FOXO flips the longevity SWItch

Ashley E. Webb and Anne Brunet

FOXO transcription factors promote longevity from worms to mammals, but the mechanisms by which FOXO extends lifespan have remained elusive. In the nematode *Caenorhabditis elegans*, FOXO is now shown to recruit the nucleosome remodelling complex SWI/SNF to its target genes, which is essential for FOXO to elicit stress resistance and longevity.

The FOXO (also known as DAF-16) family of transcription factors is a central regulator of lifespan and healthspan in metazoans[1]. FOXO factors are negatively regulated by the insulin–IGF (insulin-like growth factor) pathway, and this mechanism is well conserved across species. Reduced insulin signalling leads to lifespan extension in worms, flies and mice, and polymorphisms in the FOXO3 locus are associated with exceptional lifespan and healthspan in humans[1]. Following nutrient deprivation, FOXO transcription factors translocate to the nucleus, where they transactivate genes involved in resistance to oxidative stress and energy metabolism in worms, as well as genes implicated in DNA damage repair, glucose metabolism, autophagy, cell cycle arrest and apoptosis in mammals[2] (Fig. 1). Accordingly, FOXO factors are also involved in energy metabolism, tumour suppression and stem cell homeostasis[2]. Recent genome-wide analyses of FOXO target genes have established direct targets for this transcription factor in worms and mammalian cells[3,4]. However, the molecular mechanisms underlying FOXO-mediated transcriptional activation have remained largely unclear. In this issue, Riedel *et al.*[5] uncover an exciting mechanism of FOXO/DAF-16 gene regulation that involves the SWI/SNF (SWItch/sucrose non-fermentable) family of ATP-dependent chromatin remodellers[5].

Riedel *et al.* combined proteomic identification of the FOXO/DAF-16 complex with a functional RNA interference (RNAi) screen to discover novel partners of FOXO/DAF-16 that specifically act as FOXO co-factors to mediate gene expression and longevity. The authors report that several members of the SWI/SNF family physically interact with FOXO/DAF-16 (Fig. 1). Functional analysis revealed that members of the BAF family subclass of the SWI/SNF family are essential for FOXO/DAF-16-mediated transcriptional activation of a well-known FOXO target gene, superoxide dismutase 3 (*sod-3*). The authors extended these findings by analysing global transcriptional changes following knockdown of FOXO or SWI/SNF in worms using RNA-seq, and found that one-third of targets regulated by FOXO/DAF-16 are also regulated by the SWI/SNF BAF-like family. Consistently, chromatin immunoprecipitation followed by sequencing (ChIP-seq) showed that FOXO/DAF-16 and the BAF-like subunits of the SWI/SNF complex co-occupy the regulatory regions of FOXO target genes genome-wide. Targets that are co-regulated by FOXO/DAF-16 and SWI/SNF are enriched for genes involved in ageing, oxidative stress and cellular defence mechanisms. Furthermore, the BAF-like SWI/SNF core subunits are needed for lifespan extension and resistance to stress in response to reduced insulin signalling. The SWI/SNF complex is also required for FOXO/DAF-16-mediated dauer entry — a protective response to harsh environmental conditions — under low insulin conditions.

These results are exciting because they reveal previously unknown protein partners of FOXO that serve as co-factors for the expression of genes involved in longevity and stress resistance. Before this study, FOXO binding partners that were identified by unbiased proteomic approaches were not transcriptional regulators, and thus did not shed light on the specific mechanism of FOXO-dependent transcription[6]. Targeted candidate approaches had identified co-factors, including the deacetylase SIR-2/SIRT1, the co-factor β-catenin and the transcriptional co-regulator PGC-1 (ref. 7), but it was unclear how these co-factors actively connected with the transcription machinery. This study provides a missing link between these transcription factors and the activation of a coordinated program of genes. It will be interesting to test if FOXO binds directly to SWI/SNF members and whether the specific FOXO co-factors that were identified previously are also part of this larger complex (Fig. 1). Another important question that stems from the observations of Riedel *et al.* is whether the interaction between FOXO and SWI/SNF is modulated by environmental conditions. Given that FOXOs are heavily regulated by a variety of post-translational modifications in response to insulin and stress stimuli[7], it is possible that these modifications affect FOXO's interaction with SWI/SNF. Furthermore, SWI/SNF subunits might themselves be post-translationally modified by external stimuli. The components of the SWI/SNF complex are known to change during differentiation and development in mammals[8]. The possibility of FOXO binding to different SWI/SNF subunits at different ages or in different cell types would add layers of complexity to the interaction between FOXO and SWI/SNF in longevity and stress resistance, and could be an interesting topic for future study.

