MOLECULAR BIOLOGY

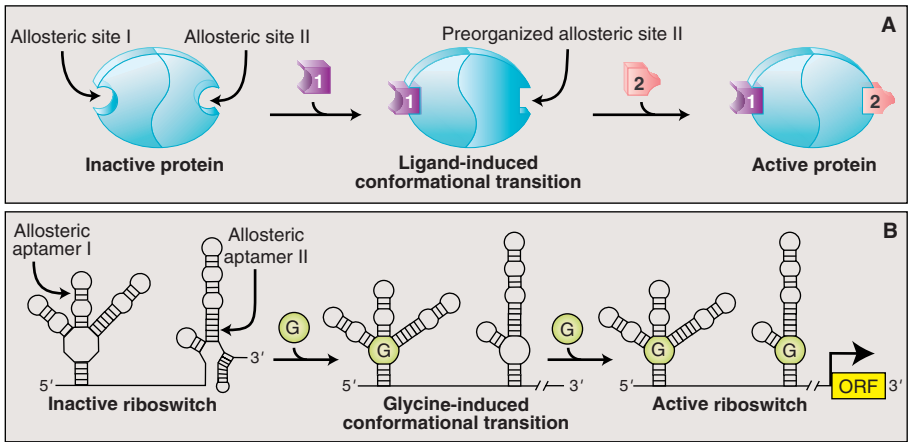
RNAs Turn On in Tandem

Michael Famulok

Proteins often carry out their cellular functions at a defined time and place, requiring that their activity be precisely regulated. An important mechanism for controlling protein activity is allosteric regulation by a small molecule that binds to the protein at a location remote from the active site. Binding of this regulatory compound to the allosteric site induces structural rearrangements in the protein that are relayed to the active site, which then becomes either stable or unstable. In allosteric proteins, multiple subunits act cooperatively: Once a regulator is bound to one subunit, another subunit responds with dramatically enhanced affinity, allowing the protein's activity to be modulated as a function of slight changes in the regulator's concentration (see the figure). On page 275 of this issue, Mandal *et al.* (*1*) report that activity regulation by cooperative binding is not restricted to proteins but also is a feature of RNA molecules. They describe a bacterial messenger RNA (mRNA) element, a so-called riboswitch, in which two allosteric RNA subunits are triggered cooperatively by the amino acid glycine. This cooperative activation regulates the expression of genes involved in glycine metabolism without the need for any additional proteins.

The glycine-responsive riboswitch joins a series of recently identified natural RNA motifs that are controlled by other small-molecule regulators (*2*). In all of them, the highly selective binding of small molecules to the RNA motifs activates or represses expression of nearby genes by inducing conformational changes in their mRNAs that interfere with transcription or translation. The RNA elements of the new glycine-responsive riboswitch are embedded within the untranslated regions of genes encoding a protein complex that enables bacteria to cleave glycine for consumption as an energy source—but only if the glycine concentration exceeds a certain level. Accordingly, the expression of the components of the glycine cleavage system must remain in an off-state when the amount of glycine is limited. If not, a resource that is indispensable for vital processes, such as maintenance of protein synthesis, would be invested in energy production, which could just as easily be accomplished with other available molecules. Conversely, if regular fuels such as carbohydrates or fats are scarce, an organism would be at a selective advantage if it could derive energy from sources that are not easily accessible to its competitors. Thus, the glycine riboswitch has to fulfill two important criteria: to act as a genetic "on-switch," and to be able to reliably sense glycine with high specificity within a narrow concentration window. Both of these abilities are far from trivial to achieve even for a protein, and thus set the new riboswitch apart from most other riboswitches. The mechanism of action of the new riboswitch is remarkable and provides a further demonstration of the power of RNA as a regulatory element.

