Cdc42? Does SipA only stabilize the filaments nucleated by SipC? Does it also stabilize the filaments induced via Bierne, H., Gouin, E., Roux, P., Caroni, P., Yin, H.L., and Cossart, SopE2, SopE, Rac, and Cdc42? Finally, it will also be important for the establishment of the definitive role of SipA during entry to decipher when this protein functions. Is SipA, like SopE and SptP, a substrate for the proteasome (Kubori and Galan, 2003), and is it degraded to facilitate disruption of actin filaments after internalization? There are still important gaps to fill to understand the coordinated spatio-temporal action of the bacterial effectors during Salmonella entry.

Pascale Cossart
Unité des Interactions Bactéries Cellules
Institut Pasteur
28 Rue du Docteur Roux
INSERM U604
Paris 75015
France

Selected Reading
Pascale Cossart

Tipping the Balance toward Longevity

Genetic experiments in C. elegans suggested that SIR2, an NAD-dependent protein deacetylase, acts through FOXO/DAF-16 transcription factor to prolong life. Recent studies show that mammalian SIR2 deacetylates FOXO, and may maximize survival by tempering cell death and increasing stress resistance.

The provocative finding that single gene mutations can dramatically extend life span in several model organisms reveals that aging is plastic, regulated, and under genetic control. At least two pivotal regulators conserved across taxa robustly extend organismal survival, forkhead transcription factor FOXO, and the SIR2 (silencing information regulator 2) protein deacetylase.

FOXO mediates the transcriptional output of insulin/IGF-1 signal transduction, which regulates the longevity of species as diverse as worms, flies, and mice (reviewed in Tatar et al., 2003). Insulin/IGF-1 signaling is pro-aging, while mild inhibition of the pathway prolongs life. When insulin/IGF-1 signaling is active, a PI3 kinase/AKT kinase cascade phosphorylates FOXO, leading to its nuclear exclusion. When insulin signaling is inhibited, unphosphorylated FOXO enters the nucleus where it promotes programs of somatic endurance, stress resistance, and longevity.

But how should blunted insulin/IGF-1 signaling promote longevity when normally it is associated with frank diabetes and premature senescence? One key is moderation: a modest downregulation of the pathway leads to healthy old age, while strong downregulation leads to frailty and morbidity. The other key is specificity. While insulin/IGF-1 signaling is traditionally known for its role in metabolic and growth control, it may be a general sensor for all kinds of environmental stress. In turn, FOXO induces a whole suite of genes, including those involved in gluconeogenesis (PEPCK), detoxification of reactive oxygen species (MnSOD), heat shock, DNA damage repair (GADD45), growth control (4EBP), cell cycle arrest (p27KIP), cell death (BIM, FAS ligand), and innate immunity (see references within Motta et al., 2004). Of all the traits controlled by FOXO, stress resistance is most tightly correlated with longevity. How then might moderation and specificity be achieved?

Here SIR2 may provide some answers. SIR2 was originally discovered in yeast as an NAD-dependent histone deacetylase with a role in silencing at the rDNA, telomeres, and mating type loci (Hekimi and Guarente, 2003). Notably, increased SIR2 dose prolongs yeast replicative life span by about 40%, while loss of function abrogates extended survival brought about by glucose limitation, a model of caloric restriction. Caloric restriction prolongs life span in numerous species, and it is proposed that SIR2 couples metabolic signals to downstream regulatory events.

Resounding evidence that SIR2 regulates animal life span came from studies in the worm, where extra doses of sir-2.1 (one of four sirtuin homologs) extend adult life by 50% (Tissenbaum and Guarente, 2001). Moreover, extension was dependent on FOXO/daf-16, bringing these pivotal regulators together in a single pathway. But the specific nature of this interaction was unknown.

