The difficulty in accounting for plate tectonics with computer simulations may be explained if plates are a self-organized system that organizes mantle convection, rather than vice versa. Upper mantle convection patterns should then be regarded as the result, not the cause, of plate tectonics. Whether the first-order features of plate tectonics emerge from this approach remains to be seen (*19*).

The mantle is usually considered as a homogeneous convecting layer that expresses itself at the surface in plate tectonics. Progress in understanding the base of the mantle, the mid-mantle, and the surface boundary layer show that this is much too simple a view. Theory shows that chemical stratification is difficult to detect with standard techniques. But a stratified mantle, along with the self-regulation of the plates, would slow down the cooling of Earth and postpone the inevitable heat death.

Thermochemical 3D convection simulations in spherical shell geometry and with self-consistent pressure-dependent thermodynamic properties and the possibility of deep undulating chemical interfaces will be required to test these ideas. If plate tectonics is a self-organizing system that also organizes mantle convection, then convection simulations need to allow multiple degrees of freedom so that all possible states can be explored.

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10. The deep mantle (below 1000 km depth) has different spatial and spectral characteristics than the upper mantle and may be isolated from it in terms of exchange of material. Density variations of the deep mantle do, however, affect surface elevation, lithospheric stress, and the geoid even if the mantle is chemically stratified. Heat will also flow upwards and may influence the thermal structure, but plate tectonics and subduction may overwhelm this signature because of their shorter time scale.
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15. The fact that Earth's mantle is split into three or four tomographically distinct regions does not necessarily imply that the mantle is chemically stratified or that the regions are convectively isolated. Phase changes and viscosity variations can also influence the flow. But layered and thermochemical calculations reproduce tomographic features (*6, 16*) and explain the geoid and dynamic topography (*20*). Even features in the deep mantle that have been attributed to slabs have alternative explanations (*16*) consistent with layered convection.
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19. At equilibrium, the structure that minimizes the free energy is selected. The existence of an equivalent principle for dynamic nonequilibrium systems is an important unsolved problem. The organizing principle for plate tectonics is unknown. Because rocks are weak under tension, the conditions for the existence of a plate probably involve the existence of lateral compressive forces. Plates have been described as rigid but this implies long-term and long-range strength. They are better described as coherent entities organized by stress fields and rheology. The corollary is that volcanic chains and plate boundaries are regions of extension. Plates probably also organize themselves to minimize dissipation.
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PERSPECTIVES: MOLECULAR BIOLOGY

Turning Gene Regulation on Its Head

Richard Losick and Abraham L. Sonenshein

Gene expression is often thought of as a binary system controlled by a series of on/off switches. New technologies for visualizing genome-wide gene expression reinforce the idea that the state of the cell can be described in terms of the sum total of the on/off states of its genes. In reality, the regulation of gene expression is more subtle. Gene expression can be modulated over a wide range in response to cues from within cells, from other cells, and from the environment. No system better illustrates this fine-tuning than the *trp* operon—the cluster of genes encoding the enzymes that make the amino acid tryptophan—in the Gram-negative bacterium *Escherichia coli*. According to Valbuzzi and Yanofsky (*1*) on page 2057 of this issue, *E. coli*'s elegant bipartite strategy for regulating expression of the *trp* operon is paralleled by an equally elegant but radically different system in the Gram-positive bacterium *Bacillus subtilis*.

