eIF4A moonlights as an off switch for TORC1

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TORC1 is actively inhibited upon amino acid withdrawal. Tsokanos et al (2016) shed light on the underlying molecular mechanism. They demonstrate that upon removal of exogenous amino acids, eIF4A inhibits TORC1 via TSC2. Thus, whereas it is well known that TORC1 regulates the translation machinery, we now know the inverse is also true.

See also: FF Tsokanos et al (May 2016)

Target of rapamycin complex 1 (TORC1) is an evolutionarily conserved regulator of cell growth and metabolism. It integrates multiple inputs (hormones, growth factors, amino acids, and cellular energy) to activate anabolic processes such as protein, lipid, and nucleotide synthesis, and to inhibit catabolic processes including autophagy. Inputs impinge on TORC1 via the TSC complex (composed of tuberous sclerosis complex 1 (TSC1), TSC2, and TBC1D7). The tumor-suppressing TSC complex is a GTPase-activating protein (GAP) for the small GTPase Rheb, a direct activator of TORC1, and thereby an upstream TORC1 inhibitor (Shimobayashi & Hall, 2014).

TORC1 regulation in response to amino acids ensures that translation is coupled to the availability of building blocks. The conserved Rag GTPases are key components of the amino acid-sensing branch upstream of TORC1. When amino acids are present, the Rag GTPases adopt an active conformation to recruit TORC1 to the lysosomal surface, where TORC1 encounters its activator Rheb (reviewed in Shimobayashi & Hall, 2016). Upon amino acid withdrawal, Rags adopt an inactive conformation that no longer engages TORC1 but rather recruits the TSC complex to the lysosome to inhibit Rheb and thereby TORC1 signaling (Demetriades et al, 2014, 2016). Thus, shutting off TORC1 upon amino acid withdrawal is not simply a dissipation of upstream activation signals, but rather an active process. The importance of the TSC complex in dampening TORC1 signaling upon amino acid removal is highlighted by the fact that mouse embryonic fibroblasts lacking the TSC complex die upon amino acid removal due to unbridled TORC1 activity (Demetriades et al, 2014).

A recent study from the Teleman lab (Tsokanos et al, 2016) provides insight on the mechanism mediating TORC1 inhibition upon amino acid withdrawal (Fig 1). In an RNAi screen in Drosophila S2 cells, the authors surprisingly identified the translation initiation factor eIF4A as an important inhibitor of TORC1 upon amino acid depletion. Upon eIF4A knockdown, cells fail to inactivate TORC1 signaling when amino acids are removed from the growth medium. eIF4A inhibits TORC1 in response to removal of different, non-overlapping subsets of amino acids, suggesting that the inhibition is not in response to the absence of a specific amino acid. Interestingly, eIF4A appears to inhibit TORC1 independently of its role in translation initiation, as knockdown of other translation initiation factors or pharmacological inhibition of translation does not affect downregulation of TORC1 signaling upon amino acid removal. The notion that eIF4A moonlights as a TORC1 inhibitor is further supported by the observation that a point mutation in eIF4A prevents TORC1 inhibition but not translation initiation. eIF4A appears to be a potent TORC1 inhibitor also in vivo since eIF4A mutant Drosophila larvae fail to inactivate

Figure 1. eIF4A inactivates TORC1 upon amino acid withdrawal.

(A) In the presence of amino acids, NAT1 binds and inhibits eIF4A, and TORC1 is active. (B) In the absence of amino acids, NAT1 releases eIF4A which then activates TSC2 to inhibit TORC1 signaling.
eIF4A regulates TORC1

