Mini-Review

A Profusion of Controls

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ANYONE who teaches knows there are two questions to steer clear of on exams: the one about evolution, to which there is no certifiable answer; and the one about gene control, to which every conceivable answer is correct. Or so it seems at times.

The profusion of transcriptional and posttranscriptional controls that have emerged in recent years can be simplified by recognizing categories. One category involves the regulation of mRNA stability. Short sequence motifs that confer sensitivity or resistance to nucleases, thereby controlling mRNA turnover, have been identified in both prokaryotes and eukaryotes (reviewed by Brawerman, 1987). Here I would like to focus attention on three other categories of gene regulation: (*a*) programmed variations in mRNA structure, particularly in ways that affect translation; (*b*) translation-linked control of mRNA metabolism; and (*c*) regulation of translation by RNA-binding proteins.

Regulated Transcriptional Events and Their Consequences for Translation in Eukaryotes

The structure of mRNA determines both the form and yield of the encoded protein. mRNA structure in turn depends on where transcription initiates and how transcripts are edited during the splicing and polyadenylation steps.

Promoter Switching

The use of alternative start sites for transcription can affect the translatability of eukaryotic mRNAs in two ways. Promoter switching may produce from one gene two forms of mRNA, one of which begins slightly farther upstream than the other. In cases where the longer transcript includes an upstream in-frame AUG codon, initiation of translation from that site will add an "extra" NH_2-terminal domain to the protein, as illustrated in Fig. 1; the biological consequences of the extra domain are significant, as indicated in Table I. An important point, predicted by theory (Kozak, 1980, 1983) and verified experimentally (see references in Table I), is that the shorter version of the protein can be synthesized only from the 5′-truncated form of mRNA even though its initiation site is present, internally, in the longer transcript. Thus, to produce two versions of the protein, two versions of mRNA are required. (There is a way to generate two proteins by initiating at the first and second AUG codons in a single mRNA, but that "leaky scanning" process requires that the upstream AUG codon occur in an unfavorable context for initiation [Kozak, 1986]; the mRNAs in Table I do not meet that requirement.)

In other cases, promoter switching does not affect the form of the encoded protein, but the 5′-noncoding sequence is changed in a way that makes translation more or less difficult. The transcription of many proto-oncogenes (Bentley and Groudine, 1986; Propst et al., 1987; Seto et al., 1988; Stanton and Bishop, 1987; Voronova et al., 1987) and other critical genes (Perlino et al., 1987; Saga et al., 1987; Stanley et al., 1985; de Thé et al., 1987) alternates between two promoters: one producing a long, GC-rich leader sequence that often has upstream out-of-frame AUG codons, while the second form of mRNA has a shorter, simpler leader sequence. The suspicion that the complicated leader sequence on the longer mRNA might impair translation has recently been confirmed for three proto-oncogenes: c-*sis* (Ratner et al., 1987; Rao et al., 1988), c-*mos* (Propst et al., 1987), and $p56^{lck}$ (Marth et al., 1988). c-*myc* is the only case in which truncating a long GC-rich leader sequence did not produce the expected improvement in translation in vivo (Butnick et al., 1985), but the notorious instability of c-*myc* mRNA might mitigate against detecting regulation at other levels.

Regulated Splicing

The phenomenon of alternative splicing is widespread and has important consequences for gene expression in eukaryotes (Breitbart et al., 1987). Whereas alternative splicing produces two or more functional forms of mRNA, recent experiments have revealed another type of control in which a transcript is either rendered functional by removing all introns or kept nonfunctional by temporarily retaining at least part of one intron. The best-characterized examples are some developmentally regulated genes in *Drosophila* (Boggs et al., 1987; Laski et al., 1986). Splicing of the *su*(w^a) gene (suppressor-of-white-apricot) is developmentally autoregulated; only in the absence of the *su*(w^a) protein are introns 1 and 2 removed to produce a translatable mRNA (Zachar et al., 1987). There is evidence of intron retention in other systems, too. The untranslated maternal "mRNAs" in sea urchin eggs, for example, appear to be incompletely spliced (Ruzdijic and Pederson, 1987). Human cells accumulate surprisingly high levels of intron-containing transcripts from the c-*fgr* proto-oncogene (Katamine et al., 1988) and the gene that encodes the U1-70K snRNP protein (Spritz et al., 1987), suggesting that the splicing of those transcripts is either constitutively inefficient or regulated.

