found for both Sec61α and Sec61γ in Eubacteria as well as in Archaea, pointing to a mechanism of protein translocation that is evolutionarily highly conserved. The Sec61 complex is required for the translocation of all substrate proteins so far tested (*10*). In addition, cross-linking studies have shown that Sec61α, the largest subunit of the complex with 10 membrane-spanning domains, is in close proximity to nascent chains throughout their translocation (*11*). The complex, isolated either from mammalian cells or yeast, can oligomerize in detergent to form a ringlike structure containing three or four copies of the complex and with an inner diameter of ~20 Å (*12*, *13*). Similar structures are seen in native membranes, providing compelling evidence that the Sec61 complex is in fact the protein-conducting channel seen in the biophysical studies.

Beckmann *et al.* (*3*) have now extended these observations by determining the EM structure of the heterotrimeric Sec61 complex bound to the ribosome, using components isolated from yeast. One key to their success was the observation that the Sec61 complex binds in a specific and saturable manner to 80S ribosomes in detergent solution, that is, under conditions that maintained the oligomerized state of the Sec61 complex. Another key was the use of three-dimensional image reconstruction, an approach used recently to produce wonderful low-resolution images of ribosomes from several organisms, including yeast. In these images, the Sec61 complex appears as a pentagon-shaped toroidal structure (or, put more simply, "a misshapen donut"), similiar in morphology to what had been observed before, with one surface of the Sec61 oligomer facing the surface of the large ribosomal subunit. The final outcome of this EM reconstruction (*3*) confirms the great insightfulness of the original model proposed in 1975 (*4*) (see figure).

Two important aspects of the mechanism of cotranslational translocation are suggested by this study. First, there is a single site of attachment between the Sec61 complex and the large ribosomal subunit. Therefore, most of the oligomeric Sec61 complex lies ~15 Å from the ribosome. Thus, as it stands, this structure cannot account for the tight seal between the ribosome and the translocon implied from fluorescence studies. It could be that additional components, either membrane or cytosolic in origin, create a "scarflike" seal around this gap. Alternatively, as the authors speculate, the nascent chain could induce a conformational change in the ribosome or the Sec61 complex so that the two structures would meet and a seal would be formed. The notion that the degree of attachment might be regulated by features in the nascent chain itself could begin to explain the dynamic behavior of the ribosome-translocon interface, which allows access of lipid to the growing chain of a transmembrane protein.

Second, the central pore of the oligomeric Sec61 complex aligns precisely with the exit site of a tunnel that runs through the large ribosomal subunit up to the subunit interface. Such a tunnel appears in other reconstructed images of ribosomes, prompting speculation that it forms the passageway for the nascent chain from its site of polymerization to the surface of the ribosome. Classic biochemical analyses showing that ribosomes protect distinct portions of the nascent polypeptide chain from proteolysis have seeded this idea (*14*). Although the existence of such a tunnel in the ribosome may still be controversial to some, the alignment of this cavity with the central opening in the Sec61 oligomer is too intriguing to discount.

These new results leave us with an image in which the translocon forms an extension of the ribosomal tunnel, connected in series to conduct the nascent chain from its site of synthesis to its final destination in the ER lumen. Indeed, ribosomes attached to translocon sites protect correspondingly larger fragments of nascent chains from proteolysis than ribosomes alone (*15*). Superimposed onto this simple conduit, however, is the likely dynamic behavior of the translocon that allows protein translocation and membrane protein integration, while strictly maintaining the permeability barrier of the membrane to other solutes. Thus, as remarkably intuitive as this new picture may appear, it is only a new beginning.

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TRANSCRIPTION

Control of the Supply Line

Jeffrey Roberts

All cells undergo orderly duplication, harnessing many complex biochemical systems in the process. As it makes a copy of itself, the cell monitors its own economy in order to provide new cell components at the right rate. How does this happen so reliably? Such mechanisms of homeostatic regulation of basic cellular components have been very elusive. For example, well-characterized elements trigger DNA replication, but it is unknown how they are modulated to initiate replication precisely when the mass of a growing cell population reaches a certain value. It has also been difficult to discern how cells determine their investment in protein-making machinery—the ribosomes—according to need: When the growth rate varies in response to a change in the availability of nutrients, the number of ribosomes increases in proportion to the growth rate (*1*), implying that the rate of ribosome synthesis increases as the square of the growth rate. The mechanism of such regulation has been a matter of prolonged controversy, but an article in this issue on page 2092 from the laboratories of R. Gourse and C. Turnbough presents a model that will crystallize our existing views (*2*).

In his pioneering work, M. Nomura identified ribosomal RNA (rRNA) synthesis as the ultimate site of regulation of ribosome synthesis; enough ribosomal protein is produced to meet the cell's need for ribosome assembly, in response to the availability of rRNA (*3*). When a critical ribosomal protein accumulates in excess, it binds its own mRNA and inhibits further translation. This regulation entails many pleasing subtleties, including a binding site in mRNA for the critical regulatory protein that mimics the

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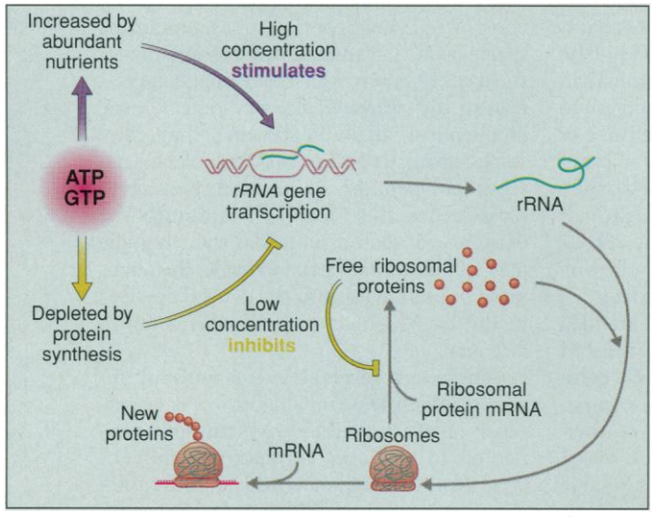
protein's binding site in rRNA, and a mechanism of sequential translation by which a single binding site at the beginning of the mRNA can control all genes of a polycistronic set.

