to determine the spatiotemporal activation pattern of RhoA effector pathways to achieve the biologically appropriate outcomes.

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FOXOphagy path to inducing stress resistance and cell survival

Marco Sandri

Nutrient deprivation and other stress stimuli elicit metabolic changes (such as the induction of autophagy and activation of FOXO transcription factors) that help an organism adapt to stressful conditions. A link between these stress response pathways is revealed by the finding that FOXO3 upregulates the expression of glutamine synthetase to promote glutamine accumulation, inhibit mTOR signalling and promote autophagy.

Under stress conditions, mammalian cells activate compensatory mechanisms to adapt to the new situation. Depending on the form of the stimuli, the adaptive response can be minimal, requiring small metabolic changes to adjust cellular homeostasis, or can involve major and sustained adjustments that need transcription-dependent adaptations. A system that is often activated in both short and prolonged stress conditions is macroautophagy — or, more simply, autophagy[1]. This is a highly conserved homeostatic mechanism used for the degradation and recycling (through the lysosomal machinery) of bulk cytoplasm, long-lived proteins and organelles[2]. The autophagy machinery generates double-membrane vesicles (autophagosomes) that engulf and sequester target cellular components[3,4]. Autophagosomes then fuse with lysosomes to degrade their contents. The autophagic process plays a crucial role in the turnover of cell components, both in constitutive conditions and in response to various stimuli such as cellular stress and nutrient deprivation. Under nutrient-poor conditions, most tissues transiently activate the autophagy pathway for a few hours or days. The quick activation of autophagy involves post-translational modifications of regulatory components of the autophagy system. In contrast, prolonged autophagic induction requires transcriptional control to replenish critical proteins that are destroyed during the fusion of autophagosomes with lysosomes[5,6]. The kinase mTOR belongs to two complexes (mTORC1 and mTORC2) that have different functions. mTOR senses availability of nutrients (especially amino acids) and is active in nutrient-rich conditions. When nutrients are available, mTORC1 inhibits autophagy by phosphorylating the kinase ULK1/2 and blocking the formation of the active ULK1/2 complex. Starvation conditions inhibit the mTORC1–ULK1/2 interaction, which de-represses ULK1/2 and induces activation of the autophagy cascade[5]. The regulation of mTORC1 activity is modulated by its subcellular localization, perhaps most notably its association with the lysosome. Accumulation of amino acids inside the lysosomal lumen generates an activating signal that is transmitted to the Rag GTPases via the vacuolar H^+-adenosine triphosphatase ATPase (v-ATPase). This recruits and activates mTORC1, which initiates protein synthesis[7] (Fig. 1) and inhibits transcription factor EB (TFEB)[8,9], a master regulator of lysosome biosynthesis with an emerging role in autophagy.

Thus, through this mechanism, mTORC1 might also regulate the transcriptional response to nutrient deprivation. However, other transcription factors, including the FOXO family members, have an established role in autophagy[10,11]. The FOXO family (FOXO1, 3, 4 and 6) is downstream of the insulin pathway and is negatively regulated by PI(3)K–PKB (also called Akt) signalling. FOXOs are well conserved and have a critical role in many cellular processes, including apoptosis, cell cycle regulation, DNA repair, glucose metabolism and the antioxidant response. Interestingly, the FOXO family has been shown to regulate autophagy in various systems, ranging from flies to mammalian cells[10,11]. In this issue of *Nature Cell Biology*, van der Vos *et al.*[12] reveal an intriguing connection between FOXO3 activity and the induction of autophagy under conditions of growth factor deprivation.

To identify FoxO target genes that regulate autophagy, the authors generated different cell lines, each ectopically expressing 4-hydroxytamoxifen (4-OHT)-conditional active variants of PI(3)K, PKB, FOXO3 and FOXO4. Gene expression profiling was performed

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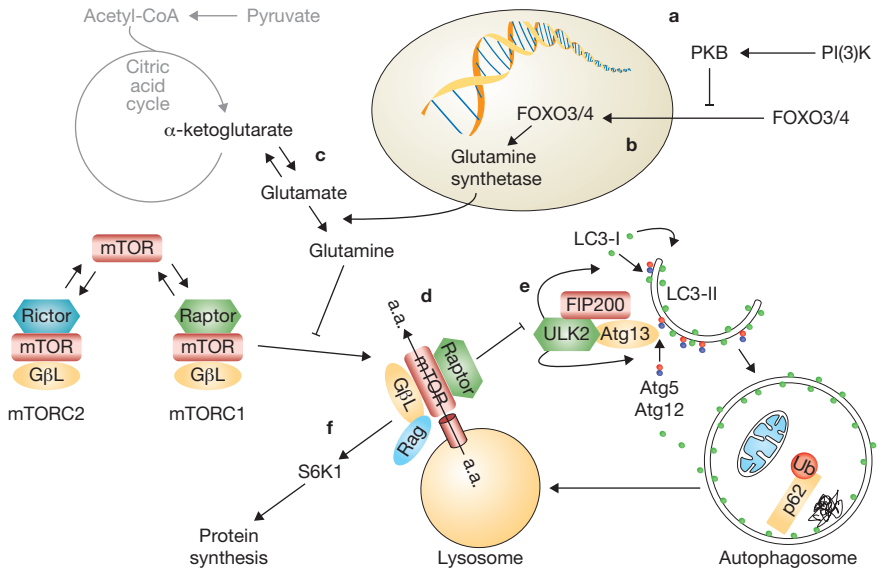