The stage-specific expression of the *Krüppel* gene in *Drosophila* is especially interesting because the intron that is subject to regulation occurs near the 5′-end of the transcript; transcripts that retain the intron have seven AUG codons up-

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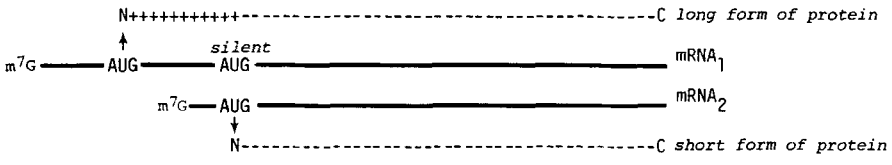

Figure 1. A scheme whereby one gene uses two promoters to produce two transcripts and thus two forms of the encoded protein, initiated respectively at the 5′ proximal AUG codon in each transcript. See Table I for examples.

stream from the major open reading frame, which probably explains their inability to synthesize the *Krüppel* protein in vivo (Gaul et al., 1987). In contrast with that in vivo result, Gaul et al. (1987) were unable to show that translation of the intron-containing mRNA was impaired in vitro, but such experiments rarely succeed when a reticulocyte translation system is used. Part of the problem is infidelity at the level of initiation, a problem that may be exacerbated when the mRNA derives from an SP6- or T7-vector; thus reticulocyte ribosomes sometimes initiate inappropriately at five or more AUG codons, only the first of which serves as an initiator codon in vivo (Gronemeyer et al., 1987; Parker et al., 1986; Ramsay et al., 1986; Rao et al., 1987; Zerial et al., 1987). In wheat germ extracts, on the other hand, translation is limited to the authentic start sites in viral and cellular mRNAs (Kozak and Shatkin, 1977; Zerial et al., 1987) and translation is inhibited in the expected way by upstream AUG codons (Khalili et al., 1987; Pachnis et al., 1988). Thus there is hope for reproducing some aspects of translational regulation in appropriate cell-free systems.

Translation-linked Control of mRNA Metabolism

In bacteria, changes in mRNA conformation during ribosome transit and the consequences for transcription are well documented (Yanofsky, 1988). In eukaryotes this is a rare phenomenon, but three systems show evidence of linkage between translation and mRNA synthesis or decay.

Case 1

The first example concerns β-tubulin autoregulation in animal cells; i.e., the ability of unpolymerized tubulins to repress new tubulin synthesis. Experiments with enucleated cells (Caron et al., 1985) revealed that autoregulation takes place in the cytoplasm and involves modulation of tubulin mRNA stability. The novelty is that only polysome-bound tubulin mRNAs are rapidly degraded in the presence of free tubulin (Pachter et al., 1987). Thus cycloheximide, which stalls ribosomes on mRNA, enhances the degradation of tubulin mRNAs in response to elevated tubulin levels; while puromycin, which releases ribosomes from mRNA, stabilizes tubulin transcripts. The sequence in β-tubulin mRNA that makes it susceptible to autoregulation is (surprisingly) not located in the 5′ or 3′ untranslated regions. Rather, the sequence that encodes the four NH_2-terminal amino acids of β-tubulin is necessary and sufficient for regulated degradation of mRNA (Yen et al., 1988). Putting those facts together, Pachter et al. (1987) suggest that ribosomes function as "cofactors" for degradation either because the nuclease is ribosome associated or because ribosome transit across the message eliminates secondary structure, thus facilitating nuclease attack. The critical requirement for free tubulin might involve binding of the protein to mRNA (in which case the location of the target site at the start of the coding region is something of a coincidence) or the free tubulin subunits might interact with the nascent tubulin polypeptide to form a multiprotein complex that stalls the ribosome. The latter mechanism is reminiscent of translational arrest by the signal

Table I. Genes that Produce "Long" and "Short" Forms of the Encoded Protein by Initiating Transcription from Two Sites, as Depicted in Fig. 1