Recently, several reports (Brunet et al., 2004; Motta et al., 2004) have explored the physical and biochemical interaction of mammalian FOXO homologs with SIRT1, the closest SIR2 homolog among the seven mammalian sirtuins. Indeed, consistent with unified action, it was shown that SIRT1 and FOXO physically interacted in coimmunoprecipitation experiments. Moreover, in response to stress FOXO was a substrate for acetylation by protein acetylases p300 and PCAF and subsequent deacetylation by SIRT1 and other deacetylases (Brunet et al., 2004; Motta et al., 2004).
Deacetylation of transcriptional complexes is generally associated with diminished transcriptional activation. Consistent with this, FOXO proapoptotic target genes, BIM and FAS ligand, were downregulated by SIRT1 (Brunet et al., 2004; Motta et al., 2004). Correlatively, apoptosis in various cell types was mitigated by greater SIRT1 activity and enhanced by SIRT1 loss of function. Similarly, SIRT1 dampened transcriptional activation from IGFBP1 and PEPCK promoters in vivo and coassembled with FOXO at the IGFBP1 promoter as shown by chromatin immunoprecipitation experiments (Motta et al., 2004).

How acetylation state influences FOXO activity is not quite so simple, however, and is apparently promoter specific. For example, SIRT1 increased transcription from the FOXO target, GADD45, suggesting that SIRT1 augments DNA repair (Brunet et al., 2004). Accordingly, SIRT1 conferred resistance to etoposide (Brunet et al., 2004), a DNA damaging agent, while thymocytes derived from SIRT1 knockout mice were more sensitive to ionizing radiation (Cheng et al., 2003). While it is yet unknown whether SIRT1 and FOXO are indeed positive effectors of mammalian longevity, the emergent hypothesis is that SIRT1 may specifically modulate FOXO activity toward maximal survival. Attenuating FOXO in some contexts may be advantageous, since overactivity could foment excessive cell death and organ decline, or lead to devastating metabolic disorders. Moreover, in quiescent or differentiated cells, FOXO may be less susceptible to regulation by growth factor signaling, deacetylation might be an important mechanism to dampen the response.

Conversely, enhancing FOXO-dependent expression of stress resistance genes could mitigate damage and promote endurance. In support of this, the overexpression of SIRT1 conferred resistance to H2O2-induced cell death, whereas loss of function increased sensitivity (Brunet et al., 2004). It is unclear, however, if these phenotypes are due to effects on apoptosis, detoxification of reactive oxygen species, or both. In the future, it will be essential to evaluate the effect of SIRT1 and FOXO on MnSOD, and other genes involved in managing oxidative stress. Finally, modestly active FOXO may prevent cellular senescence (Miyachi et al., 2004), an arrested state refractory to growth factor signals, as suggested in primary endothelial cell culture. By tempering apoptosis, increasing stress resistance, and bypassing senescence, cellular and perhaps organismal longevity could increase.

Another important player in this game may well be the p53 tumor suppressor. Like FOXO, p53 is a central regulator of cellular response to stress, genotoxic insult, and DNA damage. p53 can arrest cell cycle, invoke damage response genes, and trigger cell suicide. Notably, SIRT1 deacetylates and negatively controls p53 to augment survival under stress (Luo et al., 2001). Presumed p53 overactivity results in a live mouse with few tumors but early onset of age-related decline and a shortened life (Tyner et al., 2002). Excessive cell death, depletion of stem cell niches, and cellular senescence may contribute to shortevity. Clearly, understanding how FOXO, SIRT1, and p53 functionally interact will be important to unraveling this puzzle. Tantalizingly, p53 and FOXO were shown to coimmunoprecipitate in response to oxidative stress (Brunet et al., 2004). These exciting findings raise numerous questions. Foremost among them is how will SIRT1 and FOXO activity affect mammalian stress response and longevity in vivo, and what is their relationship to caloric restriction? Because these regulators have various roles, stage- and tissue-specific genetic manipulations are likely required. Second, how is specificity determined? FOXO could respond to various signals as growth factor deprivation and oxidative stress through insulin/IGF signaling or other inputs. SIRT1 activity could reflect the internal metabolic state of the cell, or could itself respond to distinct signals, since its activity is modulated by polyphenolics (Howitz et al., 2003). Aside from the issues of promoter and tissue specificity, FOXO may have a complex regulatory code, as five acetylated and eight phosphorylated residues were modified in response to stress (Brunet et al., 2004). Cracking this code may also help illuminate the specification of longer life.

Adam Antebi
Max-Planck-Institut für molekulare Genetik
Inhestrasse 73
D-14195 Berlin
Germany

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