Figure 1 FOXO regulates the induction of autophagy by promoting expression of glutamine synthetase. (**a**) In the presence of growth factors, the pPI(3)K–PKB pathway sequesters FOXO1, FOXO3 and FOXO4 transcription factors in the cytoplasm. (**b**) In the absence of growth factors, PKB (Akt) is inactive and so FOXOs translocate into the nucleus and transcribe target genes. (**c**) The FOXO-dependent upregulation of glutamine synthetase induces glutamine production (by acting on glutamate that is in equilibrium with citric-acid-cycle component α-ketoglutarate), which inhibits the localization of the mTORC1 complex on lysosomes. Thus, FOXO-mediated glutamine production promotes autophagy by inhibiting mTORC1. (**d**) The recruitment of the mTORC1 complex to lysosomes is mediated by Rag-GTPase, which places mTORC1 in close proximity to H^+-adenosine triphosphatase ATPase (v-ATPase). mTOR then senses and is activated by the amino acid (a.a.) flux coming from lysosomal protein breakdown. mTORC1 activity has at least two major functional implications: (**e**) it blocks autophagy by inhibiting ULK1/2 complex formation; and (**f**) it promotes protein synthesis through the S6K1 kinase. GβL, G protein β-subunit-like.

after 4-OHT-mediated activation of each factor, and data were then compared. The authors looked for genes that were downregulated by both PI(3)K and PKB activation, and upregulated by FOXO3 and FOXO4, or vice versa. This approach allowed the identification of glutamine synthetase as a FOXO3 and FOXO4 target gene. The transcriptional-dependent glutamine synthetase upregulation was confirmed by promoter studies, as well as by chromatin immunoprecipitation experiments that identified the enhancer sequence containing the FOXO binding sites. However, it is not yet clear whether FOXO3 and FOXO4 bind the glutamine synthetase promoter and drive transcription with the same efficacy, or whether the recruitment of each FOXO is dependent on context and cell type. Importantly, induction of the glutamine synthetase gene increases its enzyme activity and, therefore, glutamine production. Glutamine synthetase induction was also shown to occur downstream of DAF16, the sole FOXO orthologue in *Caenorhabditis elegans*, suggesting that this mechanism for upregulating glutamine synthetase expression is evolutionarily conserved.

This set of findings supports a previously unappreciated role for FOXO3 in influencing amino acid levels and homeostasis within the cell. Indeed, FOXO activation also promoted cellular accumulation of alanine. The idea that glutamine production is under FOXO regulation raises a new series of questions about the relevance of this process in cellular metabolic changes such as glucose homeostasis and energy production. This is especially relevant given that glutamine production induces glutamate consumption, which potentially affects α-ketoglutarate levels in the citric acid cycle and, therefore, mitochondria function and energy production[13].

The authors then went on to dissect the signalling pathways that were affected by glutamine synthetase upregulation. Increased glutamine levels inhibited mTOR activity as evidenced by the lack of phosphorylation of the S6K1 kinase, an mTORC1 downstream target. Importantly, the downregulation of mTORC1 activity was reflected in the re-localization of mTOR from the lysosomes to the cytoplasm (Fig. 1); such re-localization was previously shown to regulate mTOR activity[6]. It will be interesting to determine the mechanisms that regulate mTORC1 translocation to and from lysosomes in response to changing glutamine levels. For instance, it has been reported that FOXO can repress mTOR activity through sestrin and rictor[14,15], and it would be of interest to know whether the action of glutamine requires these or other factors, or, instead, is a direct inhibitory action on the lysosomally located Rag GTPase complex (Fig. 1).

Given the evolutionary conserved negative regulatory action of mTORC1 on the autophagy system, the authors examined whether FOXO-mediated production of glutamine synthetase modulated autophagosome formation. Indeed, by using different autophagy markers that label early and late phases of autophagosome formation, as well as measuring autophagy flux, they confirmed that FOXO-dependent glutamine synthetase activation induces autophagosome formation. They also found that glutamine alone was sufficient to induce autophagy. The induction of autophagy is an important survival mechanism for cells, and blockade of autophagy with chemical inhibitors or reduced glutamine synthetase expression decreased the viability of FOXO3-activated cells.

