ESSAY

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Size does matter

Lysosome-based signaling machine regulates cell growth

By **Liron Bar-Peled**

From *Nanoarchaeum equitans*, a microbe barely measuring 400 nm across, to *Balaenoptera musculus*, the blue whale that often exceeds 30 m in length, one of the most distinguishing characteristics of an organism is its size. Animal size is determined by total cell number, which is achieved through cell proliferation. Proliferation in turn depends on cell growth, a process regulated by both genetic and environmental factors.

When nutrients are plentiful, cells engage key programs to increase their size and mass, whereas a dearth of nutrients triggers opposing programs that release much-needed cellular building blocks to maintain homeostasis. To couple nutrient availability to cell size regulation, eukaryotic organisms rely on signaling pathways that concomitantly sense environmental nutrient availability and control downstream processes required for growth.

In the past 20 years, the mechanistic target of the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) has emerged as the central signaling pathway that regulates cellular, organ, and organismal size (*1*). mTORC1 has major roles in controlling food intake, insulin sensitivity, and life span and, when deregulated, is implicated in the pathogenesis of common cancers and diabetes. mTORC1 responds to a wide variety of stimuli, including growth factors, oxygen availability, energy, and amino acid levels to control anabolic and catabolic processes (*2*). Although amino acids are absolutely essential for mTORC1 activation, surprisingly little is known about how they are sensed and activate mTORC1.

As a first-year graduate student in David Sabatini's laboratory at MIT, I contributed to finding the first pieces of the amino acid–sensing pathway. Amino acids promote mTORC1 translocation to the lysosomal surface, where it becomes activated. This translocation is mediated by Rags, a family of small guanosine triphosphatases (GTPases) (*3*, *4*) that are localized to the lysosomal surface by a mechanism that had not been elucidated. Unique among small GTPases, Rags are obligate heterodimers: the highly related RagA and RagB (RagA/B) bind to RagC or RagD (RagC/D), which are also very similar to each other. Amino acids regulate the binding of nucleotides to RagA/B, such that amino acid stimulation increases their guanosine triphosphate (GTP) loading, which leads to the recruitment and binding of mTORC1. Thus, a critical event in the amino acid–dependent regulation of mTORC1 is the conversion of RagA/B from a guanosine diphosphate (GDP)– to a GTP-bound state (*3*–*6*). My graduate thesis focused on identifying the protein factors that positively or negatively regulate the function of Rags by controlling their nucleotide state as well as their lysosomal localization.

I hypothesized that proteins that regulate Rags would do so through a direct interaction. By purifying Rags under several conditions, we identified five proteins that form a novel complex, which we named "Ragulator" (*5*, *6*). There is a mutation in humans that partially reduces the expression of one of the Ragulator components, and patients carrying this mutation are substantially smaller in size than their peers, a characteristic of mTORC1 pathway inhibition in model organisms (*1*, *2*, *7*).

Ragulator localizes to the lysosomal surface and specifically interacts with Rags. Through loss-of-function and mislocalization studies, we determined that Ragulator is both necessary and sufficient for Rag localization, and, as expected, mTORC1 could no longer localize to lysosomes and remains inactive in cells depleted of Ragulator proteins. Furthermore, the Rag-Ragulator interaction