One fundamental aspect of the study by Riedel *et al.* is that it provides a mechanism to explain FOXO-dependent transcriptional activity. The authors show that *daf-16* mutants have reduced SWI/SNF binding at FOXO/DAF-16 targets, suggesting that FOXO/DAF-16 is responsible for recruiting SWI/SNF. This supports a model in which FOXO translocation

Ashley E. Webb and Anne Brunet are in the Department of Genetics, Stanford University, Stanford, California 94305, USA.
e-mail: abrunet1@stanford.edu

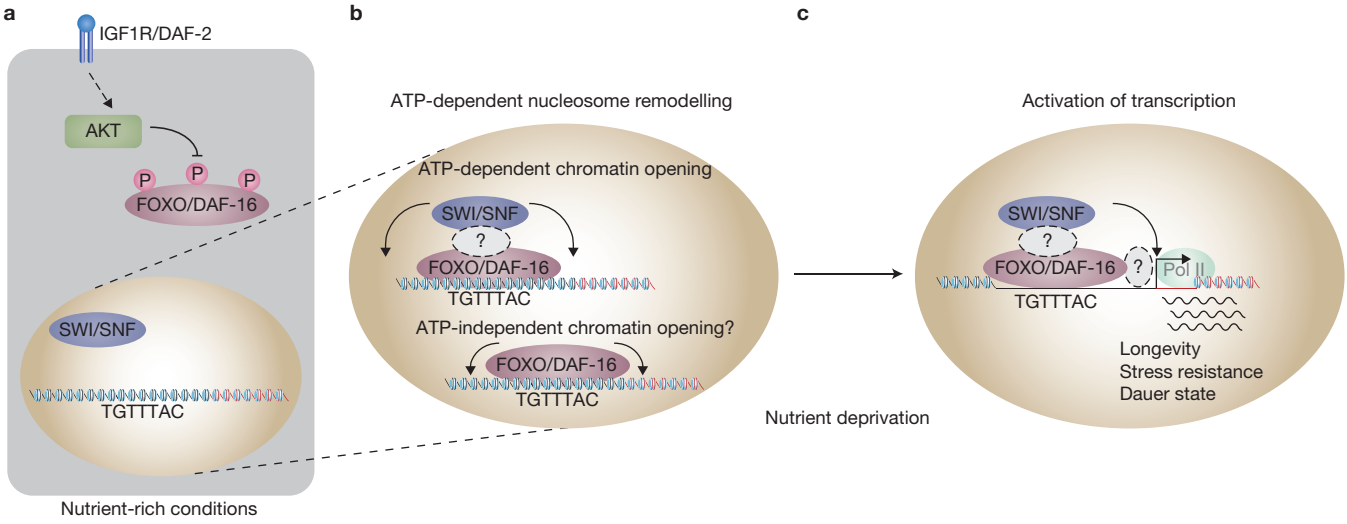

Figure 1 The role of SWI/SNF in FOXO-dependent transcription of longevity and stress-resistance genes. (**a**) When nutrients are abundant, IGF1R–DAF-2 activates the AKT pathway, leading to the phosphorylation and cytosolic sequestration of FOXO/DAF-16. In the absence of nuclear FOXO/DAF-16, SWI/SNF is not bound to FOXO/DAF-16 target genes, and chromatin remains in a compacted state at these sites. (**b**) Under low nutrient conditions, unphosphorylated FOXO/DAF-16 translocates to the nucleus and binds the consensus sequence TGTTTAC. Riedel *et al.* now report that FOXO/DAF-16 recruits the ATP-dependent chromatin-remodelling SWI/SNF complex, which would have a 'pioneering' activity and would remodel nucleosomes in the surrounding region. FOXO/DAF-16 could also open chromatin in an ATP-independent manner. (**c**) These mechanisms would result in a promoter region that is accessible to the basal transcriptional machinery as well as other co-factors, and allow the expression of genes involved in longevity, stress resistance and dauer formation. Question marks indicate potential binding partners of FOXO/DAF-16 that could bridge its interaction with members of the SWI/SNF family or the transcriptional machinery. Blue DNA indicates the promoter region and pink DNA indicates the gene region.