Riboswitches are actually natural versions of a class of artificial ligand-binding nucleic acids known as aptamers. Aptamers were first isolated from complex mixtures of trillions of synthetic sequences by in vitro evolution methods (*3*, *4*). They form binding pockets that recognize a huge variety of small organic molecules with high affinity and specificity. In various cases, different aptamer sequences have been identified for the same ligand (*5*, *6*). The glycine riboswitch consists of two different aptamer types that individually bind to a single molecule of glycine (see the figure). Interestingly, both aptamers are always joined in tandem—separated only by short linker sequences—in the genes of many different bacterial species (see the figure). An individual RNA motif requires about a 100-fold increase in glycine concentration to arrive at nearly complete saturation when starting at concentrations where binding is just about detectable. But in the complete tandem-arranged riboswitch, cooperative glycine binding narrows this concentration range to only 10-fold. Indeed, as Mandal *et al.* note (*1*), the extent of cooperation between the two glycine binding sites in the *Vibrio cholerae* riboswitch compares favorably with that of the four oxygen binding sites in hemoglobin, a star among those proteins exhibiting cooperativity.



The benefits of cooperation. (**A**) In certain proteins, two distant binding sites for positive regulator molecules 1 and 2 are conformationally coupled. Binding of molecule 1 to allosteric site I triggers conformational changes that result in reorganization of a distant second site, allosteric site II, enabling molecule 2 to bind more efficiently to this site. The protein is active when both molecules are bound. (**B**) Activation of a riboswitch, which consists of the allosteric glycine-binding RNA aptamers I and II, might occur by a similar mechanism. Here, too, binding of one glycine molecule (G) to aptamer I helps a second glycine to bind to aptamer II. Only then is the riboswitch activated, resulting in transcription of genes that encode components of the glycine cleavage system.

From an evolutionary point of view, it is fascinating that nature has evolved several different glycine-binding aptamer motifs and arranged them in tandem to function together in vivo as regulators that sense glycine within a narrower concentration window than either aptamer alone. Also remarkable is the fact that RNA molecules that comprise only 86 to 126 nucleotides are capable of binding to an organic ligand of 75 daltons with such impressive specificity that glycine can be effectively discriminated from closely related amino acids and their derivatives.

Perhaps even more notable, however, is

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the fact that for virtually every mechanism harnessed by riboswitches to control gene expression, it is possible to cite an analogous artificial system that was first constructed in the laboratory. In vitro selected aptamers that affect translation of reporter genes in cells (*7*) preceded the discovery of similar natural riboswitches (*2*). Engineered allosteric ribozymes regulated by small organic molecules (*5*) antedated the recent discovery of the first catalytic riboswitch regulated by glucosamine-6-phosphate (*8*). There is even an example of an allosteric ribozyme construct in which two aptamers specific for flavine mononucleotide and theophylline are aligned in tandem to act as a cooperative "on-switch" for ribozyme cleavage (*9*). For decades, scientists have been guided in the construction of synthetic model systems that mimic functional principles applied by the corresponding archetypes of nature. It is rewarding to see that in the case of riboswitches, engineering of functional nucleic acid molecules inspired the search for and discovery of closely related natural counterparts.

Is the principle of gene regulation by riboswitches restricted to bacteria, or is it also found in eukaryotes? These regulators appear to be widespread in bacteria, but candidates are also likely to be present in the eukaryotic genes of plants, as suggested by their evolutionary conservation (*10*). It will be interesting to see whether they can also be found in higher eukaryotes, even in humans. If so, completely new avenues of pharmaceutical research would be opened up with the exploration of RNAs as worthwhile drug targets.

