitors, and it is regulated by the transforming growth factor-β–SMAD and PI3K–FOXO pathways[98,99].

FOXOs thus have both promoting and suppressing effects on senescence, and whether FOXO-induced senescence contributes to healthy ageing or disease therefore depends on the context.

Reciprocal control of FOXO, ROS and redox signalling

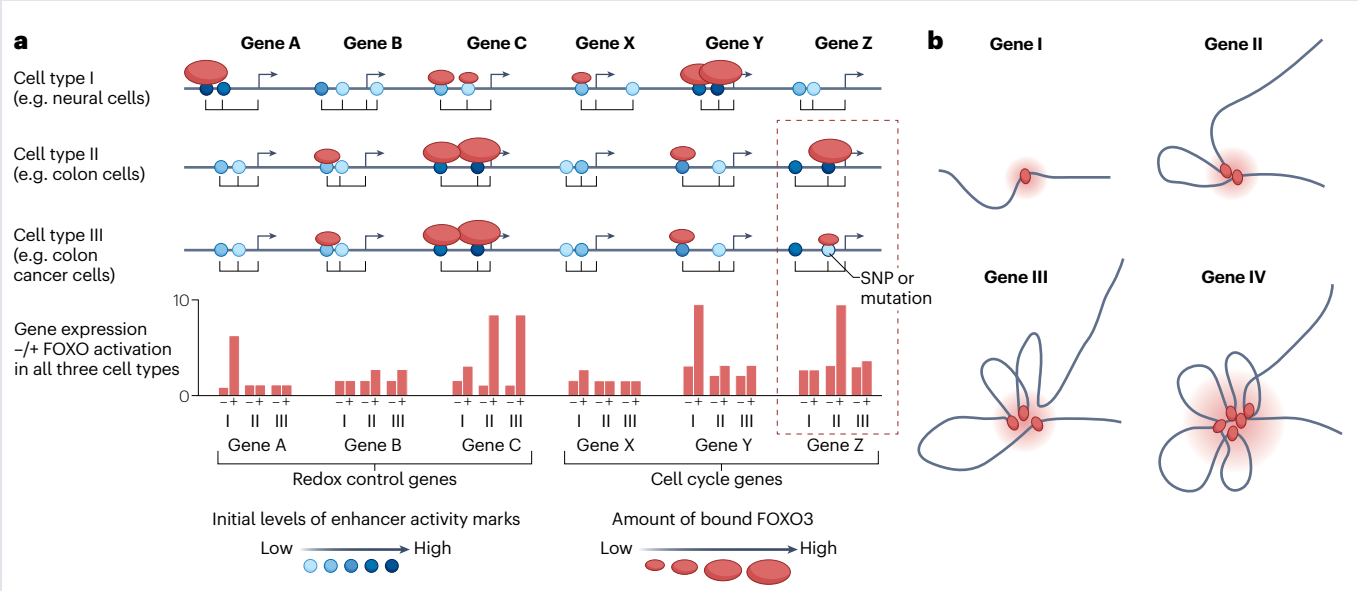
At the time when DAF-16/FOXO was discovered as the downstream regulator of long lifespan in the *C. elegans daf-2* mutant, the (mitochondrial) 'free radical theory of ageing'[100] was probably one of the most prevalent frameworks to explain age-related decline of organismal fitness. Indeed, DAF-16 was shown to transcriptionally control the expression of antioxidant enzyme genes such as *sod-3*, the orthologue of manganese superoxide dismutase (MnSOD)[101], catalase[102] and glutathione *S*-transferase; loss of the corresponding gene products also reduced lifespan extension in the *daf-2* mutant background. DAF-16 indeed also confers resistance to challenges with pro-oxidants such as

Box 2

Tuning of FOXO-mediated gene regulation by the genomic context

The results indicating that forkhead box (FOXO) may act as a pioneer factor suggest that FOXOs efficiently compete with nucleosomes for DNA binding. Pioneer function is linked to gene regulation through enhancer regions (reviewed elsewhere[27]), and FOXOs are shown to regulate gene transcription through enhancer regions as well as promoter regions. It has been shown that FOXO3 binds to pre-existing enhancers, and the level and type of enhancer activity marks, mostly H3K27 acetylation, before FOXO3 activation largely determines FOXO3 DNA binding. Furthermore, FOXO3 binding amplifies the levels of these activity marks and their absolute rather than relative changes associate best with the level of FOXO3-mediated gene expression[65] (see the figure, part **a**). Consequently, not the induction per se, but more the passing of a threshold of H3K27 acetylation determines gene expression from a locus that is within proximity (in 3D) of this histone mark. This indicates that the initial landscape of H3K27 acetylation is a highly relevant determinant for gene activation by FOXO3.

To illustrate the consequence of this mode of regulation, the location of several hypothetical enhancers (circles) and six associated target genes is depicted in the figure (see the figure, part **a**). In different cell types (for example, neuronal versus colon cells) or cellular conditions (for example, cancer versus non-cancer cells), the location as well as the level of initial activity of active enhancers can differ. For the three different situations, the active enhancer locations are shown, with varying initial activity and FOXO binding. Colours represent the relative amount of initial enhancer activity marks. The level of enhancer activity correlates with the level of FOXO3 binding. Consequently, the amount of bound FOXO reflects levels of these marks, as shown by larger FOXO symbols. Bar graphs represent expected expression levels of six genes before and upon FOXO activation in three cell types, as suggested by the observed correlation between FOXO enhancer binding and gene expression. Genes are functionally grouped, for example, genes A, B and C influence cellular redox state, genes X, Y and Z are involved in cell cycle regulation, two of the cellular processes typically affected by FOXO activation. This example now shows how FOXOs can regulate the same cellular process, such as redox state and cell cycle, in a similar manner but through different genes. In the example here, redox balance in cell type I is regulated through gene A, whereas gene C is regulated in cell type II. Cell type III harbours an SNP in an enhancer, which is associated with increased cancer incidence. This SNP does not disrupt the forkhead motif, but reduces enhancer activity. In this manner, FOXO3 binding to this enhancer is reduced and induction of the cell cycle regulator 'gene Z' is subsequently reduced. This would result in impaired ability of FOXO to regulate cell cycle arrest and hence impaired tumour suppression. Other processes which are potentially beneficial for cancer cell survival (such as target genes affecting cellular redox homeostasis) remain unaffected. This may explain the contribution of cancer-associated enhancer SNPs to the deregulation of phosphoinositide 3-kinase signalling that is frequently observed in human cancer. In addition, the 3D architecture of the genome (that is, local folding of the DNA may bring parts together that are far apart when DNA is regarded in a strictly linear fashion) affects the amplitude of target gene induction, as this can regulate the amount of bound FOXO3 nearby the transcription start site (see the figure, part **b**). Four genes are shown (I–IV) with differing amounts of bound FOXO3. Possibly, the spatial organization of the genome allows high local concentrations of FOXO3 and activating factors (indicated with fading colour surrounding FOXO3 molecules), providing an optimal environment for transcription induction.



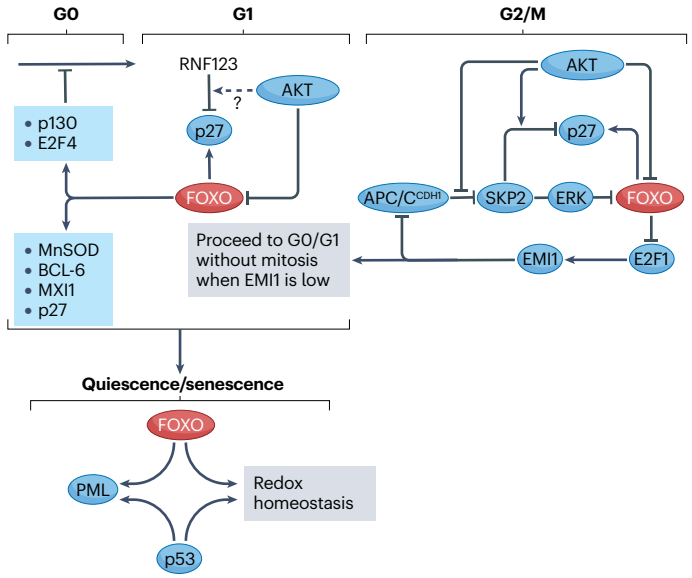


Fig. 3 | FOXO and the cell cycle. Forkhead box (FOXO) activation can halt the cell cycle both at the G1 and G2 phase. Cell cycle arrest owing to FOXO activation resulting from reduced AKT (also known as PKB) activity, for example, lack of growth factors, results in a G1 arrest. Arrest following FOXO activation by cellular stress may occur at G1 or G2. Depending on the conditions, prolonged arrest may progress into quiescence (also known as G0), senescence or alternatively cell death, when stress-induced cell cycle arrest is not accompanied by, for instance, sufficient DNA repair or restoration of redox homeostasis. Quiescence is considered to be a prolonged G1 arrest that, however, can be discriminated from G1 by expression of specific genes[90]. G0 entry is regulated by the RB family member p130 (also known as pRB2) and E2F4 (ref. 92) and prolonged FOXO activation regulates p130 (ref. 93). In addition, other genes that mark quiescence[90] are direct FOXO target genes (for example, those encoding max protein interacting 1 (MXI1), manganese superoxide dismutase (MnSOD) and B cell lymphoma 6 protein (BCL-6)). A multitude of stresses can induce senescence (for example, DNA damage, telomere attrition and oncogene-induced stress) and, although mostly p53 is considered a central pivot in establishing senescence, the sequestration of p53 in promyelocytic leukaemia (PML) protein bodies by FOXO4 is required to prevent cell death of senescent cells[97]. Recently, others[188] and we[82] have shown that mild replication stress results in activation of a G2 checkpoint, coined antephase[189]. Arrest at this cell cycle checkpoint is reversible and depends on the inhibitor early mitotic inhibitor 1 (EMI1), which is normally degraded just before mitosis. FOXOs bind to and inhibit E2F1 (ref. 190), which then can no longer transcriptionally regulate the expression of EMI1 (ref. 82). If replication stress is not resolved and the G2 arrest persists, EMI1 levels become critically low by the continued FOXO-dependent repression of its transcription. This leads to premature APC/C^{CDH1} (activator protein CDH1 (CDC20 homologue 1) of the anaphase-promoting complex (also known as the cyclosome)) activation, and cells progress directly from G2 to G0/G1 (mitotic bypass) and hence become tetraploid. In this way, FOXOs limit the time allowed in G2 to resolve stress: if it takes too long, mitosis is aborted, and this could ensure that cells do not propagate unresolved DNA damage. Depending on conditions, cells can thereafter proceed into senescence[191] or cell death[192]. Regulation of APC/C^{CDH1} activity by downregulation of EMI1 through FOXOs generates a feedforward loop, as APC/C^{CDH1} also regulates proteasomal degradation of S-phase kinase-associated protein 2 (SKP2). Lower SKP2 activity would relieve FOXOs as well as p27 from SKP2-dependent polyubiquitylation and proteasomal degradation. The phosphorylation-dependent activation of SKP2 and inhibitory phosphorylation of FOXOs by high AKT, for instance, owing to oncogenic signalling, may break this feedforward loop and allow progression from G2 to M phase, despite unresolved damage.

paraquat. Regulation of MnSOD, catalase and several other proteins involved in oxidant scavenging, and the ensuing resistance to exposure to oxidants, was also observed downstream of mammalian FOXOs (reviewed elsewhere[9]). The combined observations of stress resistance, upregulation of antioxidant genes and long lifespan by activation of DAF-16 in worms made sense, considering the free radical theory of ageing. Germline deletion of one allele of *Foxo1* and both alleles of *Foxo3* and *Foxo4* (homozygous deletion of *Foxo1* is embryonically lethal), however, had no noticeable effect on lifespan nor ageing[103].

