RNA finds a simpler way

Thomas R. Cech

Is there no end to the versatility of RNA? The latest feat to be revealed is RNA's ability to switch off genes through a neatly straightforward mechanism. So it isn't only proteins that can repress gene activity.

Organisms profit from synthesizing only those enzymes whose products are in demand. After all, if there's plenty of an amino acid around, why should a cell waste energy making the enzymes that are needed to generate it from precursors? A standard textbook mechanism by which this is achieved is repression. Repressor proteins sense when there is a build-up of a certain product, bind to the gene that encodes the enzyme that generates this product, and thereby inhibit the transcription of more messenger RNA (mRNA; Fig. 1a). On page 281 of this issue, Winkler *et al.*[1] describe a bacterial gene-regulation system with a twist. The repressor is not a protein, but instead is a switchable (on–off) self-cleavage element within the mRNA itself.

The newly discovered molecular switch involves an RNA molecule with enzymatic activity. Self-cleavage by this ribozyme is accelerated 1,000-fold in the presence of glucosamine-6-phosphate (GlcN6P), a small sugar. GlcN6P is generated by the GlmS enzyme, which is encoded by a portion of the *glmS* mRNA downstream from the ribozyme sequence. So it is easy to envisage a gene-regulatory circuit in which the *glmS* mRNA is translated into GlmS protein until the GlcN6P product accumulates; at that point, GlcN6P binds to the special catalytic element in the mRNA, causing it to self-destruct (Fig. 1b). Although the cleavage event itself leaves the coding region of the message intact, the RNA is either destabilized and subject to degradation, or its ability to be translated into functional protein is compromised.

The minimal region of the RNA that can confer this regulatory activity is roughly 75 nucleotides long, the size of a transfer RNA. The chemical mechanism by which it is cleaved during repression has previously been seen in other ribozymes, such as the hammerhead, hairpin and hepatitis delta virus ribozymes[2], but the structure of its catalytic fold appears to be distinct from these. When placed upstream of an unrelated 'reporter' gene, the *glmS* ribozyme element also repressed its expression, and this required the same sequences that are required for *glmS* self-cleavage *in vitro*[1]. Thus, the active RNA element is modular and transplantable.

Although switching gene expression in this manner is new, there are precedents for the individual components of this regulatory