Gene	Source	Function of long isoform	Function of short isoform	References
α-Isopropylmalate synthase (LEU4)	Yeast	Imported into mitochondria	Cytoplasmic	Beltzer et al., 1988
Valine-tRNA synthetase (VAS1)	Yeast	Imported into mitochondria	Cytoplasmic	Chatton et al., 1988
Histidine-tRNA synthetase (HTS1)	Yeast	Imported into mitochondria	Cytoplasmic	Natsoulis et al., 1986
tRNA-dimethyltransferase (TRM1)	Yeast	Imported into mitochondria*	Cytoplasmic	Ellis et al., 1987
Invertase (SUC2)	Yeast	Secreted	Cytoplasmic	Carlson et al., 1983
Gelsolin	Human	Secreted (plasma form)	Cytoplasmic	Kwiatkowski et al., 1988
Porphobilinogen deaminase	Human	"Housekeeping" enzyme in all nonerythroid cells	Specific to erythroid cells	Chretien et al., 1988
Surface antigen	Hepatitis B virus	Inhibits secretion (thus controlling virus assembly)	Secreted	Persing et al., 1986; Ganem and Varmus, 1987
E2	Bovine papilloma virus	Activator of transcription	Transcriptional repressor	Lambert et al., 1987
E6	Rabbit papilloma virus	Localized to nucleus; abundant in benign tumors	Cytoplasmic; predominates in malignant tumors	Barbosa and Wettstein, 1987, 1988

* In the case of N^2,N^2-dimethylguanosine tRNA methyltransferase, the NH_2-terminal extension increases the efficiency of import into mitochondria, but it is not obligatory.

recognition particle (Walter and Blobel, 1981) except, of course, that translational arrest by the signal recognition particle is not followed by mRNA degradation.

Case 2

A major class of histone mRNAs is selectively and rapidly degraded when DNA synthesis is inhibited in mammalian cells. Two types of evidence implicate a 3′ terminal stem-and-loop structure in this controlled degradation: (*a*) regulation of histone mRNA stability is lost when the 3′ hairpin is either deleted or moved to an internal position (Levine et al., 1987), and (*b*) transposing the hairpin to the 3′ end of an α-globin gene causes α-globin mRNA to be degraded in parallel with histone mRNAs (Pandey and Marzluff, 1987). Since histone mRNAs are degraded 3′ to 5′ both in vivo (Ross et al., 1986) and in vitro (Ross and Kobs, 1986), a reasonable hypothesis is that the 3′ hairpin constitutes the target for a 3′ exonuclease.

An interesting complication is that, when DNA and protein synthesis are inhibited at the same time in vivo, the accelerated degradation of histone mRNAs is prevented. Two explanations for the protein synthesis requirement have surfaced recently. Peltz and Ross (1987) postulate that histone mRNA decay is triggered by the accumulation of free histone proteins, an idea that is strongly supported by the effect of adding core histones to a cell-free system. The resulting accelerated decay was specific for histone proteins (which could not be replaced by other basic, single-stranded nucleic acid–binding proteins) and for histone mRNAs. A second explanation for the protein synthesis requirement postulates a direct role for ribosomes in mRNA turnover. Graves et al. (1987) found that histone H3 mRNA was not properly degraded in vivo when ribosomes terminated translation prematurely, as the result of frameshift mutations that introduced a terminator codon some 300–500 nucleotides upstream from the normal stop site. Thus they postulate that the length of the 3′-noncoding sequence influences the efficiency of mRNA degradation, either because the putative 3′ exonuclease is ribosome bound and must be brought close to the 3′-terminal hairpin, or because ribosomes must traverse (almost) the entire length of the message to melt secondary structure, thereby facilitating nuclease attack. The report by Capasso et al. (1987) of a mutant histone H4 mRNA that is degraded rapidly even though ribosomes terminate ~200 nucleotides upstream from the normal site does not necessarily contradict the findings of Graves et al. (1987); if the need for ribosome transit has to do with removing secondary structure from the mRNA, that requirement is likely to vary from one construct to another.