In line with the role of FOXOs as stress response factors, nuclear translocation and transcription activation is observed in response to heat and several chemical stresses and can be reversed or prevented upon treatment with antioxidant compounds such as *N*-acetylcysteine (NAC), suggesting that changes in the redox balance were at the root of these observations (reviewed elsewhere[9,104]). In addition, exposure of cells to H_2O_2 — often supraphysiological levels — to mimic elevated reactive oxygen species (ROS) levels results in extensive induction of post-translational modification of FOXOs (for example, p38 MAPK-mediated and JNK-mediated phosphorylation) (Supplementary Tables) and binding of several cofactors such as β-catenin, p300 and p53 to FOXOs (reviewed elsewhere[9]). Oxidizing conditions thereby can activate FOXOs to counteract proliferative signalling downstream of WNT and growth factor signalling (Fig. 4a), thereby preventing propagation of damage down the lineage. Although studies using exposure to exogenous H_2O_2 helped to elucidate players involved in FOXO regulation in response to oxidative stress, it can be argued that these experiments hardly recapitulate how cells respond to endogenous ROS. Within cells, H_2O_2 is produced enzymatically at various cellular locations (reviewed elsewhere[46,105]), either directly or indirectly through dismutation of superoxide anions, derived from, for example, mitochondrial respiration. It is currently unknown when and how H_2O_2 from these locations impinges on signal transduction upstream of FOXO. In addition, H_2O_2 formation by, for example, plasma membrane-bound NADPH oxidases is suggested to regulate growth factor signalling by inactivation of the catalytic cysteines of phosphatases (PTP1 and PTEN) and DUBs (reviewed elsewhere[106,107]). This can, for example, regulate the duration of the insulin signalling response, and this in return may affect FOXO transcriptional activity (see the discussion mentioned earlier).

Studies in the early 2000s using knockout and overexpression of antioxidant enzymes in several organisms started to reveal that a direct link among lifespan, ageing and ROS might be not so straightforward, questioning the validity of the free radical theory of ageing. Low levels of ROS could in some cases even lead to lifespan extension rather than rapid ageing (reviewed elsewhere[108]). Around this time, it also started to become apparent that ROS were not only damaging but could also have a role as a second messenger in what is coined redox signalling, which proceeds through the reversible oxidation of cysteine thiols side chains in redox-sensitive proteins (Supplementary Box 1). FOXO activity is also subject to reversible oxidation that can regulate interaction with their partners and regulatory proteins. Initially, it was shown that interaction of FOXO4 with p300 occurred via a cysteine disulfide-mediated binding[45]; additional studies showed that this mode of interaction is common to all FOXO isoforms[35] and that multiple cofactors interact with FOXOs through reversible oxidation of cysteine thiols side chains and cysteine disulfide formation[35]. The possibility to establish redox-dependent cysteine disulfide interactions represents an attractive mode of stabilizing protein–protein interactions only when the cellular milieu shifts to become more oxidizing. Several other

redox-sensitive proteins are regulated in this way[46], but for FOXOs the consequences of this mode of regulation for the stress response depend on the interaction partner that is involved. Disulfide-dependent binding to TNPO1 (ref. 34), IPO7 and IPO8 (ref. 35) mediates nuclear import and activation, whereas the disulfide-dependent binding to p300 is required for lysine acetylation on FOXOs and subsequent differential transcriptional target selection[45]. An overview of the regulation of FOXOs by redox signalling is provided in Fig. 4b.

Many vital physiological functions are affected by cellular redox stress and consequently influenced by redox signalling. This raises the question whether the induction of antioxidant genes by FOXOs serves to protect from damage, to modulate redox signalling or both. Loss of all five *sod* genes in *C. elegans* had no effect on median or maximum lifespan[109], but the lifespan extension in response to low levels of the superoxide generator paraquat observed in wild-type worms was lost. This could suggest that the function of SODs is not only to scavenge superoxide anions but also to provide H_2O_2 for redox signalling-induced lifespan extension. Considering this idea, the upregulation of MnSOD by FOXO might be a means to optimize redox signalling through generation of H_2O_2. Approaches using, for instance, chemogenetics[110,111] in combination with localization tags to carefully titrate localized H_2O_2 production could aid to mimic and dissect the spatiotemporal redox control of FOXO and downstream responses in the future.

Regulation of systemic metabolism

In line with its conserved role in the stress response and its ability to regulate Dauer formation in *C. elegans*, FOXOs also regulate the adaptation to limited nutrient availability in mammals. Acting downstream of insulin, FOXOs are key regulators of glucose homeostasis. Glucose is the primary energy source for all organisms, and its catabolism occurs through highly conserved mechanisms. Tight regulation of this pathway is crucial, as its dysregulation leads to diseases such as diabetes and obesity. FOXOs primarily regulate glucose metabolism by mediating the expression of enzymes of gluconeogenesis and energy metabolism (reviewed elsewhere[112]) (Fig. 5a). During fasting or exercise, FOXO induces metabolic changes that ensure the maintenance of glucose levels systemically. In the liver, FOXOs translocate to the nucleus and drive the expression of pyruvate dehydrogenase kinase 4 (PDK4) and of gluconeogenic enzymes such as glucose-6-phosphatase, fructose-1,6-biphosphatase and phosphoenolpyruvate carboxykinase. PDK4 negatively regulates the pyruvate dehydrogenase complex, thereby reducing the utilization of glucose in mitochondria. Phosphoenolpyruvate carboxykinase phosphorylates oxaloacetate to form phosphoenolpyruvate, and glucose-6-phosphatase promotes the dephosphorylation of glucose-6-phosphate, leading to the release of newly synthesized glucose into the bloodstream (reviewed elsewhere[113]). Regarding lipid metabolism, active FOXOs in the liver can induce the transcription of the apolipoprotein ApoC3, thereby elevating the levels of triglycerides in plasma[114]. Under the same conditions, but in muscle, FOXOs promote a metabolic transition from glucose catabolism to lipid oxidation (Fig. 5a). This occurs via increased expression of PDK4, of the lipoprotein lipase that promotes increased fatty acid (FA) availability and of the plasma membrane FA translocase CD36 that facilitates the uptake of FAs by muscle cells (reviewed elsewhere[115,116]). Thus, upon metabolic stress, FOXOs facilitate multiple metabolic adaptations that guarantee the maintenance of glucose homeostasis systemically.

Interestingly, FOXOs have been found to have a role in regulating feeding behaviour. FOXOs integrate signals from peripheral tissues in the hypothalamus and regulate the secretion of neuropeptides. FOXO1 induces the expression of *Agrp* and *Pomc* genes, which stimulate appetite[117–119], and FOXO1 deficiency in AGRP neurons mimics the action of insulin and leptin. This results in reduced food intake, leanness and improved glucose homeostasis in mice[120]. Therefore, next to modulating glucose maintenance systemically, it is plausible that FOXOs stimulate feeding behaviour, as another way to respond to metabolic stress.

Regulation of mitochondrial dynamics

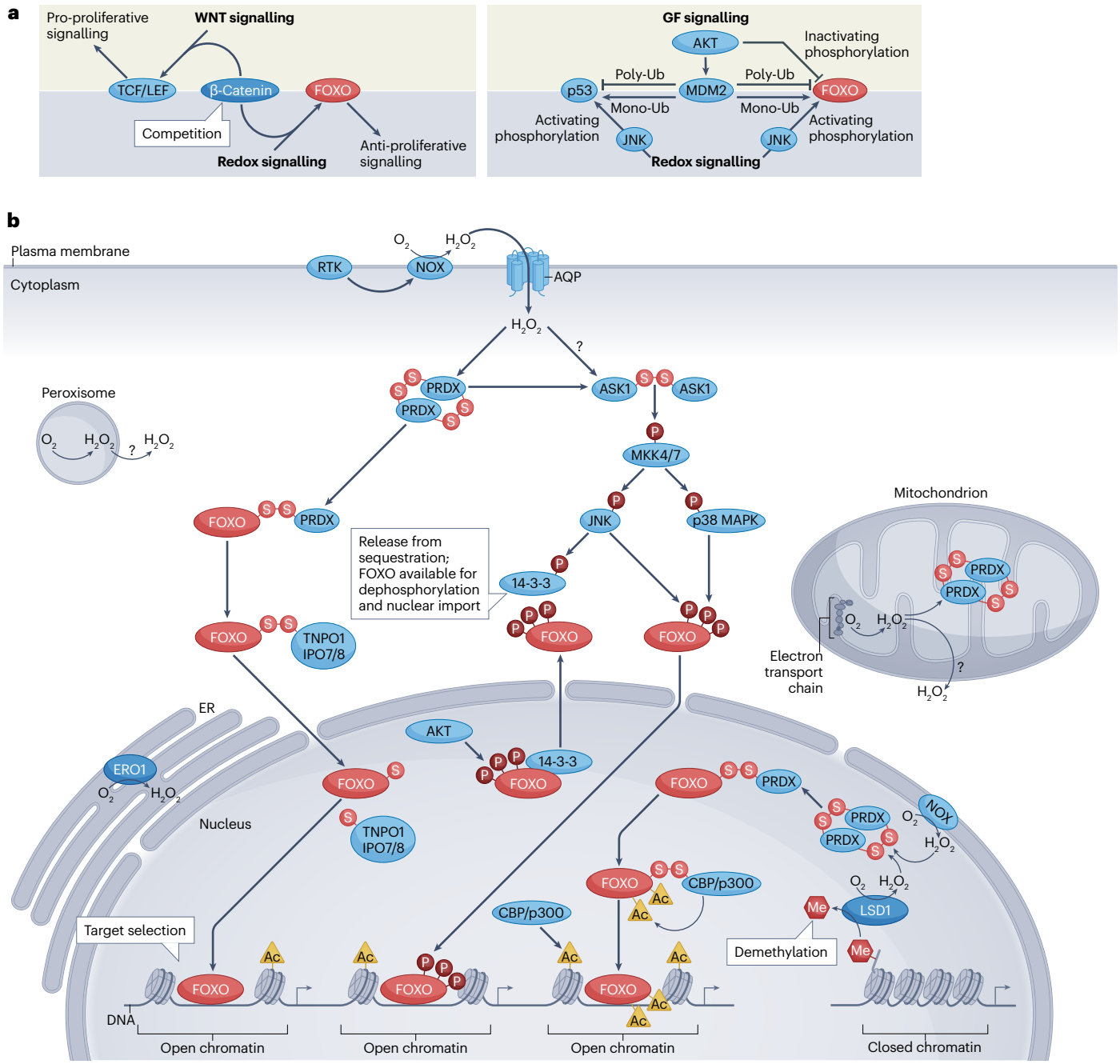
Metabolic regulation by FOXOs also extends to the regulation of mitochondrial homeostasis by controlling mitochondrial biogenesis, fission and fusion dynamics and mitophagy (Fig. 5b).

FOXOs are repressors of MYC[121], which in turn downregulates TFAM (transcription factor A mitochondrial)[122], a protein that has a key role in the transcription of mitochondrially encoded genes. Next to TFAM, several MYC-dependent nuclear-encoded mitochondrial genes decrease their expression upon FOXO activation. Therefore, despite FOXO induction of PDK4 expression, FOXO activation decreases mitochondrial mass, activity and ROS production[123] (Fig. 5b). FOXOs also regulate the expression of the mitochondrial proteins HMOX1, FXN and UROD. These proteins are shown to disrupt electron transport chain activity and to affect NAD production[124]. Reduced NAD levels decrease SIRT1 activity, which in turn can result in the inactivation of transcriptional co-activator PGC1α, which is implicated in regulating energy metabolism. As PGC1α is required for mitochondrial biogenesis, its inactivation represents an additional mechanism by which FOXOs can downregulate mitochondrial function (Fig. 5b).

Mitochondrial homeostasis also relies on mitochondrial fusion and fission dynamics. Fusion and fission define mitochondrial morphology and facilitate content exchange among mitochondria, for instance, of mtDNA and thereby contribute to mitochondrial fitness[125]. Mitochondrial fission and fusion rates are dynamic and change, for instance, during the cell cycle and are responsive to nutritional stress. It is shown that FOXOs inhibit mitochondrial fission by repressing mitochondrial elongation factor 2 and by inducing miR-484. FOXO-mediated regulation of miR-484 leads to decreased levels of FIS1, an important factor in organelle fission, thereby preventing fission of mitochondria (Fig. 5b). In the heart, this mechanism involves FOXO3 and has a protective function – inhibition of mitochondrial fission prevents apoptosis, reducing the size of myocardial infarction[126]. This mechanism of action is also observed in the intestine, where *Foxo1* and *Foxo3* knockdown leads to increased mitochondrial fission and perturbed differentiation of stem cells (discussed in the next section).

