Gene expression

Regulation by anti-sense RNA

from Andrew Travers

THE ability of two complementary strands of RNA to form a stable duplex offers a potentially powerful mode of regulating the biological function of RNA molecules. Thus the normal RNA transcript of a gene could be rendered inactive were it to form a duplex with an 'anti-sense' RNA transcript from part of the same DNA. The past year has produced some good examples of how bacteria employ such a form of control; moreover, the experimental manipulation of gene expression by double-stranded RNA in eukaryotic cells has recently been described.

In prokaryotes, a classic example of this type of control is the regulation of DNA replication of the plasmid ColE1. In this case, a short anti-sense transcript is believed to form a RNA–RNA duplex with the 5′ end of the replication primer and as a consequence to inhibit replication (Tomizawa, J. & Itoh, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1981; Lacatena, R.M. & Cesareni, G. *Nature* **294**, 623; 1981). Mutations affecting either the structure or the amount of anti-sense RNA affect both copy number and plasmid incompatibility.

One example in the control of bacterial translation is the regulation of Tn*10* transposase production (Simons, R.W. & Kleckner, N. *Cell* **34**, 683; 1983). The discovery stemmed from the observation that the presence of a multicopy plasmid containing the insertion element IS*10* inhibits *in trans* transposition of a single-copy chromosomal Tn*10* element. IS*10* is itself essential for Tn*10* activity, providing the functions necessary for Tn*10* transposition. Simons and Kleckner found that they could delete all but 75 base pairs (bp) of the transposase-coding region of IS*10* and still retain multicopy inhibition, showing that the phenomenon does not require intact transposase protein. Intriguingly, the portion of the transposase gene required contains a promoter which directs transcription in the opposite direction to the transposase gene, producing a RNA molecule which overlaps and is complementary to 36 bases at the 5′ end of transposase mRNA. To determine the target of inhibition, Simons and Kleckner constructed two sets of IS*10*–*lacZ* fusions; in one set, the amino (5′)-terminal portion of the transposase gene with its cognate promoter was fused in frame to an appropriately engineered β-galactosidase gene; in the second set, an intact *lacZ* gene was fused to transposase promoter. Multicopy IS*10*-containing plasmids reduced expression of the fusion protein by more than 10-fold but that of the intact *lacZ* gene by less than 2-fold. Assuming that transcription originated from the transposase promoter in both cases, this result strongly suggests that regulation of transposase expression is exerted primarily by modulating translation and not transcription.

A similar situation has been described for the natural regulation of the production of the outer membrane protein encoded by the *ompF* gene (Mizuno, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 1966; 1984). This protein is one of two major outer membrane proteins of *Escherichia coli* that serve as diffusion pores for small hydrophilic molecules. The total amount of these two proteins, OmpF and OmpC, remains constant but the proportion of the two varies, depending on the osmolarity of the medium. While characterizing the promoter of the *ompC* gene, Mizuno and his colleagues observed that a DNA fragment upstream of the promoter inhibited the production of OmpF protein when *ompF*$^+$ cells were transformed with a multicopy plasmid containing the fragment. They discovered that this DNA fragment encoded a small 174 base RNA, termed mic RNA (mRNA-interfering complementary RNA), which was transcribed in the opposite direction to the *ompC* gene. This RNA contains no open reading frames preceded by a ribosome-binding site and so is unlikely to encode a protein. It does, however, have extensive homology with the 5′ end of *ompF* mRNA, suggesting that the small RNA could form a stable hybrid with *ompF* mRNA. Since the region of homology includes the ribosome-binding site for *ompF* translation the obvious possibility is, again, that hybrid formation between the two RNA species inhibits translation initiation. A further characteristic of potential biological significance is the coordinate regulation of the *ompC* and mic RNA species which could provide an efficient mechanism for maintaining a constant total amount of OmpF and OmpC proteins.

For neither IS*10* transposase nor *ompF* protein production has the presence of a RNA–RNA duplex been directly demonstrated. Nevertheless, the fact that anti-sense RNA molecules can inhibit gene expression has been confirmed by engineering the production of such molecules for other genes. Coleman *et al.* (*Cell* **37**, 429; 1984) constructed plasmids that produce small RNA molecules complementary to the 5′ ends of the mRNA either for *E. coli* lipoprotein or for two other outer membrane proteins, OmpA and OmpC. In all cases expression of these anti-sense RNA species resulted in a substantial inhibition of expression of the target mRNA species. Significantly, anti-sense lipoprotein RNA also reduced expression of *ompC* mRNA and vice versa, a phenomenon consistent with the extensive homology between the 5′ regions of the two mRNA species.

The ability of anti-sense RNA to inhibit gene expression has been shown to be applicable to eukaryotes. Izant and Weintraub (*Cell* **36**, 1007; 1984) constructed a plasmid so that a promoter directed the transcription of a RNA complementary to the normal thymidine kinase (TK) transcript. When such plasmids together with a plasmid containing a normally expressed TK gene were injected into mutant mouse L cells that lacked TK, it was observed that the presence of the 'complementary' gene substantially reduced expression from the normal plasmid.

The extent to which this novel form of regulation of gene expression is utilized naturally in prokaryotes and eukaryotes remains to be established. Clearly, however, the potential for manipulating gene expression artificially by this mechanism is substantial and well worth exploiting. □

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Sedimentology

Control of dolomite formation

from M. Kastner

A PAPER on page 450 of this issue of *Nature* by Guanatilaka *et al.* describes a recent dolomite [$CaMg(CO_3)_2$] formation in the subtidal zone of a shallow hypersaline lagoon in southern Kuwait[1]. The finding is of interest not only because it provides further insight into the mechanism of dolomite formation, but also because it has economic implications, dolomite being an important hydrocarbon reservoir, a host-rock for base-metals and associated with evaporites.

Dolomite, one of the three most common sedimentary carbonate minerals, has long perplexed sedimentologists, for two reasons. The first is that there are marked irregularities in its distribution with time. Thus, dolomite is a common, extensive and widespread rock-forming mineral in ancient sediments, in particular in late Precambrian and Palaeozoic times, but it is rather scarce in the Holocene. Between the Jurassic and Cretaceous the ratio of dolomitite (sedimentary rock composed essentially of dolomite) to limestone decreases rapidly and strikingly. Early