Case 3

In the third example, the postulated role of ribosomes is once again to erase or alter secondary structures, but this time the effects are on mRNA synthesis rather than degradation. La Crosse virus is an insect-borne RNA-containing bunyavirus that replicates in the cytoplasm of infected cells. Like all negative-stranded RNA viruses, La Crosse virus carries an RNA polymerase within the virion. The problem is that the polymerase makes only short (~175 nucleotides) 5′-terminal fragments of mRNA. The solution, demonstrated both in vivo (Raju and Kolakofsky, 1987) and in vitro (Bellocq et al., 1987), is that ongoing protein synthesis relieves the transcriptional block. A clue to the mechanism is that the translational requirement can be obviated by substituting inosine for guanosine in the nascent mRNA chains (Bellocq and Kolakofsky, 1987). Because inosine substitution greatly weakens RNA secondary structures, Kolakofsky and co-workers (e.g., Bellocq and Kolakofsky, 1987; Bellocq et al., 1987) postulate that ribosome transit also disrupts base pairing. The idea is that, in the absence of ribosomes, the viral RNA polymerase senses an (as yet undefined) base-paired structure involving the 5′ end of nascent mRNA chains, and polymerase responds by terminating transcription. When translation and transcription occur simultaneously, however, ribosome transit prevents the nascent mRNA strands from folding and therefore polymerase reads through, producing full-sized mRNAs. The mechanism has obvious similarities to the attenuation phenomenon in bacteria (Yanofksy, 1988).

Control of Translation by mRNA-binding Proteins in Prokaryotes . . .

A handful of *Escherichia coli* ribosomal proteins (rp's)[1] and bacteriophage proteins are the paradigms of translational repressor proteins. The traditional list of such proteins has grown some in the past year or two (Table II) and detailed structures of many of the target sites have been deduced. The results confirm some old hypotheses and reveal some surprising twists. Many repressor binding sites involve a stem-and-loop structure, as indicated in Table II. Not every hairpin structure in mRNA is a target, of course. By mutagenesis of several target sites the features required for tight, specific binding have been identified; they include "bulged" nucleotides that protrude from the stem (Romaniuk et al., 1987; Climie and Friesen, 1987), unpaired nucleotides in an internal loop (Thomas and Nomura, 1987), and unpaired nucleotides in the hairpin loop (Romaniuk et al., 1987; Freedman et al., 1987). Many nucleotides in the stem itself can be changed without impairing regulation, provided that base pairing is maintained by compensatory sequence changes (Baughman and Nomura, 1984; Climie and Friesen, 1987); thus the function of the stem seems to be to orient the scattered unpaired bases that the protein directly touches.

The primary function of most known translational repressor proteins involves binding to nucleic acids, a circumstance that probably facilitated their recruitment to the secondary task of regulating translation. Thus Nomura et al. (1980) postulated some years back that the mRNA-binding sites for ribosomal repressor proteins might resemble their binding sites on 16S and 23S rRNA. That prediction has now been confirmed for S8, L1, and L10 (see references in Table II); in the case of S4 and L4, however, the anticipated homology between the mRNA- and rRNA-binding sites is not obvious. Threonine-tRNA synthetase is another protein, recruited secondarily to regulate translation, that probably binds to homologous sites on mRNA and, in this case, tRNA (Springer et al., 1986).

As indicated in Table II, the target site for many of the repressor proteins on mRNA overlaps the ribosome binding

1. *Abbreviations used in this paper*: RBS, ribosome binding site; rp. ribosomal protein.