Mitochondrial fission is also a prerequisite for mitophagy, which is the process of clearing dysfunctional mitochondria to ensure a healthy mitochondrial population. FOXOs regulate expression of kinase PINK1 (ref. 127), which drives mitophagy (reviewed elsewhere[128]). In addition, FOXOs can also mediate mitophagy by regulating the different stages of autophagy: mitochondrial recognition for autophagy, the formation and maturation of the autophagosome and the fusion of the autophagosome with the lysosome for cargo degradation (reviewed elsewhere[128]) (Fig. 5b).

Thus, on the one hand, active FOXOs can lead to decreased mitochondrial activity by inhibiting biogenesis and by increasing mitophagy, but on the other hand, it is also shown that FOXOs prevent mitochondrial fission, thereby potentially preventing loss of mitochondria by mitophagy. These observations reveal an intricate connection between FOXOs and mitochondria. This could relate, to some extent, to



the differential expression of MYC in various cell types and biological contexts. In line with this rationale, deletion in cardiomyocytes shows no consequences on development[129] and in the intestine, *Myc* is not expressed in all cell types and its expression is restricted to dividing progenitor cells (transit amplifying cells)[130,131].

Finally, FOXOs have been shown to localize in mitochondria and seem capable of regulation of mitochondrially encoded genes[132,133]. Thus, it is proposed that FOXOs could be important factors in the crosstalk between nucleus and mitochondria. Understanding FOXO regulation of mitochondria is particularly relevant, given that, next to ATP production and supply of metabolites, mitochondria are important hubs of redox signalling with consequences on cell fate regulation[134,135].

FOXOs in the regulation of stem cells and tissue homeostasis

The freshwater polyp *Hydra vulgaris* has an apparently eternal lifespan and shows no evidence of senescence[136,137]. The constant renewal of its tissues relies on a highly efficient maintenance of its stem cells, which is FOXO-dependent[138]. *Hydra* expresses one FOXO isoform and similar to mammals, it is under control of AKT and JNK signalling[139]. As such, *Hydra* provides an archetype example of FOXO

Fig. 4 | Redox control of FOXO transcription factors. a, Growth factors (GFs) drive proliferation through various signalling pathways that converge on activating AKT (also known as PKB) (reviewed recently[193]). WNT signalling drives proliferation by inhibiting the continuous proteasomal degradation of β-catenin induced by adenomatous polyposis coli destruction complex (reviewed recently[194]). Under oxidizing conditions, proliferation needs to be halted and forkhead box (FOXO) overrides the proliferative input. This is regulated by the redox-dependent association of FOXO with binding partners and by redox-dependent induced post-translational modification of FOXO. Proliferative WNT signalling leads to nuclear β-catenin binding to TCF/LEF and turns TCF/LEF from a transcriptional inhibitor to transcriptional activator[195]. FOXO competes with TCF/LEF for β-catenin binding especially under oxidizing conditions and thereby inhibits proliferative signalling by TCF/LEF[196–198] (left). FOXOs can also counteract active AKT signalling, which normally inactivates FOXOs by phosphorylation-dependent nuclear exclusion and proteasomal degradation (right). Redox signalling (Supplementary Box 1) induces a shift from E3 ubiquitin-protein ligase MDM2-dependent polyubiquitylation (poly-Ub), which leads to FOXO degradation to monoubiquitylation (mono-Ub), which activates FOXOs by nuclear translocation and stabilization. Oxidizing conditions also activate JUN N-terminal kinase (JNK), which activates FOXOs by phosphorylation on multiple residues (distinct from the AKT sites). The stress-dependent regulation of FOXOs by JNK and MDM2 is reminiscent of the regulation of p53, which is also stabilized by JNK-dependent phosphorylation upon redox signalling[199], leading to inhibition of MDM2-dependent breakdown and hence stabilization and activation of p53. In this manner, FOXOs can act in concert with p53 activation to mediate a robust cell cycle arrest. When p53 function is lost, as is the case in almost all cancers, redox-dependent activation of FOXOs by these mechanisms can still counteract proliferative signalling and slow down the cell cycle to allow time for repair and adaptation. **b**, Redox signalling (Supplementary Box 1) starts with the production of H_2O_2, either directly or indirectly via superoxide anions and subsequent dismutation. The diffusion range of H_2O_2 is limited because of its reactivity and efficient scavenging by the highly abundant peroxiredoxins (PRDX). The outcome of redox signalling may therefore depend on the subcellular site of H_2O_2 production, and this probably holds true for the output of redox signalling to FOXOs[200]. H_2O_2 is produced at the extracellular side of the plasma membrane by NADPH-dependent oxidases (NOX), which are activated upon receptor tyrosine kinase (RTK) ligand binding. H_2O_2 can then enter the cell through aquaporin (AQP) channels and start signalling cascades, through direct oxidation of cysteines in redox-sensitive proteins or through a PRDX oxidation and disulfide exchange-mediated redox relay in case of proteins with intrinsically unreactive cysteines (such as FOXOs). NOX enzymes are also present in the nuclear, endoplasmic reticulum (ER) and mitochondrial membrane. Mitochondria are probably the most well-established site of ROS production: this occurs in the form of superoxide anions at the electron transport chain both in the matrix and in the intermembrane space, followed by rapid dismutation to H_2O_2 by manganese superoxide dismutase (MnSOD) and Cu/ZnSOD, respectively. Endoplasmic reticulum oxidoreductin 1 (ERO1) produces H_2O_2 during oxidative folding in the ER and peroxisomes produce H_2O_2, for instance, during fatty acid β-oxidation[201]. There are also H_2O_2-producing enzymes in the nucleus, such as the histone demethylase by lysine-specific demethylase 1 (LSD1)[202] and MICAL1 (ref. 203), meaning that redox signalling to FOXO could occur within the nucleus. It is not clear how much H_2O_2 is released from the various organelles, but for mitochondria it has been shown that only very limited amounts leak out[110]. FOXO is regulated downstream of redox signalling in several ways (the figure does not show all described pathways because of space limitations). Redox-dependent dimerization and activation of apoptosis signalling kinase (ASK1) trigger JNK and p38 MAPK activity, both of which can phosphorylate and activate FOXOs[102]. 14-3-3 specifically binds to FOXO that is phosphorylated by AKT in the nucleus and subsequently facilitates nuclear export and sequestration of FOXO in the cytoplasm. JNK phosphorylates 14-3-3, preventing it from binding to FOXOs, releasing the latter from sequestration and making it available for dephosphorylation and nuclear re-entry[204]. FOXOs have also been shown to form intermolecular disulfide-dependent complexes. FOXOs are shuttled into the nucleus by disulfide formation with the nuclear import receptors transportin 1 (TNPO1) (for FOXO4)[34] and importin 7 (IPO7) or IPO8 (for FOXO3)[35]. The disulfide bond between FOXO and these importins is resolved in the nucleus before FOXO binds to the DNA[34]. Disulfide formation also covalently attaches p300 and its homologue CREB-binding protein (CBP) to the most C-terminal cysteine in FOXO, which is required for subsequent acetylation (Ac) and alteration of transcriptional target selection[44]. FOXOs have also been shown to form disulfides with PRDX[35,205], which makes it plausible that the aforementioned S-S-dependent interactions are the result of a PRDX-dependent redox relay (Supplementary Box 1), although this has not been formally shown. Most of the delineated redox signalling pathways have been elucidated using supraphysiological amounts of bolus H_2O_2 addition. Future work using more subtle methods to mimic physiological H_2O_2-dependent signalling hopefully can shed more light on the outcome of compartmentalized signalling on FOXOs[105]. Me, methylation; MKK, MAPK kinase.

function in terms of combining stress regulation with lifespan. The stem cell theory of ageing proposes that the gradual loss of stem cell populations contributes to the decline of tissue homeostasis as a driver of organismal ageing[140]. Thus, mechanisms that regulate stem cell homeostasis are highly relevant to longevity. In this section, we discuss various regulatory mechanisms by which FOXOs regulate stem cell homeostasis and the ensuing tissue homeostasis and function. These FOXO-dependent mechanisms can in large part be attributed to FOXOs earlier-described roles in cell cycle regulation, redox homeostasis and metabolism.

Regulation of the cell cycle in stem cells

Adult stem cells can be divided into two types: quiescent or proliferative (reviewed elsewhere[141]). Quiescent adult stem cells are found in the brain, muscle and the hematopoietic system, whereas proliferative stem cells are found in epithelial organs such as intestine and skin. As discussed earlier, FOXOs have roles in the regulation of cell cycle arrest. In agreement with this, FOXOs are also implicated in the maintenance of quiescence in the adult stem cell types, including neuronal stem cells[142,143], muscle stem cells[112,144–146] and haematopoietic stem cells (HSCs)[147,148]. In most cases, the involvement of FOXOs is demonstrated by a loss of stem cell maintenance or quiescence upon loss of FOXO activity. This suggests that a primary role of FOXOs in quiescent stem cells resides in regulating the timely transition from quiescence to proliferation. This function is also suggested by the observation that genetic deletion of *Foxo3* in mice results in premature ovarian failure owing to untimely and increased release of ovarian follicle cells[149]. It is noteworthy that in *C. elegans*[150] and *Drosophila*[151], the FOXO-dependent effects on lifespan are accompanied with lower reproductive rates, suggesting a conserved role for FOXO in a trade-off between soma maintenance (that is, healthy ageing) and fecundity, although the exact mechanisms may be different in these species.

In the central nervous system, FOXO3 also has an essential role in maintaining the quiescent state of neural stem cells in the adult mouse brain. *Foxo3* deletion drives premature neural stem cell differentiation, resulting in the depletion of the neural stem cell pool and brain development defects[142,143,152,153]. In muscle, FOXOs maintain the quiescent state of muscle stem cells, called satellite cells, but they are also required for myoblast differentiation, and regulate myocyte fusion into myotubes later in muscle development or during regeneration.

Satellite cell activation requires FOXO3 inhibition, and its ablation leads to the exhaustion of the satellite cell pool and impaired muscle regeneration[154].

Vascular resident stem or progenitor cells are present in all three layers of the vessel wall, and these cells are mostly quiescent[155], which is regulated by FOXOs[156,157]. In the vessels, FOXOs regulate quiescence by stimulating 2-hydroxyglutarate (2-HG) production[156] (Fig. 5c). Here, FOXO transcriptionally controls genes that encode enzymes involved in branched-chain amino acids. Branched-chain α-keto acids, which are catabolic intermediates of this pathway, are known to inhibit the activity of the mitochondrial α-ketoglutarate dehydrogenase (OGDH) complex. Thus, FOXO-mediated induction of branched-chain amino acid metabolism inhibits OGDH and leads to the build-up of its substrate 2-oxoglutarate (2-OG), which can be reduced to form 2-HG. This leads to the build-up of 2-HG, which ultimately promotes endothelial-cell quiescence. Although the mechanisms by which 2-HG limits cell cycle progression remain unknown, it is likely to be through the inhibition of 2-OG-dependent dioxygenases (reviewed elsewhere[158]) — enzymes that require O_2 and 2-OG as cofactors to catalyse reactions. Several chromatin remodellers, DNA demethylases and the prolyl hydroxylase (regulator of hypoxia-inducible factor 1) belong to this class of enzymes. Thus, abundance of 2-HG could induce hypoxia signalling and epigenetic changes that maintain quiescence.