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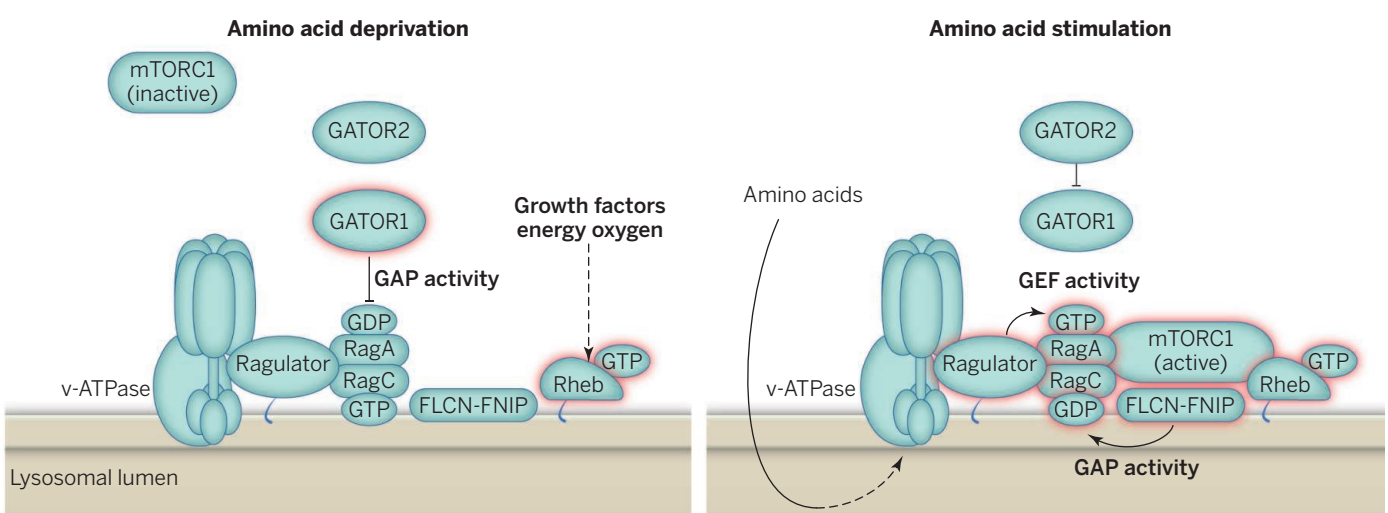

The pathway of amino acid sensing by mTORC1. (**Left**) During amino acid deprivation, Ragulator is found as an inactive complex with the v-ATPase. GATOR1 GAP activity toward RagA keeps this GTPase in an inactive GDP-bound state that cannot recruit mTORC1. (**Right**) After amino acid stimulation, GATOR1 is inhibited by GATOR2, and Ragulator and the v-ATPase undergo a conformational change releasing the guanine nucleotide exchange factor (GEF) activity of Ragulator toward RagA. At the same time, the FLCN-FNIP complex activates RagC, by promoting its GTP hydrolysis. The now-active Rag heterodimer (RagA-GTP, RagC-GDP) recruits mTORC1 to the lysosomal surface, where it interacts with and is activated by the small GTPase Rheb, which is itself controlled by growth factor, energy, and oxygen levels (*1*).

is regulated by amino acids, which suggests that Ragulator might also control the nucleotide loading of the Rag GTPases (*6*). Rags pose a particular experimental challenge for identifying factors that regulate the nucleotide state of a single Rag, as they exist as obligate heterodimers, and so we developed several methods that allowed us to analyze the nucleotide binding state of one Rag at a time. This led to the discovery that Ragulator is a guanine nucleotide exchange factor for RagA/B that promotes GTP binding and activation (*6*). Although this exchange activity of Ragulator provides a mechanism by which amino acids activate the Rag GTPases, how amino acid deprivation inactivated Rags remained a mystery.

Negative regulators of small GTPases have short-lived interactions with their cognate GTPases (*8*). Thus, to identify negative regulators of Rags, we purified them in the presence of a chemical cross-linker that preserves transiently interacting proteins. This led to the identification of a complex of eight Rag-interacting proteins that we refer to as "GATOR" [GTPase-activating protein (GAP) activity toward Rags]. GATOR is defined by two distinct subcomplexes: GATOR1 and GATOR2. GATOR1 negatively regulates mTORC1, and its loss increases cell size, whereas GATOR2 positively regulates this pathway by inhibiting GATOR1. Subsequently, we discovered that GATOR1 directly interacts with the Rag GTPases and inhibits their function through its GAP activity for RagA/B, by stimulating GTP hydrolysis (*9*).