Table II. Negative Regulation of Translation by mRNA-binding Proteins

Repressor protein	Target gene	Binding site for repressor: Structure and location*	Binding site for repressor: How identified	References
Phage R17 coat protein	Replicase	21-nucleotide hairpin; AUG is within stem‡	Saturation mutagenesis of synthetic 21-mer	Romaniuk et al., 1987
Phage Qβ replicase	Coat gene	Includes AUG initiator codon	Protected from RNase	Weber et al., 1972
Phage fl gene V	Gene II (replicase)	Unstructured sequence near AUG (see text)		Yen and Webster, 1982; Model et al., 1982
Phage T4 gene 32	Gene 32	Unstructured sequence near AUG	Deletion mutagenesis	Krisch and Allet, 1982
Phage T4 *regA*	Many T4 genes *regA*, *rIIB*, etc.	Includes AUG initiator codon	Protection from RNase	Winter et al., 1987
Phage P22 scaffold	Scaffolding protein gene	Unknown		Wyckoff and Casjens, 1985
E. coli thrS	§ *thrS*	Includes positions −10 through −40	Map spontaneous o^c mutations	Springer et al., 1986
E. coli infC	‖ *infC*	Includes AU*U* initiator codon	Mutagenesis of AUU abolishes repression	Butler et al., 1987
E. coli RNA polymerase	β/β′ genes	Unknown		Bedwell and Nomura, 1986**
E. coli rp's				
rp L1	L11 gene (L11 operon)	Hairpin in position −18 to −45‡¶	Mutagenesis	Kearney and Nomura, 1987; Thomas and Nomura, 1987
rp L4	S10 gene (S10 operon)	Hairpin in position −35 to −80‡	Deletion and point mutagenesis	Freedman et al., 1987
rp L10	L10 gene (*rif* operon)	Hairpins in positions −80 to −136, −140 to −174‡	Protection and mutagenesis	Climie and Friesen, 1987
rp S4	S13 gene (α operon)	Hairpin (−20 to −80) pseudoknotted to nucleotides +4 to +7‡	Mutagenesis; filter binding with RNA fragments	Deckman and Draper, 1987; Thomas et al., 1987
rp S8	L5 gene (*spc* operon)	Predicted hairpin has AUG within stem‡,‡‡	Mutagenesis	Olins and Nomura, 1981

* The mRNA sequence is numbered relative to the A of the AUG initiator codon, which is designated +1; the preceding nucleotide is designated −1. The Shine-Dalgarno sequence falls around position −7 to −10.
‡ These hairpin structures have been proven by generating compensatory mutations and/or by probing the mRNA with structure-specific enzymes.
§ *ThrS*, threonine-tRNA synthetase.
‖ *InfC*, translational initiation factor 3.
¶ The target site for rp L1 was defined by selecting mutants that had lost regulation but could still be translated; that approach precludes the recovery of mutations in the Shine-Dalgarno site which might or might not be included in the repressor binding site.
** Autoregulation of β/β′ has been observed in vivo, but in vitro experiments are still needed to determine if the level of regulation is (exclusively) translational.
‡‡ The location and revised secondary structure of the S8 target site on *spc* mRNA have recently been determined by site-directed mutagenesis and RNase protection experiments (Nomura, M., personal communication).

site (RBS), and those proteins probably inhibit translation by direct competition with ribosomes. L1 should probably be counted in that category, since the hairpin that comprises its target is pulled close to the RBS when the secondary structure of the whole region is taken into account (Kearney and Nomura, 1987). The target site for S4 maps surprisingly far upstream from the affected RBS, but Deckman and Draper (1987) have solved that dilemma by demonstrating the existence of a pseudoknot: the hairpin structure to which S4 binds has a single-stranded GGGC sequence in the loop that pairs with the complementary CCCG sequence just downstream from the GUG initiator codon, thus bringing the S4 protein within reach of the initiating ribosome. The binding site for L10 also maps far upstream from the start of the affected coding sequence, and again the mRNA is thought to be folded in a way that brings L10 near the RBS (Fiil et al., 1980); the tertiary interaction has not yet been defined for L10, however, as it has been for S4.

Both T4 gene 32 protein and the gene V protein of filamentous phage fl mediate DNA replication by binding to single-stranded regions of DNA; appropriately, their target sites on mRNA also seem to be unstructured. Something more than single strandedness must identify the target sites in mRNA, however, since neither of those proteins can substitute for the other as a translational repressor (Fulford and Model, 1984). A recent report suggests that the single-stranded DNA-binding protein of *E. coli* can also bind to its own mRNA and block translation (Shimamoto et al., 1987). Bacteriophage T4 *regA* protein remains the most perplexing of the translational repressors inasmuch as it inhibits the translation of a variety of phage and even some bacterial mRNAs that have no obvious common structure (Miller et al., 1987).