But if ribosomal proteins are made stoichiometrically with rRNA, what signal and mechanism regulate the RNA? An early notion that the population of ribosomes not engaged in protein synthesis is the feedback signal was disproved; thus, an excess of ribosomes made nonfunctional by mutant *rRNA* genes or by limiting translational initiation factor IF2 does not cause feedback inhibition of rRNA synthesis (*4*, *5*). However, addition of excess functional genes to the natural set of seven down-regulates them all, and deletion of some of the natural rRNA genes appropriately up-regulates the remaining ones (*6*). The regulatory signal therefore acts to maintain a constant pool of active ribosomes by monitoring the net protein synthesis capacity of this pool, not the concentration of ribosomes.

One persistent candidate for the regulatory signal has been the guanosine triphosphate (GTP) derivative ppGpp (along with the related compound pppGpp); by hypothesis, a larger amount of ppGpp signals excess capacity for protein synthesis and also, by some mechanism, inhibits rRNA synthesis. It does appear that ppGpp shuts off rRNA and transfer RNA (tRNA) synthesis upon amino acid starvation (the "stringent response"), likely by binding to RNA polymerase and inhibiting its activity at the promoters (*7*), and possibly by inhibiting the rRNA antitermination system as well (*8*). In the stringent response, amino acid starvation limits the availability of charged tRNAs and causes ribosomes to stall at the cognate codon in mRNA. The ribosome-associated protein RelA then is activated to make ppGpp nucleotides from GTP or guanosine diphosphate and adenosine triphosphate (ATP) (*7*).

But is ppGpp involved in the subtler adjustments of growth rate regulation? Its concentration is appropriately correlated to ribosomal gene transcription when the growth rate or the number of rRNA genes is changed (*9*). The definitive test of its role would be to see whether growth rate regulation occurs in cells lacking ppGpp altogether (ppGpp-0 cells) as a result of mutational inactivation of the two enzymes that can make ppGpp. However, different laboratories report contradictory results with reporter genes for the rRNA operons in ppGpp-0 cells (*10*, *11*).

The new model for growth rate regulation proposed by Gaal *et al.* relates ribosomal promoter activity directly to the energy prosperity of the cell and indirectly to the overall rate of protein synthesis, in a simple way. It is proposed that in the bacteria *Escherichia coli* the concentration of intracellular ATP and GTP determines the rate of initiation at *rRNA* promoters, so that a given nutritional state gives rise to an equilibrium in which synthesis of ATP and GTP is balanced by their use in protein synthesis (see figure). This model had two important sources of inspiration. First, *rRNA* promoters have unusual properties: They are very efficient but also form atypically unstable open complexes, so that their activity is limited by the open complex decay rate. Thus, increased concentration of the initiating nucleoside triphosphate (ATP or GTP) could increase initiation. Second, for a different promoter of *E. coli*, the concentration of the initiating nucleotide triphosphate is involved in regulating initiation of transcription (*12*).



Supplies for cell division. Regulatory circuits homeostatically control ribosome biosynthesis in *E. coli*. Increased nutrients raise the concentration of ATP and GTP, which in turn stimulates ribosome production and synthesis of new protein for cell division.

Evidence provided by Gaal *et al.* for this model is quite persuasive. First, *rRNA* promoters that initiate with ATP require a much higher ATP concentration for initiation in vitro than do most other promoters, whereas GTP-initiating promoters require correspondingly high GTP. A mutant promoter that has lost its ability to regulate growth control, so that it is still highly expressed at low cell growth rates, also shows high expression at low ATP concentrations in vitro. Second, the open complex at *rRNA* promoters is stabilized in vitro by the correct initiating nucleoside triphosphate, whereas the growth control mutant is stable at low ATP concentrations in vitro. Finally, manipulation of cells to reverse the natural increase in ATP with growth rate also reverses the response of an *rRNA* promoter reporter construct to growth rate in vivo.

How does the overall regulation work? The model implies that the rate of protein synthesis, the major energy utilizing process in the cell, is set to the maximum allowed by the nutritional state: rRNA synthesis (and hence the capacity for protein synthesis) will diminish only when protein synthesis has depleted cellular ATP enough to reduce the rate of initiation at *rRNA* promoters. In this view, stringent control would serve to inhibit futile rRNA synthesis if protein synthesis is stalled by amino acid deprivation while both ribosomes and energy are abundant.

Further work will show whether there is a relation between sequence elements required for stringent regulation (*13*) and the site affecting growth rate regulation. These sequences overlap in location—between the −10 promoter element and the start site—but are distinct by mutational analysis. Understanding both growth rate and stringent response regulation of promoters will be a healthy challenge to the notion that transcription regulation can be understood through kinetic analysis of RNA polymerase activity.

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