Upstream negative regulators of mTORC1 are commonly mutated in cancer and, indeed, inactivating mutations in GATOR1 genes are found in in a subset of glioblastoma and ovarian tumors. Moreover, the mTORC1 pathway is insensitive to amino acid starvation in cancer cell lines missing GATOR1 components and hypersensitive to treatment with a U.S. Food and Drug Administration–approved mTORC1 inhibitor, rapamycin (*9*). Thus, GATOR1 proteins may serve as useful biomarkers to help identify patients likely to respond to clinically approved pharmacological inhibitors of mTORC1. In addition to its role in cancer, the GATOR complex has also been implicated in epilepsy (*10*). Two recent studies uncovered that mutation of a GATOR1 component underlies familial focal epilepsy with multiple foci (*11*, *12*) and provides a molecular etiology for this disease.

After the identification of Ragulator and GATOR1 as the first positive and negative regulators of the Rag GTPases, respectively, additional studies highlighted the complexity of this signal transduction network with the identification of new members needed to integrate the amino acid signal with size regulation. These components include the lysosomal vacuolar adenosine triphosphatase (v-ATPase), which mediates the amino acid signal to Ragulator and is required for amino acid–dependent mTORC1 activation (*13*), as well as the folliculin–folliculin-interacting protein (FLCN-FNIP) tumor suppressor complex, which is responsible for activating RagC/D (*14*). These studies reveal the presence of a lysosome-based signaling machine that is required to sense nutrient availability for the spatiotemporal regulation of mTORC1 (see the figure), offering us a window into how the size of a cell is influenced by its environment. ■

2014 Grand Prize Winner

Cell and Developmental Biology: Liron Bar-Peled for his essay "Size does matter." Dr. Bar-Peled received his Bachelor of Science degree from the University of Georgia and his Ph.D. from the Massachusetts Institute of Technology, where he studied amino acid sensing in David Sabatini's lab. He received the 2014 Weintraub Award for Graduate Research, the 2013 Gary Bokoch memorial award from the American Society for Biochemistry and Molecular Biology, and the 2012 Abraham J. Siegel Fellowship Award from the Whitehead Institute for Biomedical Research. He is investigating how cells respond to oxidative stress in the laboratory of Benjamin Cravatt at the Scripps Research Institute in La Jolla. He is a Lallage Feazel Wall Fellow of the Damon Runyon Cancer Research Foundation.

Category Winners

Genomics and Proteomics: Dan Dominissini for his essay, "Roadmap to the epitranscriptome." Dr. Dominissini received his Bachelor of Medical Science degree from Tel-Aviv University, Israel, in 2007. He went on to study RNA posttranscriptional modifications for his Ph.D., focusing on adenosine deamination and methylation, with Gideon Rechavi at Tel-Aviv University. He is currently a Human Frontier Science Program postdoctoral fellow in the laboratory of Chuan He at the University of Chicago, where he develops novel chemistries for the study of nucleic acid modifications.

Translational Medicine: Simon C. Johnson for his essay, "A target for pharmacological intervention in an untreatable human disease." Dr. Johnson is an American Federation for Aging Research Fellow at the Albert Einstein College of Medicine in New York. He earned his Bachelor of Science degree at Oregon State University and received his Ph.D. from the University of Washington. He was a 2009 Howard Hughes Medical Institute EXROP scholar and was previously supported by the Nathan Shock Center Genetic Approaches to Aging predoctoral and Mechanisms of Cardiovascular Diseases postdoctoral competitive training grants. His work is centered on characterizing the role of genetic variation in insulin/IGF-1/mTOR signaling genes on human longevity.

Environment: Chelsea Wood for the essay, "Environmental change and the ecology of infectious disease." Dr. Wood received her Bachelor of Arts degree from Dartmouth College and her Ph.D. from Stanford University. She did postdoctoral research in Pieter Johnson's lab at the University of Colorado at Boulder, and is currently a Fellow in the Michigan Society of Fellows and an Assistant Professor in the Department of Ecology and Evolutionary Biology at the University of Michigan. She is interested in how parasites and pathogens respond to human impacts on the environment.

For the full text of all winning essays and further information, see http://scim.ag/SciLifeLab.

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