Recent studies with the cIII gene of bacteriophage λ may have uncovered the first example of a translational "activator" protein in *E. coli* (Altuvia et al., 1987). The cIII gene is preceded by an RNase III cleavage site and cIII is translated efficiently only in cells that have RNase III. That is not an uncommon situation; the usual explanation is that cleavage by RNase III relieves some conformational constraint and thereby facilitates initiation. Altuvia et al. (1987) discovered, however, that a mutant cIII gene that cannot be cleaved by RNase III still requires RNase III for efficient translation. Thus they postulate that the mere binding of RNase III to mRNA induces the requisite change in conformation, and that subsequent cleavage of (wild-type) cIII mRNA actually inactivates it for translation. The translation of lambda gene cII is also positively regulated by a host protein, but in a less direct fashion than gene cIII (Mahajna et al., 1986).

. . . But Not Yet in Eukaryotes

Specific mRNA-binding proteins that modulate translation have not yet been identified in eukaryotes. Autoregulation by free tubulin subunits and histones might involve the binding of those proteins to their respective mRNAs, but that has not yet been shown and, in any case, the effect is on mRNA stability rather than translation. The signal recognition particle does arrest translation, but it interacts with the nascent polypeptide (Krieg et al., 1986) rather than with the mRNA.

Some experiments with HIV, the etiological agent of AIDS, suggest that the "transactivating" protein *tat* might facilitate translation (Cullen, 1986; Feinberg et al., 1986; Knight et al., 1987; Wright et al., 1986), but direct binding of the protein to viral mRNA has not been demonstrated, and the phenomenology varies from experiment to experiment (Kao et al., 1987; Muesing et al., 1987; Peterlin et al., 1986; Rice and Mathews, 1988). Whereas the *tat* gene product of HIV is postulated to enhance translation, the *rep* gene product of adeno-associated virus is postulated to suppress the synthesis of viral capsid proteins, apparently at the level of translation (Trempe and Carter, 1988). The inhibitory effect of *rep* protein shows a puzzling lack of specificity, however, which makes it unlikely that the protein binds directly to the affected mRNAs.

Unlike prokaryotes, the balanced accumulation of ribosomal proteins in eukaryotes is not accomplished by translational autoregulation. Test after test in yeast (Abovich et al., 1985), mammalian cells (Bowman, 1987), and *Xenopus* oocytes (Pierandrei-Amaldi et al., 1985*b*) has failed to detect feedback regulation by ribosomal proteins at the level of translation. The only exceptions were two yeast ribosomal protein genes, L3 and L29, that were at first thought to be translationally controlled (Pearson et al., 1982; Warner et al., 1985). A careful followup study, however, revealed no change in polysome distribution when L3 and L29 mRNAs were overproduced (Maicas et al., 1988); instead, the discrepancy between mRNA levels and protein accumulation was traced to rapid degradation of the newly synthesized L3 and L29 proteins (see below). The possibility that a few rp genes might be regulated at the level of transcriptional processing has also been suggested (Dabeva et al., 1986; Caffarelli et al., 1987) but has not yet been confirmed by using an in vitro splicing system. The most frequent and most compelling finding is that the synthesis of rp's is not balanced in eukaryotes; rather, each protein is made in proportion to its mRNA concentration, and excess proteins that cannot be incorporated into ribosomes are rapidly degraded (Abovich et al., 1985; Bowman, 1987; Maicas et al., 1988; Pierandrei-Amaldi et al., 1985*a*; Warner, 1977). Inefficient as that may seem, it nevertheless accomplishes the same balanced accumulation of rp's as the elegant bacterial mechanism. Parenthetically, the reliance on proteolysis to balance rp accumulation in eukaryotes is not a complete surprise; a similar mechanism disposes of excess β-spectrin (Woods and Lazarides, 1985) and β-tubulin (Whitfield et al., 1986) which, like rp's, are subunits of multicomponent protein structures.

Conclusion

Although many genes are regulated by simply switching their transcription on and off, there is growing evidence of the importance and variety of posttranscriptional controls. The three levels of control highlighted in this review were chosen because they are areas of recent progress and because they represent regulatory devices that are used again and again. The examples cited reveal how minor shifts in mRNA structure can have major consequences for translation, how ongoing translation can control the synthesis or stability of mRNA, and how mRNA-binding proteins regulate translation in prokaryotes but not (so far) in eukaryotes.

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