Interestingly, it was also shown that FOXOs can induce production of 2-HG by direct transcriptional regulation of mutant isocitrate dehydrogenase 1 (IDH1) enzyme in cancer[159] (Fig. 5c). *IDH1* and *IDH2* mutations are found in 70% of lower-grade gliomas, in acute myeloid leukaemia (AML) and in glioblastoma. Wild-type IDH1 catalyses the conversion of isocitrate to 2-OG with the concomitant reduction of NADP to NADPH, and the latter is required to maintain redox homeostasis. Mutant IDH1 and IDH2 convert 2-OG to 2-HG and oxidize NADPH back to NADP, resulting in the loss of redox balance. Accumulation of 2-HG in cancers with mutated IDH1 also contributes to the alteration of the epigenome by inhibition of 2-OG-dependent dioxygenases, leading to a proliferative stem cell state of tumour cells, thereby greatly contributing to tumour development.

Regulation of stem cell redox homeostasis

Several mouse models show that increased ROS levels correlate with impaired stem cell function. Causality of the increased ROS level in stem cell impairment is usually inferred from the use of antioxidants such as NAC. For example, deletion of the ataxia telangiectasia mutated

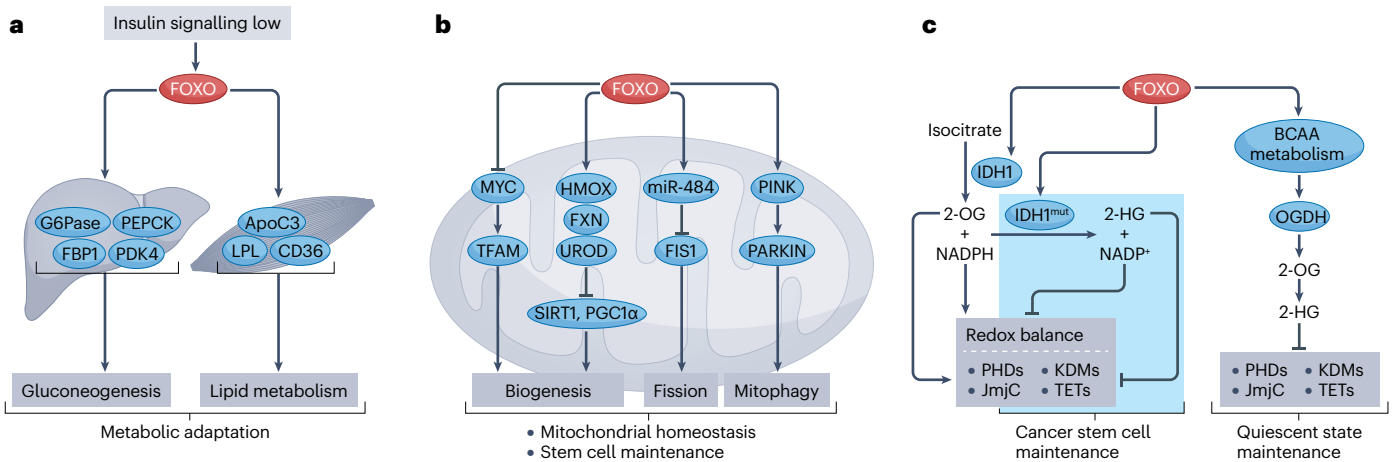


Fig. 5 | FOXO and the regulation of metabolism and cell fate. a, Forkhead box (FOXO) regulates systemic metabolism. Upon low insulin signalling, FOXOs are activated and upregulate the expression of gluconeogenic enzymes (glucose-6-phosphate dehydrogenase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4)) and of genes involved in lipid metabolism (apolipoprotein C-III (ApoC3), lipoprotein lipase (LPL) and fatty acid translocase CD36). In this way, FOXO activity in liver and muscle supports metabolic adaptation in response to low blood glucose. **b**, FOXOs regulate mitochondrial homeostasis. FOXOs inhibit mitochondrial biogenesis, prevent mitochondrial fission and induce mitophagy. The regulation of mitochondrial abundance and activity by FOXOs is important for, but not restricted to, stem cell maintenance and cardiomyocyte function. FOXOs induce the expression of miR-484, which subsequently reduces FIS1 levels, leading to the inhibition of mitochondrial fission. Loss of FOXOs in the small intestine induces mitochondrial fission leading to intestinal stem cell differentiation into secretory cells. In the heart, inhibition of mitochondrial fission by FOXOs prevents apoptosis and reduces the size of myocardial infarction. **c**, FOXOs increase the levels of the metabolites 2-oxoglutarate (2-OG) and 2-hydroxyglutarate (2-HG) by transcriptional regulation of isocitrate dehydrogenase 1 (IDH1) (wild type or mutated (R132H)) and of enzymes of the branched chain amino acid (BCAA) catabolic pathway. Cytosolic IDH1 converts isocitrate to 2-OG with the concomitant reduction of NADP to NADPH, which is the key-reducing equivalent to maintain redox homeostasis. In cancer cells, heterozygous expression of mutant IDH1 (R132H) converts 2-OG into the oncometabolite 2-HG with the concomitant oxidation of NADPH to NADP, leading to redox imbalance. Although 2-OG is required for the catalytic function of 2-OG-dependent dioxygenases, such as propyl hydroxylase domain (PHD) proteins (that regulate the turnover of hypoxia-inducible factor 1α (HIF1α)), ten–eleven translocation methylcytosine dioxygenase (TET) epigenetic regulators and Jumonji-C (JmjC) histone demethylases (KDMs), 2-HG inhibits their activity. Inhibition of these enzymes by 2-HG formation by the mutant IDH1 alters the epigenome, preventing the cell differentiation programme, thereby inducing a cancer stem cell proliferative state. In untransformed endothelial cells, on the other hand, FOXO maintains quiescence by inducing 2-HG. FOXO regulates the transcription of enzymes that regulate BCAA catabolism. This leads to the accumulation of intermediates of BCAA catabolism, which inhibit mitochondrial α-ketoglutarate dehydrogenase (OGDH), an enzyme that converts 2-OG to succinyl-CoA. OGDH inhibition leads to the build-up of 2-OG and facilitates its conversion to 2-HG. In endothelial cells, this results in the maintenance of the quiescent state, potentially through the inhibition of 2-OG-dependent dioxygenases. ACADSB, short/branched chain specific acyl-CoA dehydrogenase; BCKDHB, branched-chain keto acid dehydrogenase E1 subunit-β; DBT, dihydrolipoamide branched chain transacylase.

kinase results in increased levels of ROS in HSCs[160] and HSC dysfunction. Indeed, NAC treatment restores HSC function. In addition, it has been shown that redox-dependent activation of stress kinases such as p38 MAPK limits the self-renewal capacity of ataxia telangiectasia mutated-deficient HSCs[161]. Loss of FOXO3 in the haematopoietic compartment similarly led to elevated ROS levels, p38 MAPK activation and loss of stem cell quiescence and impaired HSC renewal[11,147]. Studies in mice with deletions in *Foxo1*, *Foxo3* and *Foxo4* indicated that HSC depletion owing to loss of these factors in the haematopoietic compartment in 4–5-week-old mice could be rescued by providing NAC to the mice, suggesting that boosting antioxidant capacity and restoring redox homeostasis are key functions for FOXOs in stem cell maintenance[11]. Although increased ROS levels are deleterious for stem cell function, the reverse, namely, that reduced ROS levels improve stem cell function is less clear. Interestingly, others and we have shown that low, physiological levels of ROS are required for proper stem cell maintenance in the small intestine[162,163] and in neuronal stem cells[164]. This would argue that reduced ROS levels would also result in impaired stem cell function.

Metabolic regulation in stem cells

As discussed in the previous sections, FOXOs tightly interplay with metabolic cues, which is relevant to stem cell regulation. Severe metabolic stresses such as fasting, diabetes and cancer can induce muscle atrophy in a FOXO-dependent manner. Starvation leads to the protein degradation primarily by the ubiquitin–proteasome pathway. FOXOs have a critical role in muscle loss by the direct regulation of atrogin-1 — an E3 ubiquitin ligase that drives ubiquitin-mediated protein degradation in skeletal muscle[165]. Previously, it was reported that calorie restriction can increase numbers and activity of satellite cells in young and old mice, indicating that energy deprivation might be beneficial for muscle homeostasis and growth[166]. However, more recent research indicates that fasting slows down muscle regeneration by inducing a deep quiescent state and resilience in satellite cells mediated by ketone bodies — compounds released by the liver during starvation serve as an alternative energy source to glucose. Specifically, ketone body β-hydroxybutyrate promotes quiescence by inhibiting class I and II histone deacetylases and consequent activation of p53 (ref. 167). A potential explanation for the discrepancy between these studies could be that caloric restriction and fasting elicit different metabolic phenotypes (for instance, activation of ketogenesis in the case of fasting but not by caloric restriction). As mentioned previously, FOXOs do induce a metabolic reprogramming to shift from glucose to FA oxidation during fasting (Fig. 5a); however, direct evidence of FOXOs regulating satellite cell function through metabolism requires further research.

During bone healing, skeletal progenitor cells may differentiate into either osteogenic or chondrogenic cells. Osteogenic differentiation is promoted by lipid availability, supplied by the proximity of blood vessels, and the subsequent increase in FA oxidation. Skeletal progenitors in poorly vascularized regions are deprived of lipids and prompt to chondrocyte differentiation — a process driven by FOXOs[168]. Upon bone fracture, FOXOs translocate to the nucleus in poorly vascularized regions, and this can be prevented by stimulation of FA signalling, showing that FOXO activation is dependent on lipid deprivation. Activation of FOXOs induces SOX9 transcription factor, which dictates chondrocyte differentiation and downregulates FA oxidation to support survival under the lipid-scarce metabolic environment. Accordingly, reduced FOXO levels have been associated with osteoarthritis (degeneration of the cartilage around the bones)[169]. Although the detailed mechanism of the signalling cascade needs to be elucidated, this study shows that FOXOs can sense nutrient availability in the local environment and define cell-type specification in response.

Intestinal stem cells are highly proliferative and give rise to two main cell types: absorptive and secretory cells[170]. This balance is tightly regulated by the differential activity of numerous signalling pathways. Intestinal stem cells heavily rely on mitochondrial metabolism to carry out their functions, whereas secretory Paneth cells, which are located adjacent to stem cells, have a reduced number of mitochondria despite sharing the same metabolic niche[163,171]. This implies that over the process of stem cell differentiation towards a secretory cell, a metabolic transition takes place leading to decreased mitochondrial abundance. In the small intestine, stem cells display high levels of FOXO, and depletion of FOXOs in the intestinal epithelium leads to skewed differentiation as reflected by an increase in secretory cell number[172]. FOXO depletion also decreased mitochondrial abundance, activity and increased mitochondrial fission by promoting microRNA-484. Thus, FOXOs maintain the intestinal stem cell state and loss of FOXOs drives stem cells to secretory differentiation by increasing mitochondrial fission and decreasing mitochondrial abundance (Fig. 5b).

In the colon, FOXO3 mediates the inhibitory effect of the bacterial metabolite, butyrate, on stem cell proliferation[173]. FOXOs also control gut barrier integrity of the intestine by regulating mucus secretion and commensalism with the gut microbiome[174]. The key function of the secretory cells — called goblet cells — is to produce and secrete mucus to protect the intestinal epithelium from commensal bacteria and pathogens. Foxo1 deletion in the mouse intestine leads to reduced mucus secretion by Goblet cells. Interestingly, the mechanism of action of FOXOs does not seem to involve transcription factor activity, as Foxo1 nuclear reconstitution fails to restore this phenotype. Rather, the role of FOXOs in this context has been linked to the regulation of autophagy proteins and subsequent ROS. This agrees with earlier observations that suggest cytosolic rather than nuclear FOXOs in the regulation of autophagy[175]. Mechanistically, the defect in mucus secretion does not seem to alter the gut barrier integrity directly. Instead, altered mucus secretion leads to dysbiosis (altered microbiome) and perturbation of microbial metabolites, which seems responsible for the enhanced permeability of the gut barrier in the *Foxo1* null mice. Importantly, FOXO function in this model is particularly relevant under stress, for example, when the epithelial barrier is already perturbed. Moreover, although young mice with intestinal *Foxo1* deletion show a normal tissue morphology in unperturbed conditions, aged mice display increased immune-cell infiltrates throughout the intestine resulting in inflammation. This highlights that although under basal conditions FOXO activity or the lack of FOXO activity does not yield observable major phenotypes, it does become key in response and adaptation to stress and in ageing.