Thus, these data provide an intriguing link between FOXO transcription factors and the induction of autophagy, and reveal that glutamine production is a key intermediary between these two processes. It is tempting to speculate that FOXO activation could also indirectly activate the TFEB transcription factor, as both TFEB and FOXO have been described to control expression of several autophagy and lysosomal genes. The potential interaction or cooperation of these transcription factors should be considered in future work. Furthermore, the physiological and pathological implications of this glutamine-dependent regulation of autophagy are important and need to be established. For instance, the most abundant source of glutamine in the human body is skeletal muscles. In catabolic conditions, amino acids are released from the muscles as a consequence of increased protein breakdown of contractile proteins to sustain energetic and metabolic demands of organs. Interestingly, glutamine synthetase is strongly upregulated in skeletal muscles during cancer cachexia, and belongs to a list of atrophy-related genes that are induced during muscle loss[16]. Tumorigenesis affects glutamine metabolism and induces glutamine release

from muscles. Importantly, an increase in glutamine synthetase expression apparently cannot match the increased rate of efflux, and so leads to glutamine depletion in muscles. Therefore, it is possible that glutamine production would induce excessive activation of autophagy, and thus the development of severe atrophy and cachexia. Alternatively, reduction in glutamine levels might affect mitochondrial function, resulting in energy imbalance that can contribute to the development of cachexia. Lastly, both FOXO activation and mTOR inhibition have been related to life-span extension, whereas autophagy is reported to decline during ageing[17–19]. Therefore, understanding the interrelationships between FOXO and mTOR has major implications in metabolism, ageing and regulation of cell growth that will certainly keep scientists occupied well into the future.

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Brassinosteroids, gibberellins and light-mediated signalling are the three-way controls of plant sprouting

Yvon Jaillais and Grégory Vert

The steroid hormones found in plants, the brassinosteroids, were originally genetically identified about 15 years ago as critical regulators of seedling photomorphogenesis. Two studies now shed light on the molecular mechanisms behind this observation. Brassinosteroids control seedling morphogenesis through direct interaction with master transcriptional regulators downstream of growth-promoting hormones and light signalling.

Plants are photosynthetic organisms that rely on light to produce energy. Because they are sessile, they must constantly adapt their development to accommodate changing light environments[1]. One of the most dramatic plant responses to light occurs just after germination, when the embryonic leaves (named cotyledons) emerge from the soil[2]. When exposed to light, a young seedling undergoes a process called photomorphogenesis (Fig. 1a). The cotyledons rapidly turn green and expand to maximize photosynthesis, the hypocotyl (the embryonic stem) stops elongating, and the root grows rapidly to anchor the young plant to the soil and to forage for nutrients (Fig. 1a). In contrast, when a seedling germinates in the dark (for example, in the soil), a completely different developmental program is established: etiolation (Fig. 1b). Etiolation dedicates all the resources of the plant towards elongation growth of the hypocotyl to search for light. This developmental program, often called skotomorphogenesis, is associated with small unopened and non-photosynthetic cotyledons, a fast elongating hypocotyl and a short primary root. Furthermore, the top of the hypocotyl forms an apical hook believed to protect the stem cell niche responsible for post-embryonic organogenesis from being damaged during progression through the soil. In this issue of *Nature Cell Biology*, two studies from Wang and colleagues now shed light on how seedling growth is controlled by the intricate integration between light signalling and the brassinosteroid and gibberellin growth-promoting-hormone transduction pathways[3,4].

Plants perceive light quality, intensity and duration using a battery of photoreceptors. Phytochromes (Phy) are a class of red- and far-red-light-absorbing photoreceptors that play a major role in triggering photomorphogenesis[2]. Under red light, phytochromes are converted from a red-absorbing form (Pr) to a far-red-absorbing conformation (Pfr), which promotes their translocation from the cytosol to the nucleus (Fig. 1a). Phy(Pfr) limits stem growth by promoting the degradation of the transcriptional regulators phytochrome-interacting factors (PIFs; here, PIF4) that are positive regulators of the etiolated state. In the dark, phytochromes are mostly in their inactive Pr form, and thus restricted to the cytosol. Under these conditions, PIFs are stable and promote hypocotyl growth (Fig. 1b). A quadruple mutant that is invalidated for *PIF1, PIF3, PIF4* and *PIF5* (named *pifq)* shows constitutive photomorphogenesis (or de-etiolation) in the dark, displaying a short hypocotyl, expanded cotyledons and expression of photosynthetic genes[2].

Brassinosteroids are a class of steroid hormones that negatively regulate photomorphogenesis. Brassinosteroid biosynthetic and signalling mutants indeed show de-etiolated phenotypes in the dark[5]. They are perceived by a transmembrane receptor kinase, BRI1

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