In *E. coli*, the *trp* operon genes are transcribed from a single promoter. The tryptophan produced is loaded onto its specific transfer RNA ($tRNA^{Trp}$) by the enzyme tryptophanyl tRNA synthetase and is then transferred by the $tRNA^{Trp}$ to growing polypeptide chains emanating from ribosomes, the cell's protein synthesis factories. In classic experiments, Yanofsky and co-workers established that *E. coli* regulates transcription of the *trp* operon in two ways: by repressing transcription in response to an increase in the cellular concentration of tryptophan, and by attenuating transcription in response to a rise in uncharged $tRNA^{Trp}$ (that is, $tRNA^{Trp}$ without tryptophan attached) (*2*). At high tryptophan concentrations, the tryptophan repressor protein is activated and it binds to the operon, preventing initiation of transcription; at low tryptophan concentrations, the repressor is unable to bind to DNA, hence RNA polymerase has unfettered access to the operon's promoter and transcription ensues. However, transcription of the *trp* operon is also regulated by sequences located between the 5′ end of the mRNA and the first enzyme coding sequence of the operon. This leader region has domains that can fold into alternative and mutually exclusive stem-loop (hairpin) structures. One stem-loop acts as a transcription terminator, the other as an antiterminator. The leader sequence also includes a tiny coding region containing two tryptophan codons. When tryptophan is abundant, ribosomes are able to move across and translate this short sequence, interfering with antiterminator formation and thereby favoring terminator formation resulting in the termination (attenuation) of transcription. When the cell is starved of tryptophan and the amount of charged $tRNA^{Trp}$ plummets, ribosomes stall at the tryptophan codons in the leader sequence, the antiterminator forms, and terminator formation is blocked. Thus, *E. coli* adjusts transcription of the *trp* operon by relying on two sensors: the repressor, which measures the cellular concentration

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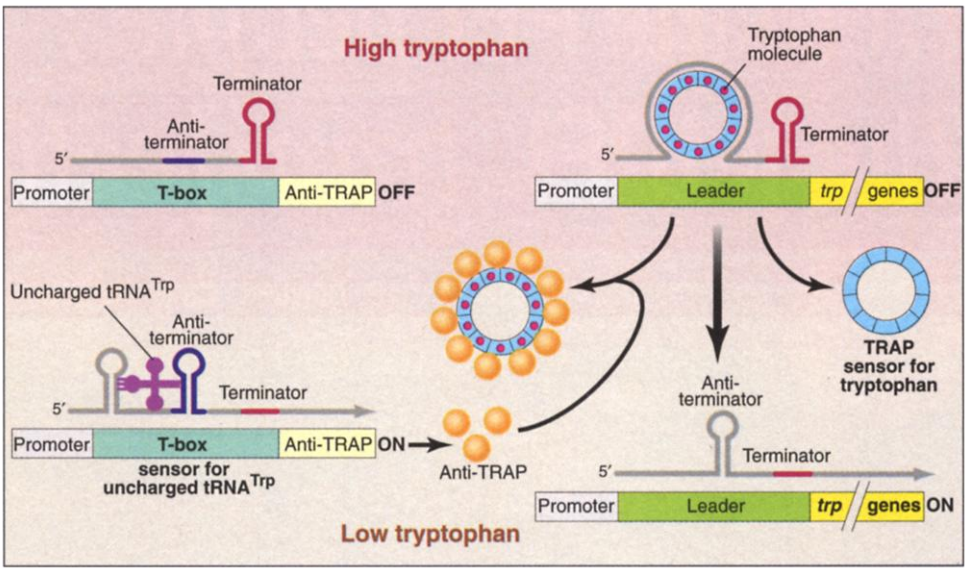
of tryptophan, and the ribosome, which monitors the amount of uncharged tRNA^{Trp} in the cell. Fine-tuning is possible because molecules of mRNA that escape repression are still subject to attenuation.

But do *E. coli*'s distant cousins control the transcription of their tryptophan biosynthetic genes in a like manner? Nature's logic often becomes more transparent when the same problem is addressed in different biological systems, even though bacteria are notorious for having devised multiple solutions to the same problem. Yanofsky and co-workers have shown that *B. subtilis* uses the same enzymes as *E. coli* to make tryptophan. As in *E. coli*, the *B. subtilis trp* operon is regulated by attenuation: A leader RNA sequence forms either a terminator or antiterminator stem-loop structure (*3*). Remarkably, however, the *B. subtilis* leader RNA is not translated by ribosomes; instead, attenuation is governed by a protein, TRAP, that senses the intracellular concentration of tryptophan rather than the amount of uncharged tRNA^{Trp}. TRAP—for tryptophan-activated *trp* RNA binding attenuation protein—consists of 11 subunits in a circular arrangement (see the figure) (*4*). Each subunit binds to a molecule of tryptophan. The leader RNA wraps around the tryptophan-bound TRAP like a belt around a wheel, such that it sequesters the antiterminator-forming sequence and exposes the terminator (see the figure). When tryptophan concentrations are high, TRAP promotes transcriptional termination. In contrast to *E. coli*, which senses tryptophan through a DNA binding protein that inhibits transcription initiation, *B. subtilis* relies on a tryptophan-sensing, RNA binding protein that terminates transcription.