to the nucleus recruits SWI/SNF, which in turn induces chromatin remodelling and transcriptional activation (Fig. 1). This observation also provides a potential molecular mechanism for the 'pioneer' activity of FOXO transcription factors — that is, their ability to bind to closed chromatin and open it to the binding of specific transcription factors[9]. However, in mammals, FOXO can act as a 'pioneer factor' and open compacted nucleosomes *in vitro* in the absence of ATP, suggesting that the pioneer activity of FOXO can also occur in the absence of ATP-dependent chromatin remodelers such as SWI/SNF (ref. 9). Thus, some FOXO/DAF-16 target genes may require ATP-dependent chromatin remodellers for activation, whereas other target genes may be directly remodelled by FOXO/DAF-16 in an ATP-independent manner. The two mechanisms need not be mutually exclusive: FOXO/DAF-16 may first bind and open regions of highly compacted chromatin and subsequently recruit SWI/SNF chromatin remodellers to extend or maintain the open conformation (Fig. 1). This mechanism could also extend to other Forkhead transcription factors, such as FOXA (PHA-4 orthologue), which is known to be involved in liver function in mammals and dietary-restriction-induced longevity in worms[10,11]. It would be interesting to test if FOXO's ability to induce chromatin remodelling paves the way for the binding of other transcription factors and, if so, which ones.

This study is also important as it identifies chromatin remodellers as regulators of longevity downstream of insulin signalling. This is interesting in light of the recent discovery that chromatin modifiers can regulate longevity in invertebrates. For example, the histone methylation complexes involved in trimethylation of histone H3 at lysine 4 (H3K4me3) or lysine 27 (H3K27me3) regulate lifespan in *C. elegans* and *Drosophila melanogaster*[12–14]. However, the precise mechanism of lifespan regulation by chromatin modifiers is still unknown. Chromatin modification enzymes have been suggested to impact ageing by affecting transcription generally, thereby modifying the maintenance of overall tissue integrity. In contrast, the work by Riedel *et al.* demonstrates a genome-wide interaction between a pro-longevity transcription factor, FOXO/DAF-16, and a global chromatin remodeller, SWI/SNF. This work suggests that, at least in the case of SWI/SNF, chromatin remodelling promotes longevity through activation of specific target genes and not just by affecting global transcription. FOXO is not required for the effect of H3K4me3 regulators on worm lifespan[12], but it is required for the effect of H3K27me3 regulators (for example, UTX)[13]. It would be interesting to test whether SWI/SNF and UTX genetically interact to regulate lifespan, and whether SWI/SNF can also modulate the deposition of specific histone marks.

Is the mechanism of FOXO-dependent transcription unravelled by Riedel *et al.* in *C. elegans* also at work in other species? The FOXO binding site (TGTTTAC) and several FOXO target genes are conserved from worms to mammals, raising the possibility that the regulation of FOXO-dependent transcription may be conserved across species. A key question is whether FOXO also physically and functionally interacts with BAF-like SWI/SNF factors in other species, and whether some specific FOXO target genes — perhaps those that are most conserved — are particularly susceptible to SWI/SNF action. As the SWI/SNF complex has been implicated in a wide range of processes in mammals, including development, immunity, cancer, stem cell function and cellular reprogramming[15], it is possible that some of these processes involve FOXO factors. Conversely, given the important role of the insulin–FOXO pathway in mammalian longevity, a provocative question is whether SWI/SNF subunits also modulate longevity in mammals.

