PERSPECTIVES: CELL BIOLOGY

Surviving Starvation

Susan Gottesman and Michael R. Maurizi

Cells engage in a delicate tightrope act: They must balance energy-efficient growth with the ability to adapt rapidly to sudden changes in their environment. For example, in an environment rich in amino acids, cells do not expend energy making enzymes required for amino acid synthesis. However, if the environment becomes depleted of amino acids (nutritional downshift), cells will be caught lacking both the enzymes required to make amino acids and the amino acids required to make these enzymes. To solve this dilemma, cells in nutrient-poor environments must use their own proteins as sources of amino acids. Once amino acid biosynthetic enzymes start to accumulate, the cell is able to produce its own amino acids, and a new growth phase begins. On page 705 of this issue, Kuroda *et al.* (*1*) describe the molecular components that enable cells to adapt to an environmental downshift, when a period of feast abruptly ends and leaner times roll around.

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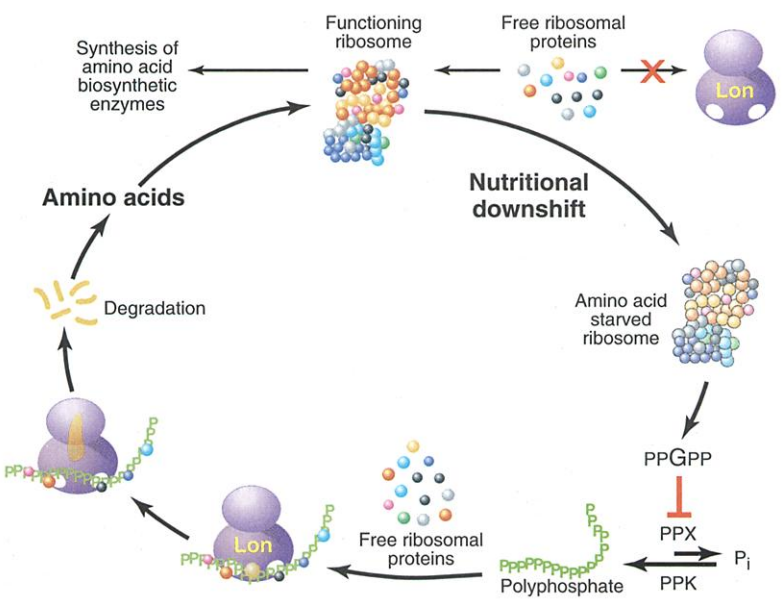
In all cells, there is increased degradation of otherwise stable proteins during times of starvation (*2*). In prokaryotes, such as the bacterium *Escherichia coli*, starvation-induced proteolysis is an energy-dependent process requiring hydrolysis of the energy-releasing molecule adenosine triphosphate (ATP). The proteases and regulatory factors responsible for protein degradation during starvation have not been identified, and little is known about which proteins are degraded. Kuroda *et al.* (*1*) present convincing evidence that protein degradation in *E. coli* during a nutritional downshift depends on the ATP-dependent proteases Lon and Clp. Moreover, protein degradation in this bacterium seems to be triggered by accumulation of an unusual molecule: a string of hundreds of inorganic phosphate residues (polyphosphate). Finally, certain very abundant ribosomal proteins in *E. coli* are the sacrificial substrates targeted for degradation during a transition from nutrient-rich to nutrient-poor conditions.

Polyphosphate accumulates in cells in response to a variety of stresses including depletion of amino acids. If the polyphosphate kinase gene (*ppk*) is missing and polyphosphate cannot accumulate, cells fail to recover from a shift to a nutrient-poor medium; however, addition of amino acids enables the mutant cells to resume growth (*3*). Kuroda *et al.* show that mutations in both the Lon and Clp proteases produce the same phenotype as *ppk* mutations—cells fail to overcome a nutritional downshift, and addition of amino acids overcomes the block. Their biochemical analyses suggest that polyphosphate stimulates Lon protease to degrade specific proteins. Of the many cellular proteins screened as potential substrates, ribosomal proteins (not associated with intact ribosomes) were found to be preferentially targeted by the Lon-polyphosphate complex. Polyphosphate associates not only with Lon protease but also with ribosomal proteins, suggesting that it may be a specificity factor. The accumulation of polyphosphate in response to starvation and its ability to mediate degradation of substrate proteins by Lon (and possibly Clp) provides a model to explain how cells cope with a sudden depletion of the amino acid pool (see the figure).