The aforementioned illustrates the intricate nature of FOXO regulation of metabolism, especially in relation to stem cell control and cell specification. Notably, in line with being master regulators of stress adaptation, FOXO maintenance of integrity and function become particularly important when tissues age, are under stress or undergo healing and repair.

Conclusions and perspective

In normal cells, the primary purpose of the stress response is to alleviate the disruption and prevent transition towards a dysfunctional state. However, impaired function can come in several flavours. First, cells can lose only in part their optimal normal function, or cells can

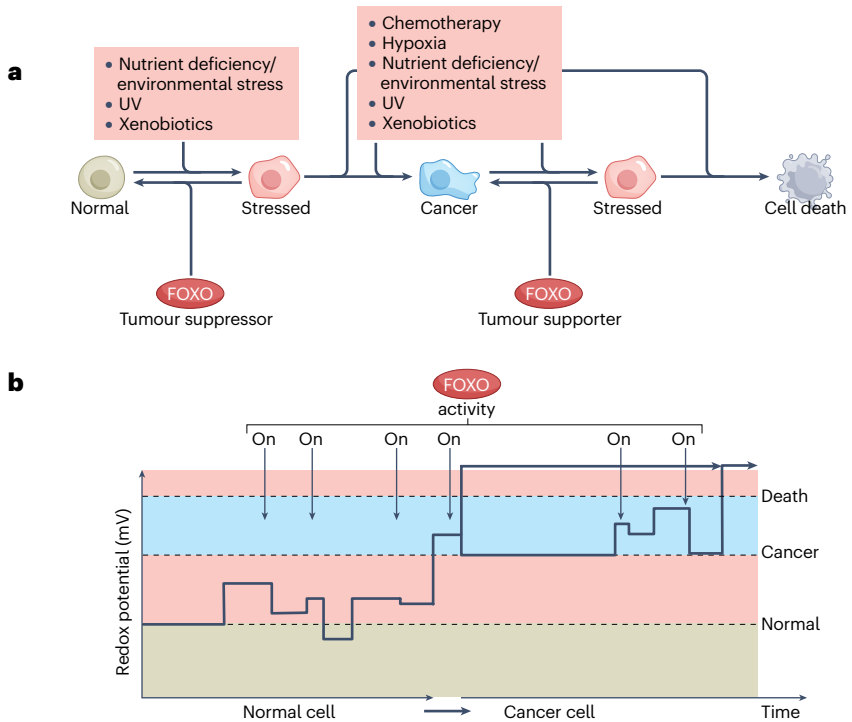


Fig. 6 | A model illustrating how FOXO-dependent stress survival could sustain both healthy and pathological conditions. a, To illustrate how stress survival downstream of forkhead box (FOXO) applies both in normal and pathological conditions, we use cancer as an example. Cells are frequently challenged by stresses, such as excessive levels of reactive oxygen species, and limited availability of nutrients or exogenous sources such as ultraviolet (UV) and xenobiotics. This pushes cells into a stressed state that leads to FOXO activation, which in turn increases the expression of genes that lower the stress input (antioxidant enzymes and metabolic rewiring) and counteract consequences of stress (repair of damage). When the stress is resolved, FOXO is switched off again, hence cells oscillate between normal and stressed states where FOXO mediates reversion to the normal, unstressed state, thereby maintaining organismal homeostasis and fitness. When stress is insufficiently counteracted, this can result in, for example, permanent damage (DNA mutations). Unless cleared by apoptosis, the surviving damaged cell represents a new ground state that can contribute to disease owing to its dysfunctional properties, such as derailed proliferation in the case of cancer. The cancer cell, however, is challenged by the same stresses as normal cells but faces additional challenges such as hypoxia and anticancer therapies. This generates a new oscillation, now between the 'normal' and the stressed cancer cells and FOXOs can contribute to reversion to the unstressed state in the same manner as in normal cells, promoting cancer cell survival with pathological consequences for the organism. If the stress cannot be resolved, for instance, owing to (partial) FOXO inactivation downstream of oncogenic signalling or at levels of stress that lead to irreparable damage, cells may ultimately encounter a level of stress that results in cell death. Note that permanent activation of FOXO would lead to cell cycle arrest, meaning that (partial) FOXO inactivation is likely required for proliferation and cancer progression. **b**, FOXOs are best known for responding to and controlling cellular redox homeostasis and to illustrate the model of FOXO contribution to stress management in the transition from a healthy to a cancer cell in part **a**, we show here how changes in the cellular redox state overlay the process of stress adaptation by FOXOs. Upon a rise in redox potential either because of enhanced reactive oxygen species production or loss of sufficient NADPH regeneration to provide reductive capacity, FOXOs are switched 'on' and FOXO activity can lower the redox potential again. FOXOs may be unable to sufficiently counteract a change in redox potential, for instance, owing to (partial) FOXO inactivation by upstream signalling or limited availability of cofactors or nutrients, or simply because the stress level outcompetes FOXO-dependent mechanisms counteracting stress. In this case, the cell enters a new ground state (stressed cell), at which the basal redox potential is chronically increased compared with the normal cell or engages apoptosis. What determines exactly whether apoptosis is engaged may be stochastic or dependent on the apoptotic threshold, which is cell-type-dependent and context-dependent. But importantly, FOXOs now aid in survival of these stressed cells with increased basal redox potential. These oscillations between enhanced stress and FOXO-dependent stress relief repeat, resulting eventually in the emergence of cancer cells, which are known to have a higher basal redox potential[206]. Further increases in stress in these cells, for instance, owing to chemotherapy, will again activate FOXO, which now contributes to stress relief and therapy resistance in the tumour, thus sustaining the pathological condition. But, the already higher redox potential in cancer cells also makes them more prone to succumb to cell death when FOXO-dependent stress survival insufficiently counteracts an additional oxidative insult.

become fully dysfunctional and transit, for example, to a senescent phenotype. Cells can also become dysfunctional by adopting a new functionality, for example, the transition of a normal cell to a cancer cell, or cells become dysfunctional but consequently are removed by, for example, a form of programmed cell death, possibly followed by regeneration. The FOXO-induced stress response primarily acts to prevent the transition to these dysfunctional states. This is best illustrated by the observation that FOXOs are acclaimed bona fide tumour suppressors as broad somatic deletion of three *Foxo* isoforms establishes a progressive cancer-prone condition characterized by thymic lymphomas and haemangiomas[103]. However, in a mouse breast cancer model, both short hairpin RNA-mediated loss of FOXOs and

overexpression of a constitutively active FOXO3 mutant (FOXO3.A3) suppressed tumour growth and metastasis, whereas overexpression of wild-type FOXO3 had no effect[176]. This indicates that FOXO activity is also relevant during tumour progression, as either loss or constitutive activation may be deleterious. Also, in AML, it has been shown that in approximately 40% of the patient samples FOXOs are active and that loss of FOXO in AML cells hampered leukaemic cell growth and improved animal survival in a mouse model for AML[177]. Thus, the FOXO stress response is not only directed at preventing the transition to a dysfunctional (cancer) cell but also contributes to the survival of the dysfunctional cancer cell, as suggested by its tumour-promoting role. Clearly, mechanisms of stress resistance are not restricted to normal cells and dysfunctional cells, as cancer cells also endure stress. From the perspective of FOXOs as being activated by stress and acting to preserve and protect cells from stress, it follows that FOXOs will react and act in the dysfunctional cells similarly (Fig. 6a). This support of aberrant-cell survival is not restricted to cancer and may apply to other human diseases. In chronic (fatty) liver disease, recurrent FOXO gain-of-function mutations (Ser22Ala) are observed[178]. Here, it is argued that FOXOs enable survival of the dysfunctional cells by inducing gene expression that helps in coping with high lipid levels.

A major stress input for FOXOs derives from changes in cellular redox status (reviewed previously[46,105]) and, as discussed, FOXOs can increase transcription of redox regulators to lower cellular redox stress. Normal cells have a lower basal redox potential compared with cancer cells[179] and, thus, the only difference with respect to FOXO function in normal versus cancer cells could be to maintain a low versus higher redox potential, respectively (Fig. 6b). As many types of stress (nutrient, genotoxic and so on) function at least in part through changing the cellular redox balance and a plethora of diseases have been linked to enhanced oxidative damage, this universal role of maintaining a defined cellular redox potential through the FOXO stress response may contribute to many of the age-related diseases.

Finally, we observed that FOXOs transcriptionally control the expression of wild-type but also mutant IDH1 (ref. 159). In this case, the physiological role of FOXOs is repurposed in cancer to fuel epigenetic changes associated with tumorigenesis.

The regulation of the stress response by FOXOs and how this results in not only preventing but also sustaining disease are certainly not unique to FOXOs. NRF2 and p53 are important other transcription regulators of the stress response and for both similar apparently paradoxical outcomes have been described, especially in the context of cancer initiation and progression. Similar to FOXOs, NRF2 integrates cellular stress signals to protect from oxidative stress through transcription regulation. Also, for NRF2, it has been questioned whether NRF2 is a tumour suppressor or, conversely, a tumour supporter (discussed in ref. 180). This controversy can also be settled by considering that NRF2 similar to FOXOs regulates the stress response in both normal and malignant cells (discussed for NRF2 in ref. 181). The importance of context also accounts for p53 function in the stress response. p53 was originally identified as an oncogene[182], and only later it became the archetypical tumour suppressor gene. But more recently, studies have shown that wild-type p53 function can also protect some cancers from, for instance, metabolic and redox stress[183].

Of note, prevention of age-related disease does not translate per se into an extended maximum lifespan. It does translate, however, to the period of our lives that we live in good health (healthy lifespan). Thus, it is important to discriminate between the role (roles) of FOXOs in maximum lifespan versus healthy lifespan. We propose that part of the paradox, which may originate from studying FOXOs function in extended lifespan versus delayed onset of ageing, comes from the fact that the role of FOXOs in mediating stress adaptation is ubiquitous among cell types. In long-lived species, FOXOs eventually will provide the same survival benefit to malignant and dysfunctional cells and thereby actually may contribute to the loss of bodily functions, with cancer progression probably as the best example.