The Riedel *et al.* study raises several further questions worth exploring. SWI/SNF subunits are expressed ubiquitously, and ChIP-seq on

whole worms does not reveal tissue-specific binding. Is SWI/SNF playing a role in specific tissues to promote longevity? Does SWI/SNF participate in the regulation of the cell non-autonomous targets of FOXO/DAF-16 (ref. 1)? Also, it is worth noting that several FOXO/DAF-16-regulated genes were not affected by SWI/SNF perturbation. Is there another chromatin remodelling complex involved in the regulation of these target genes, or is the ATP-independent pioneer activity of FOXO/DAF-16 sufficient to activate these genes? And what about genes at which FOXO acts as a transcriptional repressor — is a different complex involved at these genes? Finally, which SWI/SNF–FOXO/DAF-16 targets have the greatest impact on longevity? Are these genes involved in mammalian healthspan? This exciting study by Riedel *et al.* expands our mechanistic understanding of how pro-longevity transcription factors such as FOXO/DAF-16 work together with chromatin remodellers such as SWI/SNF, which should provide valuable insight into the mechanisms of longevity in mammals, including humans.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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A nexus for receptor recycling

Suzanne R. Pfeffer

Sorting nexin proteins (SNXs) and the cargo-selective retromer complex play key roles in receptor recycling from endosomes to the cell surface. A global proteomics analysis reveals a collection of cell surface proteins that rely on SNX27 and the retromer complex for their cell surface localization at steady state.

The mechanisms by which receptors are recycled from early endosomes to the cell surface have broad implications for our understanding of normal cell physiology and the molecular basis of several disease states. SNXs represent a large family of proteins that are implicated in receptor recycling[1,2], and SNX27 is involved in the recycling of the Kir3 potassium channel[3] and the β2 adrenergic receptor[4,5]. In this issue, Steinberg *et al.*[6] use a sophisticated proteomics approach to catalogue the cohort of proteins that bind to SNX27, and those whose surface expression is altered following loss of SNX27 from cells. This work shows that SNX27 is important for the sorting and stability of a significant number of cell surface proteins, and documents a discrete recognition pathway for the localization of proteins needed for glucose and ion transport in mammalian cells.

SNXs contain a PHOX-homology (PX) domain that enables them to bind phosphatidylinositol-3-phosphate (PtdIns(3)P) on early endosomes, where most PtdIns(3)P is localized. Twelve mammalian SNX proteins also contain a bin–amphiphysin-rvs (BAR) domain and are thus called SNX–BAR proteins. BAR domains dimerize to form a curved surface that interacts with membranes and stabilizes membrane curvature[7]. BAR-domain proteins are important for the tubulation of endosomal membranes that accompanies (and may even drive) the process by which receptors are sorted for recycling.

Certain SNX proteins work in concert with the highly conserved retromer complex that is critical for endosomal protein recycling[8,9]. In yeast, retromer is comprised of a trimer of subunits encoded by the Vps (vacuolar protein sorting) genes VPS35, VPS29 and VPS26 (which mediate cargo selection), together with a dimer of two SNX–BAR proteins, Vps5p and Vps17p. In mammals, the Vps5p homologues SNX1 and SNX2 dimerize with a Vps17p orthologue, SNX5 or SNX6. Retromer was first shown to be important for the recycling of proteins from endosomes back to the Golgi complex[8]. More recent work has shown a role for the retromer in recycling of certain receptors from endosomes back to the cell surface[9]. Retromer also recruits the macromolecular WASH (Wiskott-Aldrich syndrome protein and SCAR homologue) complex to endosomes to promote the formation of branched actin networks[9]. The WASH complex may help to couple actin polymerization to drive tubule formation from the early endosome compartment. The term, retromer, will be used here to refer to a complex of VPS35, VPS29 and VPS26 proteins.

Certain SNX proteins recognize cargo directly: a PDZ domain in SNX27 enables it to bind to PDZ domain binding sites in the C-termini of the Kir3 potassium channel[3] and the β2 adrenergic receptor[4]; SNX17 binds to β_1 integrins through its so-called FERM-like (4.1–ezrin–radixin–moesin) domain[10]. Like SNX17, SNX27 also contains a FERM-like domain. These SNX–receptor interactions are critical for the endosomal recycling of their binding partners[3–5].

Given the importance of SNX proteins in receptor recycling, characterization of their cargoes can provide mechanistic detail to our understanding of a broad swath of mammalian cell functions. To this end, Steinberg *et al.*[6] first used SILAC (stable isotope labelling by amino acids in culture) to identify interaction partners

Suzanne R. Pfeffer is in the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305-5307, USA.
e-mail: pfeffer@stanford.edu