This model provides the missing link between protein degradation and a phenomenon known as the "stringent response." The stringent response depends on guanosine tetraphosphate (ppGpp), a key signaling molecule in starving or nutritionally stressed cells. Synthesis of ppGpp depends on the presence of idling ribosomes and uncharged transfer RNAs (tRNAs without their attached amino acids). This molecule integrates many of the transcriptional effects of amino acid starvation: It shuts down transcription of genes encoding ribosomal proteins and proteins involved in rapid growth, and switches on genes required for biosynthetic pathways needed to replenish depleted metabolites (*4*).

Intriguingly, ppGpp is also required for the accumulation of polyphosphate and for the increase in degradation of otherwise stable proteins during starvation. The Kuroda *et al.* work shows that these effects are all related. Polyphosphate is made by PPK and is broken down by exopolyphosphatase (PPX). Both enzymes are constitutively expressed, but PPX activity is inhibited by ppGpp. Consequently, when ppGpp builds up in the cell after a nutritional downshift, a decrease in PPX activity results in accumulation of polyphosphate (*5*). The ppGpp-dependent increase in protein degradation now can be explained by the ability of newly made polyphosphate to bind to ribosomal proteins, making them available to Lon pro-



Switching gears. Nutrient depletion induces protein degradation. During amino acid depletion in *E. coli*, there is increased production of ppGpp and consequently of polyphosphate. The binding of polyphosphate to a subclass of free ribosomal proteins and to the Lon protease stimulates Lon-dependent degradation of ribosomal proteins. Amino acids are then made available to the cell by cytoplasmic peptidases that chop up the short peptides released by Lon. The cell is then able to adjust to nutrient-poor conditions by making biosynthetic enzymes from the released amino acids. In vivo, both Lon and Clp proteases appear to contribute to recovery of bacteria from a nutrient downshift.

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tease for degradation (see the figure). The ribosome thus acts as a "starvation sensor," signaling through ppGpp to the cell that it needs to tap into amino acid reserves.

How are ribosomal proteins made available for degradation? Kuroda *et al.* found that polyphosphate does not destabilize intact ribosomes, although they did not rule out other factors causing ribosomal disassembly during a nutritional downshift. If ribosomes are not disassembled during a downshift, then the usual regulation of ribosomal protein synthesis and assembly must be sufficiently flexible in rapidly growing cells to ensure that a store of accessible amino acids (in the form of free ribosomal proteins) is always available. Even if polyphosphate-dependent degradation is not restricted to ribosomal proteins, the increase in protein turnover during the transition phase following a nutritional downshift may be more selective than previously thought.

The adaptation of cells to a decrease in amino acid availability may differ from long-term adaptation to true starvation—in the signaling molecules used, the proteins degraded, and the proteases involved. Cells deprived of essential elements such as carbon, nitrogen, sulfur, phosphate, or metal ions, or permanently deprived of an amino acid through mutation of its biosynthetic enzyme, must enter a holding phase during which no increase in cell mass is possible. In contrast, cells undergoing a nutritional downshift need only readjust their metabolism to begin exploiting less-efficient sources of essential nutrients. It remains to be seen whether other sacrificial substrates degraded under starvation conditions are targeted by polyphosphate.