Published online: 14 September 2023

References

1. Gems, D. et al. Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. *Genetics* **150**, 129–155 (1998).
2. Murphy, C. T. et al. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* **424**, 277–283 (2003).
3. Lee, S. S., Kennedy, S., Tolonen, A. C. & Ruvkun, G. DAF-16 target genes that control *C. elegans* life-span and metabolism. *Science* **300**, 644–647 (2003).
4. Jenkins, N. L., McColl, G. & Lithgow, G. J. Fitness cost of extended lifespan in *Caenorhabditis elegans*. *Proc. Biol. Sci.* **271**, 2523–2526 (2004).
5. Kenyon, C. The plasticity of aging: insights from long-lived mutants. *Cell* **120**, 449–460 (2005).
   This is one of the first papers to show that a single specific genetic mutation (*daf-2*) can increase lifespan and can be reverted by a second mutation (*daf-16*), revealing a connection between insulin signalling and lifespan.
6. Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. A. *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464 (1993).
7. Willcox, B. J. et al. FOXO3A genotype is strongly associated with human longevity. *Proc. Natl Acad. Sci. USA* **105**, 13987–13992 (2008).
8. Santo, E. E. et al. FOXO3A-short is a novel regulator of non-oxidative glucose metabolism associated with human longevity. *Aging Cell* **22**, e13763 (2023).
9. Eijkelenboom, A. & Burgering, B. M. FOXOs: signalling integrators for homeostasis maintenance. *Nat. Rev. Mol. Cell Biol.* **14**, 83–97 (2013).
10. Liang, R. & Ghaffari, S. Stem cells seen through the FOXO lens: an evolving paradigm. *Curr. Top. Dev. Biol.* **127**, 23–47 (2018).
11. Tothova, Z. et al. FoxOs are critical mediators of hematopoietic stem cell resistance physiologic oxid. stress. *Cell* **128**, 325–339 (2007).
    This study shows the redundancy of FOXO1, FOXO3 and FOXO4 in HSC maintenance and that antioxidant defence downstream of FOXO is a key driver of stem cell maintenance.
12. Shimokawa, I. et al. The life-extending effect of dietary restriction requires Foxo3 in mice. *Aging Cell* **14**, 707–709 (2015).
13. Hwang, I. et al. FOXO protects against age-progressive axonal degeneration. *Aging Cell* **17**, e12701 (2018).
14. Du, S. et al. FoxO3 deficiency in cortical astrocytes leads to impaired lipid metabolism and aggravated amyloid pathology. *Aging Cell* **20**, e13432 (2021).
15. Hedrick, S. M., Hess Michelini, R., Doedens, A. L., Goldrath, A. W. & Stone, E. L. FOXO transcription factors throughout T cell biology. *Nat. Rev. Immunol.* **12**, 649–661 (2012).
16. Calissi, G., Lam, E. W. & Link, W. Therapeutic strategies targeting FOXO transcription factors. *Nat. Rev. Drug Discov.* **20**, 21–38 (2021).
17. Brown, A. K. & Webb, A. E. Regulation of FOXO factors in mammalian cells. *Curr. Top. Dev. Biol.* **127**, 165–192 (2018).
18. Calnan, D. R. & Brunet, A. The FoxO code. *Oncogene* **27**, 2276–2288 (2008).
19. Franz, F. et al. The transcriptional regulation of FOXO genes in thyrocytes. *Horm. Metab. Res.* **48**, 601–606 (2016).
20. Urbanek, P. & Klotz, L. O. Posttranscriptional regulation of FOXO expression: microRNAs and beyond. *Br. J. Pharmacol.* **174**, 1514–1532 (2017).
21. Asmamaw, M. D., Liu, Y., Zheng, Y. C., Shi, X. J. & Liu, H. M. Skp2 in the ubiquitin–proteasome system: a comprehensive review. *Med. Res. Rev.* **40**, 1920–1949 (2020).
22. Brenkman, A. B., de Keizer, P. L., van den Broek, N. J., Jochemsen, A. G. & Burgering, B. M. Mdm2 induces mono-ubiquitination of FOXO4. *PLoS ONE* **3**, e2819 (2008).
23. Huang, H. & Tindall, D. J. Regulation of FOXO protein stability via ubiquitination and proteasome degradation. *Biochim. Biophys. Acta* **1813**, 1961–1964 (2011).
24. Wang, X., Wang, J. & Jiang, X. MdmX protein is essential for Mdm2 protein-mediated p53 polyubiquitination. *J. Biol. Chem.* **286**, 23725–23734 (2011).
25. Grossman, S. R. et al. Polyubiquitination of p53 by a ubiquitin ligase activity of p300. *Science* **300**, 342–344 (2003).
26. Zhou, B. P. et al. HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nat. Cell Biol.* **3**, 973–982 (2001).
27. Mayo, L. D. & Donner, D. B. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc. Natl Acad. Sci. USA* **98**, 11598–11603 (2001).
28. van der Horst, A. et al. FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. *Nat. Cell Biol.* **8**, 1064–1073 (2006).

29. Heimbucher, T. & Hunter, T. The *C. elegans* ortholog of USP7 controls DAF-16 stability in insulin/IGF-1-like signaling. *Worm* **4**, e1103429 (2015).
30. Kops, G. J. et al. Direct control of the forkhead transcription factor AFX by protein kinase B. *Nature* **398**, 630–634 (1999).
31. Brunet, A. et al. Akt promotes cell survival by phosphorylating and inhibiting a forkhead transcription factor. *Cell* **96**, 857–868 (1999).
This study and the study by Kops et al. (1999) are the first to show that the regulation of FOXOs, the orthologues of DAF-16 in mammalians, are directly controlled by AKT and PI3K signalling, thereby showing evolutionary conservation.
32. Brownawell, A. M., Kops, G. J., Macara, I. G. & Burgering, B. M. Inhibition of nuclear import by protein kinase B (Akt) regulates the subcellular distribution and activity of the forkhead transcription factor AFX. *Mol. Cell Biol.* **21**, 3534–3546 (2001).
33. Essers, M. A. et al. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J.* **23**, 4802–4812 (2004).
34. Putker, M. et al. Redox-dependent control of FOXO/DAF-16 by transportin-1. *Mol. Cell* **49**, 730–742 (2013).
35. Putker, M. et al. Evolutionary acquisition of cysteines determines FOXO paralog-specific redox signaling. *Antioxid. Redox Signal.* **22**, 15–28 (2015).
36. Zaret, K. S. & Carroll, J. S. Pioneer transcription factors: establishing competence for gene expression. *Genes Dev.* **25**, 2227–2241 (2011).
37. Clark, K. L., Halay, E. D., Lai, E. & Burley, S. K. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature* **364**, 412–420 (1993).
38. Wang, F. et al. Biochemical and structural characterization of an intramolecular interaction in FOXO3a and its binding with p53. *J. Mol. Biol.* **384**, 590–603 (2008).
39. Bourgeois, B. et al. Multiple regulatory intrinsically disordered motifs control FOXO4 transcription factor binding and function. *Cell Rep.* **36**, 109446 (2021).
40. Obsil, T. & Obsilova, V. Structural basis for DNA recognition by FOXO proteins. *Biochim. Biophys. Acta* **1813**, 1946–1953 (2011).
41. Li, J. et al. Mechanism of forkhead transcription factors binding to a novel palindromic DNA site. *Nucleic Acids Res.* **49**, 3573–3583 (2021).
42. Psenakova, K. et al. Forkhead domains of FOXO transcription factors differ in both overall conformation and dynamics. *Cells* **8**, 966 (2019).
43. Sugase, K., Dyson, H. J. & Wright, P. E. Mechanism of coupled folding and binding of an intrinsically disordered protein. *Nature* **447**, 1021–1025 (2007).
44. Shoemaker, B. A., Portman, J. J. & Wolynes, P. G. Speeding molecular recognition by using the folding funnel: the fly-casting mechanism. *Proc. Natl Acad. Sci. USA* **97**, 8868–8873 (2000).
45. Dansen, T. B. et al. Redox-sensitive cysteines bridge p300/CBP-mediated acetylation and FoxO4 activity. *Nat. Chem. Biol.* **5**, 664–672 (2009).
This study is among the first to show that redox signalling, similar to growth factor signalling, proceeds through protein–protein interactions that are enforced by redox-sensitive cysteine disulfide bridges.
46. Sies, H. & Jones, D. P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* **21**, 363–383 (2020).
47. van der Horst, A. et al. FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2. *J. Biol. Chem.* **279**, 28873–28879 (2004).
48. Yoshimochi, K., Daitoku, H. & Fukamizu, A. PCAF represses transactivation function of FOXO1 in an acetyltransferase-independent manner. *J. Recept. Signal Transduct. Res.* **30**, 43–49 (2010).
49. Adamowicz, M., Vermezovic, J. & d'Adda di Fagagna, F. NOTCH1 inhibits activation of ATM by impairing the formation of an ATM-FOXO3a-KAT5/Tip60 complex. *Cell Rep.* **16**, 2068–2076 (2016).
50. Brunet, A. et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303**, 2011–2015 (2004).
This study, together with van der Horst et al. (2004) provides a mechanistic link between FOXO and SIRT, which were independently shown to affect lifespan.
51. Daitoku, H. et al. Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. *Proc. Natl Acad. Sci. USA* **101**, 10042–10047 (2004).
52. Tseng, A. H., Wu, L. H., Shieh, S. S. & Wang, D. L. SIRT3 interactions with FOXO3 acetylation, phosphorylation and ubiquitinylation mediate endothelial cell responses to hypoxia. *Biochem. J.* **464**, 157–168 (2014).
53. Mihaylova, M. M. et al. Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. *Cell* **145**, 607–621 (2011).
54. Daitoku, H., Sakamaki, J. & Fukamizu, A. Regulation of FoxO transcription factors by acetylation and protein–protein interactions. *Biochim. Biophys. Acta* **1813**, 1954–1960 (2011).
55. Riedel, C. G. et al. DAF-16 employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. *Nat. Cell Biol.* **15**, 491–501 (2013).
56. Webb, A. E. & Brunet, A. FOXO flips the longevity SWItch. *Nat. Cell Biol.* **15**, 444–446 (2013).
57. Mattila, J., Kallijarvi, J. & Puig, O. RNAi screening for kinases and phosphatases identifies FoxO regulators. *Proc. Natl Acad. Sci. USA* **105**, 14873–14878 (2008).
58. Liu, J. et al. Targeting the BRD4/FOXO3a/CDK6 axis sensitizes AKT inhibition in luminal breast cancer. *Nat. Commun.* **9**, 5200 (2018).
59. Webb, A. E., Kundaje, A. & Brunet, A. Characterization of the direct targets of FOXO transcription factors throughout evolution. *Aging Cell* **15**, 673–685 (2016).
60. Hao, N. & O'Shea, E. K. Signal-dependent dynamics of transcription factor translocation controls gene expression. *Nat. Struct. Mol. Biol.* **19**, 31–39 (2011).
61. Demirbas, B. et al. Control of *C. elegans* growth arrest by stochastic, yet synchronized DAF-16/FOXO nuclear translocation pulses. Preprint at *bioRxiv* https://doi.org/10.1101/2023.07.05.547674 (2023).
62. Lasick, K. A. et al. FOXO nuclear shuttling dynamics are stimulus-dependent and correspond with cell fate. *Mol. Biol. Cell* **34**, ar21 (2023).
63. Hatta, M. & Cirillo, L. A. Chromatin opening and stable perturbation of core histone: DNA contacts by FoxO1. *J. Biol. Chem.* **282**, 35583–35593 (2007).
64. Hatta, M., Liu, F. & Cirillo, L. A. Acetylation curtails nucleosome binding, not stable nucleosome remodeling, by FoxO1. *Biochem. Biophys. Res. Commun.* **379**, 1005–1008 (2009).
65. Eijkelenboom, A., Mokry, M., Smits, L. M., Nieuwenhuis, E. E. & Burgering, B. M. FOXO3 selectively amplifies enhancer activity to establish target gene regulation. *Cell Rep.* **5**, 1664–1678 (2013).
66. Newman, J. R. et al. Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature* **441**, 840–846 (2006).
67. Allgayer, J., Kitsera, N., Bartelt, S., Epe, B. & Khobta, A. Widespread transcriptional gene inactivation initiated by a repair intermediate of 8-oxoguanine. *Nucleic Acids Res.* **44**, 7267–7280 (2016).
68. Desai, R. V. et al. A DNA repair pathway can regulate transcriptional noise to promote cell fate transitions. *Science* **373**, eabc6506 (2021).
69. Raser, J. M. & O'Shea, E. K. Noise in gene expression: origins, consequences, and control. *Science* **309**, 2010–2013 (2005).
70. Munsky, B., Neuert, G. & van Oudenaarden, A. Using gene expression noise to understand gene regulation. *Science* **336**, 183–187 (2012).
71. Wong, V. C. et al. NF-kappaB-chromatin interactions drive diverse phenotypes by modulating transcriptional noise. *Cell Rep.* **22**, 585–599 (2018).
72. Comandante-Lou, N., Baumann, D. G. & Fallahi-Sichani, M. AP-1 transcription factor network explains diverse patterns of cellular plasticity in melanoma cells. *Cell Rep.* **40**, 111147 (2022).
73. Bahar, R. et al. Increased cell-to-cell variation in gene expression in ageing mouse heart. *Nature* **441**, 1011–1014 (2006).
74. Somel, M., Khaitovich, P., Bahn, S., Paabo, S. & Lachmann, M. Gene expression becomes heterogeneous with age. *Curr. Biol.* **16**, R359–R360 (2006).
75. Rangaraju, S. et al. Suppression of transcriptional drift extends *C. elegans* lifespan by postponing the onset of mortality. *eLife* **4**, e08833 (2015).
76. Cheung, P. et al. Single-cell chromatin modification profiling reveals increased epigenetic variations with aging. *Cell* **173**, 1385–1397.e14 (2018).
77. Burgess, D. J. Human epigenetics: showing your age. *Nat. Rev. Genet.* **14**, 6 (2013).
78. Booth, L. N. & Brunet, A. The aging epigenome. *Mol. Cell* **62**, 728–744 (2016).
79. Fielenbach, N. & Antebi, A. *C. elegans* Dauer formation and the molecular basis of plasticity. *Genes Dev.* **22**, 2149–2165 (2008).
80. Medema, R. H., Kops, G. J., Bos, J. L. & Burgering, B. M. AFX-like forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1. *Nature* **404**, 782–787 (2000).
This study links FOXO function to inhibition of the cell cycle, thereby suggesting a role for FOXOs in tissue homeostasis and cancer.
81. Furukawa-Hibi, Y., Yoshida-Araki, K., Ohta, T., Ikeda, K. & Motoyama, N. FOXO forkhead transcription factors induce G(2)-M checkpoint in response to oxidative stress. *J. Biol. Chem.* **277**, 26729–26732 (2002).
82. Hornsveld, M. et al. A FOXO-dependent replication checkpoint restricts proliferation of damaged cells. *Cell Rep.* **34**, 108675 (2021).
83. Sekimoto, T., Fukumoto, M. & Yoneda, Y. 14-3-3 Suppresses the nuclear localization of threonine 157-phosphorylated p27(Kip1). *EMBO J.* **23**, 1934–1942 (2004).
84. Blain, S. W. & Massague, J. Breast cancer banishes p27 from nucleus. *Nat. Med.* **8**, 1076–1078 (2002).
85. Gao, D. et al. Phosphorylation by Akt1 promotes cytoplasmic localization of Skp2 and impairs APCCdh1-mediated Skp2 destruction. *Nat. Cell Biol.* **11**, 397–408 (2009).
86. Huang, H. et al. Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. *Proc. Natl Acad. Sci. USA* **102**, 1649–1654 (2005).
87. Shtivelman, E., Sussman, J. & Stokoe, D. A role for PI 3-kinase and PKB activity in the G2/M phase of the cell cycle. *Curr. Biol.* **12**, 919–924 (2002).
88. Kamura, T. et al. Cytoplasmic ubiquitin ligase KPC regulates proteolysis of p27(Kip1) at G1 phase. *Nat. Cell Biol.* **6**, 1229–1235 (2004).
89. Ou, L. et al. Incomplete folding upon binding mediates Cdk4/cyclin D complex activation by tyrosine phosphorylation of inhibitor p27 protein. *J. Biol. Chem.* **286**, 30142–30151 (2011).
90. Coller, H. A., Sang, L. & Roberts, J. M. A new description of cellular quiescence. *PLoS Biol.* **4**, e83 (2006).
91. Grana, X., Garriga, J. & Mayol, X. Role of the retinoblastoma protein family, pRB, p107 and p130 in the negative control of cell growth. *Oncogene* **17**, 3365–3383 (1998).
92. Smith, E. J., Leone, G., DeGregori, J., Jakoi, L. & Nevins, J. R. The accumulation of an E2F-p130 transcriptional repressor distinguishes a G0 cell state from a G1 cell state. *Mol. Cell Biol.* **16**, 6965–6976 (1996).
93. Kops, G. J. et al. Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors. *Mol. Cell Biol.* **22**, 2025–2036 (2002).
94. Campisi, J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* **120**, 513–522 (2005).