Like *E. coli*, *B. subtilis* is able to fine-tune the expression of its *trp* operon over a wide range of tryptophan concentrations. But a single protein (TRAP) with a set affinity for tryptophan seems inadequate to explain this fine-tuning. Moreover, the TRAP story, as beautiful as it is, fails to account for an old observation that seemed destined to rest in perpetual obscurity. The late William Steinberg reported in 1974 that a mutation in the gene for tryptophanyl tRNA synthetase causes overexpression of the *B. subtilis trp* operon, even when cells contain excess amounts of tryptophan (*5*). Perhaps *B. subtilis* senses uncharged tRNA^{Trp} after all, but if it does, how does it do so? This is where the new work from Yanofsky's laboratory comes in (*1*). Reinvestigating the tRNA^{Trp} of *B. subtilis*, this group reports the discovery of a protein they dub anti-TRAP, which binds to TRAP and prevents it from interacting with RNA. Anti-TRAP is encoded within an operon whose transcription is induced by uncharged tRNA^{Trp} (*6*). Just how this induction occurs is explained by the work of Grundy and Henkin, who discovered that aminoacyl tRNA-synthetase genes and certain other operons in *B. subtilis* are transcribed in response to the concentration of specific tRNAs—the uncharged tRNA pairs with the secondary structure (T-box) of the leader RNA that precedes the gene (*7*). Both the tryptophanyl tRNA synthetase gene and the operon containing the gene for anti-TRAP are preceded by leader RNAs that specifically interact with uncharged tRNA^{Trp}. This interaction is believed to stabilize the formation of an antiterminator and thereby to promote transcription. Conversely, when tryptophan is abundant and the amount of uncharged tRNA^{Trp} is low, the leader instead forms a terminator hairpin that attenuates transcription and hence impedes synthesis of the synthetase and anti-TRAP. Thus, in *B. subtilis*, fine-tuning of *trp* operon expression depends on anti-TRAP—synthesized when uncharged tRNA^{Trp} accumulates—which limits the activity of TRAP.

B. subtilis has turned *E. coli*'s regulatory system on its head, using a tryptophan-sensing protein to control attenuation and a tRNA-sensing RNA to control the synthesis of a regulatory protein. Yet, at a deeper level, the principles of the two systems are the same. Both bacteria rely on separate sensors for tryptophan and uncharged tRNA^{Trp} and, in both cases, the alternative formation of terminators and antiterminators in RNA secondary structures lies at the heart of transcriptional control. Bacterial *trp* operons offer two additional lessons. First, we still have only a superficial understanding of the intricacies of regulation of most bacterial genes, and, second, studying bacterial systems continues to provide insight into the breadth and depth of gene regulation in all organisms.



Two sensors are better than one. Two sensors govern the expression of genes for tryptophan biosynthesis in *B. subtilis*. **(Right)** When the cellular concentration of tryptophan is high, the leader RNA for the *trp* operon is wrapped around the 11-subunit TRAP protein, which is bound to tryptophan. This arrangement promotes the formation of a terminator that blocks transcription of the *trp* operon. **(Left)** Meanwhile, the T-box RNA forms a terminator that turns off transcription of the operon containing the gene for anti-TRAP. When the tryptophan concentration decreases, the *trp* operon is switched on in two ways. First, TRAP molecules from which tryptophan has dissociated are unable to bind to the leader RNA. This allows formation of the antiterminator in some mRNA molecules, thereby partially turning on the *trp* operon. Second, uncharged tRNA^{Trp} binds to the T-box RNA, blocking formation of the terminator and turning on the gene for anti-TRAP. The anti-TRAP protein produced then binds to tryptophan-activated TRAP, preventing it from associating with *trp* leader RNA. Thus, expression of the *trp* operon over a broad range of tryptophan concentrations is achieved by the T-box RNA, a sensor for uncharged tRNA^{Trp}, and by TRAP, a sensor for tryptophan.

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