In eukaryotes, proteins to be degraded are first bound to ubiquitin, a delivery tag that targets the substrate to 26*S* proteasomes or to lysosomes for degradation. Selection of substrates for ubiquitination is under the control of a panoply of ubiquitin protein ligases, each associated with adaptor proteins. These adaptors recruit different families of proteins to the ligase depending on the presence of specific binding motifs (*6*). Although ubiquitin tagging is absent in prokaryotes, other adaptor proteins have evolved alternative ways to recruit specific proteins to any of five known ATP-dependent proteases. For example, phosphorylation of a response regulator that interacts with both the sigma factor RpoS and the ClpXP protease results in degradation of RpoS (*7*); degradation of SsrA-tagged proteins by ClpXP is accelerated by yet another adaptor, SspB (*8*).

Polyphosphate is particularly suitable as an adaptor during nutritional downshifts because its synthesis does not require amino acids. How does polyphosphate promote protein degradation? Perhaps the proximity of protein substrate and protease is sufficient to allow capture and degradation of the bound protein. Alternatively, the Lon protease may recognize a motif on the bound protein or some other region that becomes exposed once the protein interacts with polyphosphate.

Polyphosphate is found in all cells, including those of mammals. As it also accumulates when cells are under nonnutrient stress, its interactions with certain target proteins may turn out to be unrelated to protein degradation. The Kuroda *et al.* work should spur new investigations into this ubiquitous polymer and its importance in protein degradation and other stress responses.

References and Notes

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PERSPECTIVES: VOLCANOLOGY

Predicting Volcanic Eruptions

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In the past decade, some major explosive volcanic eruptions have been forecast successfully, for example, at Pinatubo, Philippines, in 1991, Rabaul, New Guinea, in 1994, and Soufriere Hills, Montserrat, in 1995. Anomalous geophysical signals were correctly interpreted as early warnings of an impending eruption, and cities at the foot of these volcanoes were evacuated several hours to weeks before the renewal of explosive activity. Nevertheless, volcanic eruptions and their secondary effects, such as lahars or mudflows, still claim many lives, for example, in the eruptions of Nevado del Ruiz, Colombia, in 1985 and Lake Nyos, Cameroon, in 1986, both of which caused thousands of deaths.

The reason for this discrepancy is that no rigorous methodology exists for predicting an impending eruption and the evolution of eruptive activity. Early detection of volcanic activity is mainly based on the observation of an anomalous increase in seismic activity and ground deformations. Substantial variations in these factors, which are a result of increased pressure of the magmatic system and magma motion, certainly constitute a basic tool for issuing a warning (*1*), but not all volcanoes behave as expected, and existing data are too limited for reliable prediction on a global scale.

That may soon change. In the past two decades, the temporal and spatial resolution of ground deformation data has increased rapidly through use of the Global Positioning System (GPS) and satellite radar interferometry. In addition, seismological observations now provide information about the underground structure and the magmatic chambers at a scale of a few hundred meters. Waveform inversion from broadband seismic instruments is emerging as a powerful tool for modeling the source geometry of the magma feeding system and its dynamics. An excellent example is the report by Kumagai *et al.* on page 687 of this issue (*2*). The wealth of data provided by these techniques and by other broadband instruments such as strainmeters (see the figure) is beginning to put eruption prediction on a quantitative footing.

The medium- and long-term behavior of high-risk explosive volcanoes remains, however, quite puzzling. Many eruptions of explosive volcanoes have occurred in the past two decades and were consequently relatively well monitored (*3*, *4*). Most of them were preceded by relevant geophysical phenomena, allowing successful forecasting and early warnings. On the other hand, Usu, in Japan, exhibited different eruptive and preeruptive behavior during its four eruptions in the 20th century. The behavior of other volcanoes also does not always follow a clear pattern.

One part of the problem is that systematic geophysical monitoring is performed on only a small percentage (about 10%) of the 1300 active or potentially active volcanoes worldwide. Successful prediction of impending activity has been achieved on basaltic volcanoes such as Kilauea (Hawaii) (*5*) and Sakurajima (Japan) (*6*) and generally for volca-

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