95. Nogueira, V. et al. Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. *Cancer Cell* **14**, 458–470 (2008).
96. de Keizer, P. L. et al. Activation of forkhead box O transcription factors by oncogenic BRAF promotes p21cip1-dependent senescence. *Cancer Res.* **70**, 8526–8536 (2010).
97. Baar, M. P. et al. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell* **169**, 132–147.e16 (2017).
This paper shows that FOXOs can be a target for the specific elimination of senescent cells in order to mitigate age-related decline.
98. Munoz-Espin, D. et al. Programmed cell senescence during mammalian embryonic development. *Cell* **155**, 1104–1118 (2013).
99. Storer, M. et al. Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell* **155**, 1119–1130 (2013).
100. Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11**, 298–300 (1956).
101. Honda, Y. & Honda, S. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J.* **13**, 1385–1393 (1999).
This is the first study to show that DAF-16 regulates the expression of antioxidant enzymes, providing a link between the free radical theory of ageing and DAF-16-dependent lifespan extension.
102. Honda, Y. & Honda, S. Life span extensions associated with upregulation of gene expression of antioxidant enzymes in *Caenorhabdms elegans*; studies of mutation in the AGE-1, PI3 kinase homologue and short-term exposure to hyperoxia. *J. Am. Aging Assoc.* **24**, 179–186 (2001).
103. Paik, J. H. et al. FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell* **128**, 309–323 (2007).
104. Klotz, L. O. et al. Redox regulation of FoxO transcription factors. *Redox Biol.* **6**, 51–72 (2015).
105. Sies, H. et al. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat. Rev. Mol. Cell Biol.* **23**, 499–515 (2022).
106. Netto, L. E. S. & Machado, L. Preferential redox regulation of cysteine-based protein tyrosine phosphatases: structural and biochemical diversity. *FEBS J.* **289**, 5480–5504 (2022).
107. Snyder, N. A. & Silva, G. M. Deubiquitinating enzymes (DUBs): regulation, homeostasis, and oxidative stress response. *J. Biol. Chem.* **297**, 101077 (2021).
108. Wang, Y. & Hekimi, S. Mitochondrial dysfunction and longevity in animals: untangling the knot. *Science* **350**, 1204–1207 (2015).
109. Van Raamsdonk, J. M. & Hekimi, S. Superoxide dismutase is dispensable for normal animal lifespan. *Proc. Natl Acad. Sci. USA* **109**, 5785–5790 (2012).
110. Hoehne, M. N. et al. Spatial and temporal control of mitochondrial H(2) O(2) release in intact human cells. *EMBO J.* **41**, e109169 (2022).
111. Saeedi Saravi, S. S. et al. Differential endothelial signaling responses elicited by chemogenetic H(2)O(2) synthesis. *Redox Biol.* **36**, 101605 (2020).
112. Gross, D. N., van den Heuvel, A. P. & Birnbaum, M. J. The role of FoxO in the regulation of metabolism. *Oncogene* **27**, 2320–2336 (2008).
113. Postic, C., Dentin, R. & Girard, J. Role of the liver in the control of carbohydrate and lipid homeostasis. *Diabetes Metab.* **30**, 398–408 (2004).
114. Altomonte, J. et al. Foxo1 mediates insulin action on apoC-III and triglyceride metabolism. *J. Clin. Invest.* **114**, 1493–1503 (2004).
115. Bastie, C. C. et al. FoxO1 stimulates fatty acid uptake and oxidation in muscle cells through CD36-dependent and -independent mechanisms. *J. Biol. Chem.* **280**, 14222–14229 (2005).
116. Kamei, Y. et al. A forkhead transcription factor FKHR up-regulates lipoprotein lipase expression in skeletal muscle. *FEBS Lett.* **536**, 232–236 (2003).
117. Belgardt, B. F. et al. PDK1 deficiency in POMC-expressing cells reveals FOXO1-dependent and -independent pathways in control of energy homeostasis and stress response. *Cell Metab.* **7**, 291–301 (2008).
118. Kim, M. S. et al. Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. *Nat. Neurosci.* **9**, 901–906 (2006).
119. Kitamura, T. et al. Forkhead protein FoxO1 mediates Agrp-dependent effects of leptin on food intake. *Nat. Med.* **12**, 534–540 (2006).
120. Ren, H. et al. FoxO1 target Gpr17 activates AgRP neurons to regulate food intake. *Cell* **149**, 1314–1326 (2012).
121. Peck, B., Ferber, E. C. & Schulze, A. Antagonism between FOXO and MYC regulates cellular powerhouse. *Front. Oncol.* **3**, 96 (2013).
122. Li, F. et al. Myc stimulates nuclearly encoded mitochondrial genes and mitochondrial biogenesis. *Mol. Cell Biol.* **25**, 6225–6234 (2005).
123. Ferber, E. C. et al. FOXO3a regulates reactive oxygen metabolism by inhibiting mitochondrial gene expression. *Cell Death Differ.* **19**, 968–979 (2012).
124. Cheng, Z. et al. Foxo1 integrates insulin signaling with mitochondrial function in the liver. *Nat. Med.* **15**, 1307–1311 (2009).
125. Chan, D. C. Mitochondrial dynamics and its involvement in disease. *Annu. Rev. Pathol.* **15**, 235–259 (2020).
126. Wang, K. et al. miR-484 regulates mitochondrial network through targeting Fis1. *Nat. Commun.* **3**, 781 (2012).
127. Mei, Y. et al. FOXO3a-dependent regulation of Pink1 (Park6) mediates survival signaling in response to cytokine deprivation. *Proc. Natl Acad. Sci. USA* **106**, 5153–5158 (2009).
128. Cheng, Z. FoxO transcription factors in mitochondrial homeostasis. *Biochem. J.* **479**, 525–536 (2022).
129. Munoz-Martin, N., Sierra, R., Schimmang, T., Villa Del Campo, C. & Torres, M. Myc is dispensable for cardiomyocyte development but rescues Mycn-deficient hearts through functional replacement and cell competition. *Development* **146**, dev170753 (2019).
130. Muncan, V. et al. Rapid loss of intestinal crypts upon conditional deletion of the Wnt/Tcf-4 target gene c-Myc. *Mol. Cell Biol.* **26**, 8418–8426 (2006).
131. Sansom, O. J. et al. Myc deletion rescues Apc deficiency in the small intestine. *Nature* **446**, 676–679 (2007).
132. Lettieri-Barbato, D. et al. FoxO1 localizes to mitochondria of adipose tissue and is affected by nutrient stress. *Metabolism* **95**, 84–92 (2019).
133. Caballero-Caballero, A. et al. Mitochondrial localization of the forkhead box class O transcription factor FOXO3a in brain. *J. Neurochem.* **124**, 749–756 (2013).
134. Spinelli, J. B. & Haigis, M. C. The multifaceted contributions of mitochondria to cellular metabolism. *Nat. Cell Biol.* **20**, 745–754 (2018).
135. Chakrabarty, R. P. & Chandel, N. S. Mitochondria as signaling organelles control mammalian stem cell fate. *Cell Stem Cell* **28**, 394–408 (2021).
136. Klimovich, A. et al. Non-senescent hydra tolerates severe disturbances in the nuclear lamina. *Aging* **10**, 951–972 (2018).
137. Martinez, D. E. Mortality patterns suggest lack of senescence in hydra. *Exp. Gerontol.* **33**, 217–225 (1998).
138. Boehm, A. M. et al. FoxO is a critical regulator of stem cell maintenance in immortal Hydra. *Proc. Natl Acad. Sci. USA* **109**, 19697–19702 (2012).
139. Bridge, D. et al. FoxO and stress responses in the cnidarian *Hydra vulgaris*. *PLoS ONE* **5**, e11686 (2010).
This is the first paper to show that FOXO is expressed in the cnidarian *H. vulgaris*, and that FOXO-dependent adaptation to stress was introduced early in animal evolution.
140. Schultz, M. B. & Sinclair, D. A. When stem cells grow old: phenotypes and mechanisms of stem cell aging. *Development* **143**, 3–14 (2016).
141. Li, L. & Clevers, H. Coexistence of quiescent and active adult stem cells in mammals. *Science* **327**, 542–545 (2010).
142. Paik, J. H. et al. FoxOs cooperatively regulate diverse pathways governing neural stem cell homeostasis. *Cell Stem Cell* **5**, 540–553 (2009).
143. Renault, V. M. et al. FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell* **5**, 527–539 (2009).
144. Garcia-Prat, L. et al. FoxO maintains a genuine muscle stem-cell quiescent state until geriatric age. *Nat. Cell Biol.* **22**, 1307–1318 (2020).
145. Wang, G. et al. p110alpha of PI3K is necessary and sufficient for quiescence exit in adult muscle satellite cells. *EMBO J.* **37**, e98239 (2018).
146. Yue, F. et al. Pten is necessary for the quiescence and maintenance of adult muscle stem cells. *Nat. Commun.* **8**, 14328 (2017).
147. Miyamoto, K. et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* **1**, 101–112 (2007).
148. Tothova, Z. & Gilliland, D. G. FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. *Cell Stem Cell* **1**, 140–152 (2007).
149. Castrillon, D. H., Miao, L., Kollipara, R., Horner, J. W. & DePinho, R. A. Suppression of ovarian follicle activation in mice by the transcription factor Foxo3a. *Science* **301**, 215–218 (2003).
This study establishes a link between FOXO function and fecundity in mice, thereby establishing a FOXO-dependent evolutionary conserved trade-off between fecundity and lifespan.
150. Tissenbaum, H. A. & Ruvkun, G. An insulin-like signaling pathway affects both longevity and reproduction in *Caenorhabditis elegans*. *Genetics* **148**, 703–717 (1998).
151. Giannakou, M. E. et al. Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* **305**, 361 (2004).
152. Schaffner, I. et al. FoxO function is essential for maintenance of autophagic flux and neuronal morphogenesis in adult neurogenesis. *Neuron* **99**, 1188–1203.e6 (2018).
153. Yeo, H. et al. FoxO3 coordinates metabolic pathways to maintain redox balance in neural stem cells. *EMBO J.* **32**, 2589–2602 (2013).
154. Gopinath, S. D., Webb, A. E., Brunet, A. & Rando, T. A. FOXO3 promotes quiescence in adult muscle stem cells during the process of self-renewal. *Stem Cell Rep.* **2**, 414–426 (2014).
This paper, together with Garcia-Prat et al. (2020), shows the role of FOXOs and redox regulation in quiescent adult (muscle) stem cells.
155. Zhang, L., Issa Bhaloo, S., Chen, T., Zhou, B. & Xu, Q. Role of resident stem cells in vessel formation and arteriosclerosis. *Circ. Res.* **122**, 1608–1624 (2018).
156. Andrade, J. et al. Control of endothelial quiescence by FOXO-regulated metabolites. *Nat. Cell Biol.* **23**, 413–423 (2021).
157. Wilhelm, K. et al. FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* **529**, 216–220 (2016).
158. Islam, M. S., Leissing, T. M., Chowdhury, R., Hopkinson, R. J. & Schofield, C. J. 2-Oxoglutarate-dependent oxygenases. *Annu. Rev. Biochem.* **87**, 585–620 (2018).
159. Charitou, P. et al. FOXOs support the metabolic requirements of normal and tumor cells by promoting IDH1 expression. *EMBO Rep.* **16**, 456–466 (2015).
160. Ito, K. et al. Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. *Nature* **431**, 997–1002 (2004).
161. Kim, J. & Wong, P. K. Loss of ATM impairs proliferation of neural stem cells through oxidative stress-mediated p38 MAPK signaling. *Stem Cell* **27**, 1987–1998 (2009).