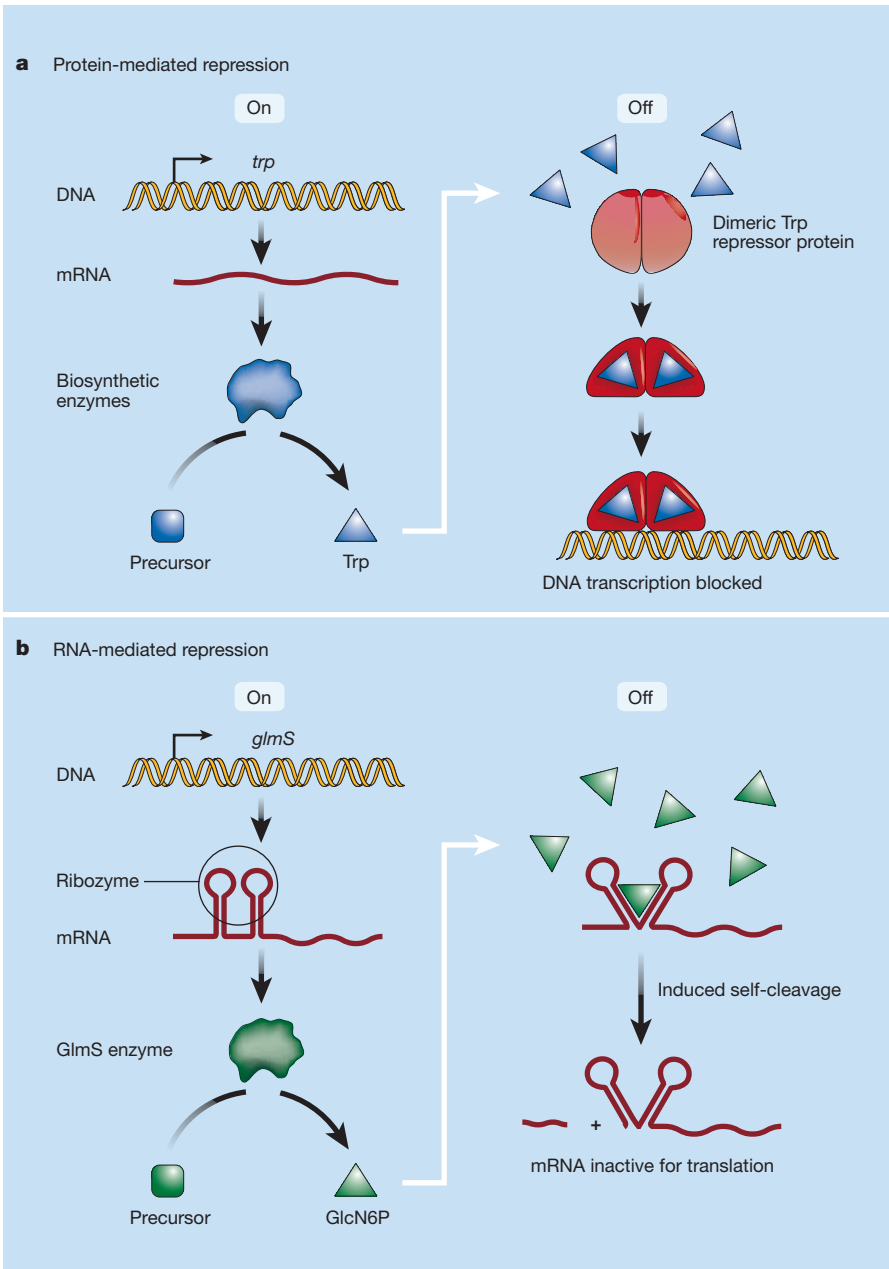

Figure 1 **Repression systems.** a, **Classic repression of the genetic unit involved in production of the amino acid tryptophan. Transcription of the DNA encoding the biosynthetic enzymes for tryptophan produces messenger RNA, which is translated to make the enzymes themselves. When enough tryptophan (blue triangles) has been produced, the tryptophan binds to the dimeric Trp repressor protein, inducing a conformational change that enables it to bind the DNA, thereby blocking transcription.** b, **Repression of a gene at the RNA level by metabolite-activated ribozyme cleavage, as described by Winkler *et al.*[1]. The GlmS enzyme, which is involved in the synthesis of GlcN6P (green triangle), is encoded by the *glmS* mRNA. Also in this RNA is a ribozyme sequence. In the presence of GlcN6P, the self-cleavage activity of the ribozyme is markedly enhanced; ribozyme self-destruction renders the *glmS* mRNA non-functional, thereby inhibiting further production of GlcN6P.**

©2004 Nature Publishing Group

circuit. First, consider GlcN6P binding. Even though it once seemed unlikely that RNA, with its limited diversity of chemical groups and its high negative charge, could specifically bind small molecules, we now know that some naturally occurring RNAs do just that. For example, a particular group of ribozymes forms a pocket that binds guanosine monophosphate, one of the four monomer building-blocks of RNA[3]. And a specific region of RNA from the human immunodeficiency virus binds a derivative of the amino acid arginine[4]. More recently, short (<100 nucleotides) RNA 'aptamers' have been identified that specifically bind everything from hydrophobic amino acids to small organic molecules to metal ions[5,6]. In terms of specificity, an RNA aptamer can even distinguish the plant alkaloid theophylline from the closely related molecule caffeine[7].

As a second precedent, aptamers found within some natural mRNAs bind small molecules as part of gene-regulatory feedback circuits. In the bacterium *Escherichia coli*, coenzyme B12 binds directly to, and thereby represses translation of, the mRNA coding for the protein that transports its precursor, cobalamin[8]. In *Bacillus* species, the synthesis of thiamin and riboflavin involves discrete genetic units. These operons are controlled by direct binding of thiamin pyrophosphate and flavin mononucleotide to leader sequences of the corresponding mRNAs, resulting in transcription coming to an early finish[9]. Although structured RNAs had previously been found to regulate gene expression at multiple levels — during gene transcription, splicing of the RNA and the translation of mRNA into protein — these new systems are different in that they consist entirely of RNA. No protein seems to be required for either sensing the concentration of the metabolic end-product or switching off gene expression.

Finally, several groups had previously engineered artificial riboswitches — RNA aptamers containing ribozyme sequences that, on binding small molecules, induce ribozyme-mediated cleavage of the RNA[10]. These findings might have prompted some to wonder why nature failed to use, or perhaps to retain, such an elegant mechanism. But it's clear now that this perhaps-ancient talent of RNA is alive and well, and is currently used by at least some bacteria to control their *glmS* genes. An exciting question is how widespread these control elements are in biology.