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TORC1 when transferred to food lacking amino acids.
eIF4A activates translation as part of the so-called eIF4F complex that also contains eIF4E and eIF4G. Both eIF4E and eIF4G, but not other translation initiation factors, collaborate with eIF4A also in regulating TORC1, although neither eIF4E nor eIF4G was identified in the original RNAi screen. As revealed by immunoprecipitation and proximity ligation assays, eIF4A interacts directly with Raptor (a core TORC1 subunit) in the cytoplasm and not on the lysosome. This interaction is not impaired in the absence of amino acids suggesting that binding alone is not sufficient for the inhibitory effect of eIF4A on TORC1. Indeed, eIF4A inhibits TORC1 indirectly by interacting with and activating TSC2. Surprisingly, eIF4A knockdown does not appear to affect subcellular localization of TSC2. This suggests that upon amino acid withdrawal, eIF4A acts on TSC2 via a mechanism other than recruiting it to the lysosome.

To identify the mechanism by which eIF4A is regulated by amino acid availability, Tsokanos et al (2016) investigated proteins that co-immunoprecipitate with eIF4A in the presence or absence of amino acids. The most prominent hit was NAT1, the fly ortholog of mammalian initiation factor EIF4G2. Binding of NAT1 to eIF4A is high in the presence of amino acids and decreases upon amino acid removal. Knockdown of NAT1 resulted in decreased TORC1 activity, and epistasis analyses demonstrated that NAT1 acts upstream of eIF4A to inhibit TORC1. How NAT1 senses amino acid availability to inhibit eIF4A remains to be determined.

Does eIF4A inhibit TORC1 in mammals as in flies? As in S2 cells, eIF4A1 and eIF4A2 knockdown in HeLa cells prevents down-regulation of mammalian TORC1 (mTORC1) signaling upon amino acid removal. However, in contrast to TORC1 in Drosophila cells, cycloheximide prevents inhibition of mTORC1 in response to amino acid deprivation. This experiment is complicated by the fact that cycloheximide normally activates TORC1/mTORC1 by increasing pools of free amino acids. In any event, the contrasting effects of cycloheximide in mammalian and fly cells could stem from differences in amino acid efflux or autophagy rates. Furthermore, the Telemann lab recently reported that unlike other mammalian cells, HeLa cells display constitutively high lysosomal TSC2 localization, even when amino acids are present (Demetriades et al, 2016). Thus, HeLa cells may not be the best mammalian system to study the functional link between the protein synthesis machinery and mTORC1. It is also possible that, in mammalian cells, eIF4A affects mTORC1 activity via two separate mechanisms—one translation-dependent and one translation-independent.

Tsokanos et al (2016) confirm earlier observations that TORC1 signaling components interact directly with translation initiation complexes (Holz et al, 2005; Harris et al, 2006; Csibi et al, 2010). They demonstrate that various proteins involved in translation are enriched in Rag GTPases immunoprecipitates. Second, they show a direct interaction between Raptor and eIF4A and co-localization of these two proteins in the cytoplasm. These observations raise many intriguing questions. How is TORC1 recruited to the translation machinery? What is the mechanism by which eIF4A activates the TSC complex to inactivate TORC1? How is the binding of NAT1 to eIF4A controlled? How does NAT1 prevent eIF4A from inactivating TORC1? Answering these questions will significantly improve our understanding of TORC1 regulation. TORC1 substrates mediate translation (S6K, 4E-BP) and other cellular processes (S6K, Ulk1, Grb10). It would be interesting to know whether eIF4A affects TORC1 activity toward all substrates or only toward substrates involved in translation regulation.

Increased TORC1 activity is observed in multiple human cancers and in organs affected by metabolic dysfunction (Laplante & Sabatini, 2012). In particular, mutations in the amino acid-sensing branch of TORC1 have recently been shown to be the underlying cause of increased TORC1 signaling in multiple cancer cell lines (Shimobayashi & Hall, 2016). It would be very valuable to extend the studies of NAT–eIF4A–TORC1 interaction to various human pathologies. In particular, it would be interesting to know whether manipulating NAT1 and/or eIF4A can reduce TORC1 activity in cells with preexisting TORC1 hyperactivation and translate this into clinical benefit.

References