162. Jones, R. M. et al. Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J.* **32**, 3017–3028 (2013).
163. Rodriguez-Colman, M. J. et al. Interplay between metabolic identities in the intestinal crypt supports stem cell function. *Nature* **543**, 424–427 (2017).
One of the first studies that shows metabolic crosstalk between niche and stem cells, thereby showing that next to growth factors, metabolites also act as crucial signalling molecules in stem cell maintenance.
164. Le Belle, J. E. et al. Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. *Cell Stem Cell* **8**, 59–71 (2011).
165. Sandri, M. et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117**, 399–412 (2004).
166. Cerletti, M., Jang, Y. C., Finley, L. W., Haigis, M. C. & Wagers, A. J. Short-term calorie restriction enhances skeletal muscle stem cell function. *Cell Stem Cell* **10**, 515–519 (2012).
167. Benjamin, D. I. et al. Fasting induces a highly resilient deep quiescent state in muscle stem cells via ketone body signaling. *Cell Metab.* **34**, 902–918.e6 (2022).
168. van Gastel, N. et al. Lipid availability determines fate of skeletal progenitor cells via SOX9. *Nature* **579**, 111–117 (2020).
This study shows how FOXO can sense the metabolic environment and, in response, specify cell differentiation.
169. Matsuzaki, T. et al. FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. *Sci. Transl Med* **10**, eaan0746 (2018).
170. Beumer, J. & Clevers, H. Cell fate specification and differentiation in the adult mammalian intestine. *Nat. Rev. Mol. Cell Biol.* **22**, 39–53 (2021).
171. Schell, J. C. et al. Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism. *Nat. Cell Biol.* **19**, 1027–1036 (2017).
172. Ludikhuize, M. C. et al. Mitochondria define intestinal stem cell differentiation downstream of a FOXO/Notch axis. *Cell Metab.* **32**, 889–900.e7 (2020).
173. Kaiko, G. E. et al. The colonic crypt protects stem cells from microbiota-derived metabolites. *Cell* **165**, 1708–1720 (2016).
174. Chen, Z. et al. Foxo1 controls gut homeostasis and commensalism by regulating mucus secretion. *J. Exp. Med.* **218**, e20210324 (2021).
175. Zhao, Y. et al. Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. *Nat. Cell Biol.* **12**, 665–675 (2010).
176. Hornsveld, M. et al. FOXO transcription factors both suppress and support breast cancer progression. *Cancer Res.* **78**, 2356–2369 (2018).
177. Sykes, S. M. et al. AKT/FOXO signaling enforces reversible differentiation blockade in myeloid leukemias. *Cell* **146**, 697–708 (2011).
This is the first study to show that FOXOs not only act as tumour suppressor but also promote tumorigenesis.
178. Ng, S. W. K. et al. Convergent somatic mutations in metabolism genes in chronic liver disease. *Nature* **598**, 473–478 (2021).
179. Sullivan, L. B. & Chandel, N. S. Mitochondrial reactive oxygen species and cancer. *Cancer Metab.* **2**, 17 (2014).
180. Kensler, T. W. & Wakabayashi, N. Nrf2: friend or foe for chemoprevention. *Carcinogenesis* **31**, 90–99 (2010).
181. Sporn, M. B. & Liby, K. T. NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer* **12**, 564–571 (2012).
182. O'Reilly, D. R. p53 and transformation by SV40. *Biol. Cell* **57**, 187–196 (1986).
183. Levine, A. J. p53: 800 million years of evolution and 40 years of discovery. *Nat. Rev. Cancer* **20**, 471–480 (2020).
184. Furuyama, T., Nakazawa, T., Nakano, I. & Mori, N. Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. *Biochem. J.* **349**, 629–634 (2000).
185. Brunet, A. et al. 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. *J. Cell Biol.* **156**, 817–828 (2002).
186. Wang, F. et al. Structures of KIX domain of CBP in complex with two FOXO3a transactivation domains reveal promiscuity and plasticity in coactivator recruitment. *Proc. Natl Acad. Sci. USA* **109**, 6078–6083 (2012).
187. Wang, F. et al. Synergistic interplay between promoter recognition and CBP/p300 coactivator recruitment by FOXO3a. *ACS Chem. Biol.* **4**, 1017–1027 (2009).
188. Feringa, F. M. et al. Hypersensitivity to DNA damage in antephase as a safeguard for genome stability. *Nat. Commun.* **7**, 12618 (2016).
189. Chin, C. F. & Yeong, F. M. Safeguarding entry into mitosis: the antephase checkpoint. *Mol. Cell Biol.* **30**, 22–32 (2010).
190. Shats, I. et al. FOXO transcription factors control E2F1 transcriptional specificity and apoptotic function. *Cancer Res.* **73**, 6056–6067 (2013).
191. Krenning, L., Feringa, F. M., Shaltiel, I. A., van den Berg, J. & Medema, R. H. Transient activation of p53 in G2 phase is sufficient to induce senescence. *Mol. Cell* **55**, 59–72 (2014).
192. Krenning, L., van den Berg, J. & Medema, R. H. Life or death after a break: what determines the choice. *Mol. Cell* **76**, 346–358 (2019).
193. Hoxhaj, G. & Manning, B. D. The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat. Rev. Cancer* **20**, 74–88 (2020).
194. Rim, E. Y., Clevers, H. & Nusse, R. The wnt pathway: from signaling mechanisms to synthetic modulators. *Annu. Rev. Biochem.* **91**, 571–598 (2022).
195. Daniels, D. L. & Weis, W. I. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nat. Struct. Mol. Biol.* **12**, 364–371 (2005).
196. Hoogeboom, D. et al. Interaction of FOXO with beta-catenin inhibits beta-catenin/T cell factor activity. *J. Biol. Chem.* **283**, 9224–9230 (2008).
197. Almeida, M., Han, L., Martin-Millan, M., O'Brien, C. A. & Manolagas, S. C. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box O-mediated transcription. *J. Biol. Chem.* **282**, 27298–27305 (2007).
198. Liu, H. et al. Wnt signaling regulates hepatic metabolism. *Sci. Signal.* **4**, ra6 (2011).
199. Shi, T., van Soest, D. M. K., Polderman, P. E., Burgering, B. M. T. & Dansen, T. B. DNA damage and oxidant stress activate p53 through differential upstream signaling pathways. *Free Radic. Biol. Med.* **172**, 298–311 (2021).
200. Fuentes-Lemus, E. & Davies, M. J. Effect of crowding, compartmentalization and nanodomains on protein modification and redox signaling — current state and future challenges. *Free Radic. Biol. Med.* **196**, 81–92 (2023).
201. Sies, H., Berndt, C. & Jones, D. P. Oxidative stress. *Annu. Rev. Biochem.* **86**, 715–748 (2017).
202. Marabelli, C., Marrocco, B. & Mattevi, A. The growing structural and functional complexity of the LSD1/KDM1A histone demethylase. *Curr. Opin. Struct. Biol.* **41**, 135–144 (2016).
203. Bai, J. et al. Actin reduction by MsrB2 is a key component of the cytokinetic abscission checkpoint and prevents tetraploidy. *Proc. Natl Acad. Sci. USA* **117**, 4169–4179 (2020).
204. Yoshida, K., Yamaguchi, T., Natsume, T., Kufe, D. & Miki, Y. JNK phosphorylation of 14-3-3 proteins regulates nuclear targeting of c-Abl in the apoptotic response to DNA damage. *Nat. Cell Biol.* **7**, 278–285 (2005).
205. Hopkins, B. L. et al. A peroxidase peroxiredoxin 1-specific redox regulation of the novel FOXO3 microRNA target let-7. *Antioxid. Redox Signal.* **28**, 62–77 (2018).
206. Kong, H. & Chandel, N. S. Regulation of redox balance in cancer and T cells. *J. Biol. Chem.* **293**, 7499–7507 (2018).
207. Bansal, A. et al. Transcriptional regulation of *Caenorhabditis elegans* FOXO/DAF-16 modulates lifespan. *Longev. Healthspan* **3**, 5 (2014).
208. Guertin, D. A. et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. *Dev. Cell* **11**, 859–871 (2006).

Acknowledgements

The authors apologize for not being able to cite all relevant studies owing to space constraints. The authors thank T. Madl (University Graz, Austria) for discussion on FOXO structure. The authors also thank all members of their respective laboratories for discussion. Research performed in the laboratories of the authors is financially supported by grants from the Dutch Cancer Foundation (KWF) (B.M.T.B., T.B.D. and M.J.R.-C.), Dutch Research Council (NWO) (M.J.R.-C.), Health Holland (B.M.T.B. and T.B.D.) and Oncode Institute (B.M.T.B.).

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41580-023-00649-0.

Peer review information *Nature Reviews Molecular Cell Biology* thanks Andrew Paek, Jihye Paik and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Related links

PhosphoSitePlus: https://www.phosphosite.org