In their eloquent and prescient article on genetic regulatory mechanisms, François Jacob and Jacques Monod[11] thought that genetic repressors might be RNA. They nonetheless much preferred the idea of a protein repressor, as, in their own words "the capacity to form stereospecific complexes with small molecules appears to be a privilege of proteins". When many repressors were subsequently identified as proteins, the idea that RNA repressors existed faded away. But more recently, the concept that RNA can in fact bind small molecules with high specificity and affinity has been established. In a sense, the recent discoveries of naturally occurring 'RNA biosensors' have gone full-circle back to Jacob and Monod — RNA can be the active element that switches off repressible genes in response to the concentration of a cellular metabolite. ■

Thomas R. Cech is at the Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, Maryland 20815, and in the Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309, USA.
e-mail: thomas.cech@colorado.edu

1. Winkler, W. C., Nahvi, A., Roth, A., Collins, J. A. & Breaker, R. R. *Nature* **428,** 281–286 (2004).
2. Doudna, J. A. & Cech, T. R. *Nature* **418,** 222–228 (2002).
3. Michel, F., Hanna, M., Green, R., Bartel, D. P. & Szostak, J. W. *Nature* **347,** 578–580 (1989).
4. Puglisi, J. D. *et al. Science* **257,** 76–80 (1992).
5. Ellington, A. D. & Szostak, J. W. *Nature* **346,** 818–822 (1990).
6. Gold, L., Polisky, B., Uhlenbeck, O. & Yarus, M. *Annu. Rev. Biochem.* **64,** 763–797 (1995).
7. Zimmermann, G. R., Jenison, R. D., Wick, C. L., Simorre, J. P. & Pardi, A. *Nature Struct. Biol.* **4,** 644–649 (1997).
8. Nahvi, A. *et al. Chem. Biol.* **9,** 1043–1049 (2002).
9. Mironov, A. S. *et al. Cell* **111,** 747–756 (2002).
10. Silverman, S. K. *RNA* **9,** 377–383 (2003).
11. Jacob, F. & Monod, J. *J. Mol. Biol.* **3,** 318–356 (1961).

Astronomy

We can see clearly now…

Nicholas White

The mystery of the diffuse γ-ray glow that pervades the Milky Way has been solved, thanks to a space telescope with the power to resolve compact γ-ray sources in the Galaxy.

The Milky Way glows brightly at γ-ray wavelengths. Although this glow was discovered more than three decades ago, its origin has been a mystery. Now the Milky Way has been observed at these wavelengths with new clarity, by astronomers using the European Space Agency (ESA) INTEGRAL observatory. In this issue (*Nature* **428,** 293–296; 2004), F. Lebrun *et al.* report that the soft γ-ray glow is the sum of radiation from previously unresolved point sources, many of them black holes and neutron stars, buried in gas and dust. This discovery points to a future in which soft γ-rays will become a powerful tool for finding black holes in otherwise obscured regions.

Radiation such as γ-rays and their less energetic cousins, X-rays, is used in everyday applications to see inside objects — for example, in medical scanners and for security screening at airports. This same penetrating power makes them a useful astronomical probe for finding and studying objects that may be buried deep in obscuring clouds of dust and gas. By the same token, the capability of γ-rays to penetrate rather than be reflected makes it challenging to design γ-ray telescopes — until recently these telescopes have been little more than crude light buckets, with poor angular resolution (a few degrees, equivalent to many times the diameter of the Moon).

Despite their penetrating power, γ-rays are eventually stopped and absorbed in the extreme depth of Earth's atmosphere — which is fortunate for life on Earth! Consequently, the γ-ray window is closed to Earth-bound telescopes; only with the start of the space age was it opened up for astronomical exploration. The first γ-ray telescopes, launched in the 1970s, detected many bright cosmic sources,

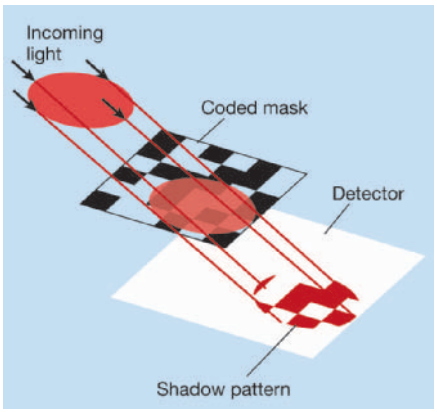

Figure 1 **The coded-mask technique. In optical telescopes, mirrors or lenses are used to focus light, but these same lenses would absorb γ-rays. The coded-mask technique is similar to the principle of a pinhole camera. The mask partly covers the opening of the telescope and casts a shadow on the detector below. Because the position of the shadow depends on the orientation of the mask with respect to the source, it is possible to determine the position and intensity of the source (or sources) and thus produce an image of the γ-ray sky.**

including the γ-ray glow of the Milky Way.

The origin of these soft γ-rays was puzzling. If it is truly a diffuse glow, then the energy being released is huge and would have ionized the molecules in the interstellar medium. The most promising explanation was that this emission originates from an unknown population of point sources, which is spread out on the sky but separated by less than the angular resolution of the γ-ray telescopes available at the time. Confirming this theory required a new large telescope with much sharper imaging.

©2004 Nature Publishing Group