References

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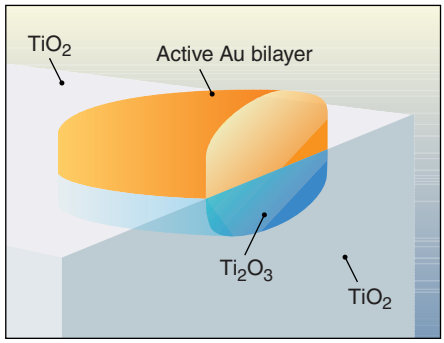
PHYSICS

The Active Site in Nanoparticle Gold Catalysis

Charles T. Campbell

Most pollution from U.S. automobiles is emitted in the first 5 min after startup. This is because Pt- or Pd-based catalysts currently used in automobile exhaust cleanup are inactive below about 200°C. Gold-based catalysts present a potential solution to this cold-startup problem. Gold nanoparticles dispersed across the surfaces of certain oxides have been shown to be amazingly active and selective as catalysts for a variety of important reactions. There is intense interest in these catalysts for carbon monoxide oxidation, because they are active at room temperature. Interestingly, the low-temperature gold catalysts are totally inactive unless the gold is in the form of particles smaller than ~8 nm in diameter (*1*–*3*). Though gold nanoparticles have been perhaps the most widely studied catalyst system in the last 2 to 3 years, the structure of the active site has remained elusive. On page 252 in this issue, Chen and Goodman (*4*) take an important step toward characterizing the active site.

The problem has been that the active sites are on or near tiny gold particles that are themselves difficult to structurally characterize, and the gold-coated surface is very heterogeneous and thus structurally ill-defined. Chen and Goodman (*4*) have produced a highly active model gold catalyst where the gold is incorporated in a crystalline film, spread uniformly over a Ti_2O_3 surface like icing on a cake. The coated surface is therefore amenable to structural elucidation with quantitative low-energy electron diffraction and other surface crystallographies. The gold appears to be a pure, crystalline film, two atomic layers thick, with an epitaxial relationship to the underlying oxide support, itself a crystalline thin film of Ti_2O_3. The authors prepared this Ti_2O_3 as an ultrathin film on the (112) surface of a molybdenum single crystal using elegant synthetic strategies pioneered previously (*5*–*7*). The very high catalytic turnover rate for this gold film raises the exciting possibility of an ~50-fold improvement in the performance of realistic, high-area catalysts.



Gold that works: Proposed structure of active gold nanoparticles in which reduced TiO_2 accumulates under gold islands, as first shown in (*19*).

Previous work on oxide-supported gold nanoparticle catalysts has provided evidence used to support a wide range of active-site structures. Some researchers have proposed that the active sites are on the surface of the oxide (usually defects), possibly modified by the presence of nearby gold, and function together with sites on the gold nanoparticles (*8*–*10*). Others attribute the catalytic activity entirely to the presence of neutral gold atoms on the gold nanoparticles. These neutral atoms differ from atoms on bulk gold in three ways that might enhance their catalytic activity: (i) They have fewer nearest-neighbor atoms (that is, a high degree of coordinative unsaturation) (*11*–*14*) and also possibly a special bonding geometry to other gold atoms that creates a more reactive orbital (*15*). (ii) They exhibit quantum size effects that alter the electronic band structure of gold nanoparticles (*3*). (iii) They undergo electronic modification by interactions with the underlying oxide that cause partial electron donation to the gold cluster (*16*). Another proposal is that positively charged gold ions on the oxide support are the key to the catalytic activity of these gold catalysts (*17*, *18*).

The Chen and Goodman study (*4*) marks an important step toward identification of the active site. Although the atomic resolution crystal structure of this highly active gold thin film has not yet been determined, the authors have provided strong evidence for the broad features of its structure using a powerful combination of surface analysis techniques (qualitative low-energy electron diffraction, x-ray photoelectron and Auger electron spectroscopies, high-resolution electron energy loss spectroscopy, and low-energy ion-scattering spectroscopy). Their results imply that the active site, at least for low-temperature CO oxidation, involves gold atoms that are nearly electrically neutral and bound to the surface via Au-Au covalent bonds and Au-Ti bonds. The authors find that the gold film completely covers the oxide and that reactants are sterically hindered from chemical bonding directly with the underlying Ti_2O_3.

This exciting work raises a number of important questions. What is the crystal structure of this Au/Ti_2O_3 film? Why are the gold atoms in this thin gold film different from the atoms on bulk gold? Will this

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