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CONTROL OF TRANSLATION INITIATION IN ANIMALS

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ABSTRACT

Regulation of translation initiation is a central control point in animal cells. We review our current understanding of the mechanisms of regulation, drawing particularly on examples in which the biological consequences of the regulation are clear. Specific mRNAs can be controlled via sequences in their 5′ and 3′ untranslated regions (UTRs) and by alterations in the translation machinery. The 5′UTR sequence can determine which initiation pathway is used to bring the ribosome to the initiation codon, how efficiently initiation occurs, and which initiation site is selected. 5′UTR-mediated control can also be accomplished via sequence-specific mRNA-binding proteins. Sequences in the 3′ untranslated region and the poly(A) tail can have dramatic effects on initiation frequency, with particularly profound effects in oogenesis and early development. The mechanism by which 3′UTRs and poly(A) regulate initiation may involve contacts between proteins bound to these regions and the basal translation apparatus. mRNA localization signals in the 3′UTR can also dramatically influence translational activation and repression. Modulations of the initiation machinery, including phosphorylation of initiation factors and their regulated association with other proteins, can regulate both specific mRNAs and overall translation rates and thereby affect cell growth and phenotype.

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INTRODUCTION

The importance of the translation machinery and its fidelity have been apparent since the discovery of the genetic code. However, the broad impact of translational regulation in eukaryotic cells has emerged explosively only in the last few years. This new appreciation of translational regulation has been propelled by work in systems as diverse as budding yeast and human tumors, and approaches that range from genetics to biochemistry and cell biology. It is the diversity of these controls, their biological implications, and increasing access to key regulators that prompts this review.

There are two general forms of translational control: In one, a specific mRNA or subset of mRNAs is regulated. Such regulation can be quantitative, determining the amount of protein produced; this may be all-or-none, or graded. Specific regulation can also be qualitative, enabling a single mRNA to produce several different proteins. In the second form, regulation is global and modulates rates and patterns of protein synthesis, thereby contributing to the overall regulation of cell growth and metabolism. These two forms of regulation are not mutually exclusive.

Translational control is important throughout development but nowhere more so than in the oocyte and early embryo. This is not entirely surprising: Early embryos are commonly transcriptionally inactive, or nearly so, yet require rapid changes in the proteins they contain in order to regulate key developmental decisions. Consequently, translation during early development has been the subject of intense genetic and biochemical scrutiny. The earliest steps in pattern formation in *Drosophila* emphasize the importance and intricacy of translational control (St Johnston & Nüsslein-Volhard 1992, Wharton 1992, Curtis et al 1995, Macdonald & Smibert 1996). The circuitry is sophisticated enough to rival any in molecular biology (Figure 1). Formation of the anterior-posterior axis requires a cascade of translational regulation in which, for example, the translational activation of one mRNA generates a protein product that in turn represses or activates the translation of another. Similarly, in *Caenorhabditis elegans*, translational controls contribute to the determination of cell fates, pattern formation, and the timing of development events (Schnabel & Priess 1997, Wickens et al 1996). We discuss these processes and many of the interactions depicted in Figure 1 in detail below. Here, we emphasize two perspectives. First, genetic analysis has revealed that these controls are indispensable and often require regulatory elements in the 3′UTR. Yet in virtually none of these genetically defined instances of 3′UTR-based control has the molecular mechanism been elucidated. This stands as a primary and immediate goal.

Second, our understanding of the basic mechanisms of translation initiation, as well as 5′UTR-mediated and global forms of control, has obtained a greater degree of molecular detail. Much of this knowledge has been obtained biochemically and completing this picture is an immediate objective. Determining the precise biological role of such events in the intact organism is an equally pressing and formidable challenge.

We discuss the regulation of translation initiation in eukaryotes, considering both mRNA-specific and global controls. In general, we focus on examples from multicellular animals, emphasizing cases with particular relevance to developmental biology. We refer to the translation of yeast, plant, and viral mRNAs only as needed. Several reviews of related topics that we do not consider here include the regulation of elongation (Proud 1994) and termination (Tuite & Stansfield

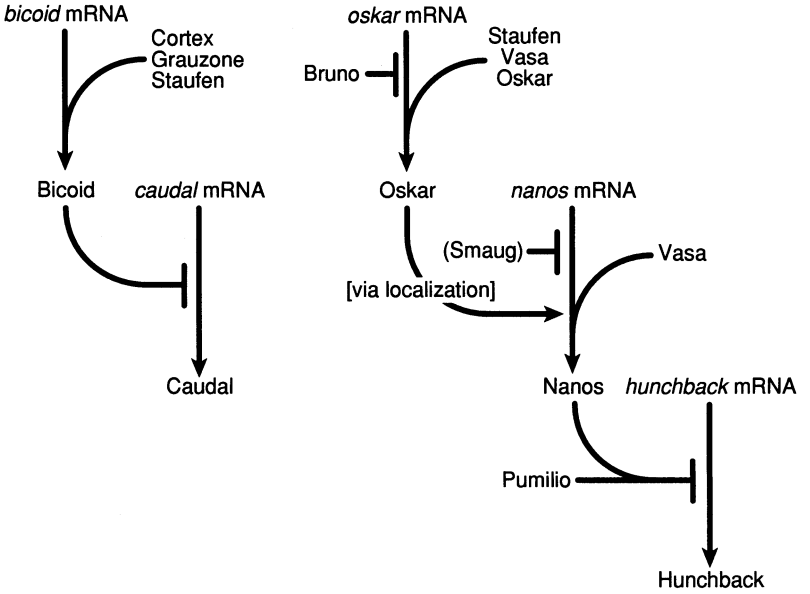


Figure 1 Specific RNA-protein interactions that control anterior-posterior axis formation in *Drosophila*. A series of opposing protein gradients help determine the anterior-posterior axis of *Drosophila* and are established by a regulated cascade involving mRNA localization and translation (see text for references; St Johnston et al 1992). *Arrowheads* depict positive events, and *blunt ends* indicate repressive events. All of these events occur either in the growing oocyte or in syncitial early embryo. mRNAs produced in nurse cells enter the growing oocyte from the presumptive anterior end; some mRNAs must move across the oocyte to the presumptive posterior. Activation of *bicoid* mRNA, which is localized to the anterior end and repressed during oogenesis, requires Cortex, Grauzone, and Staufen proteins. Bicoid protein then represses the translation in the anterior of uniformly distributed *caudal* mRNA, establishing a gradient of Caudal protein. In the posterior end, the initial event is localized expression of Oskar. Translation of *oskar* mRNA during its transit from the anterior end of the oocyte is repressed by Bruno, and its localization and full activation require Staufen, Vasa, and Oskar proteins. *nanos* mRNA is also localized to the posterior pole, a process that requires the presence of Oskar. Its mis-localized expression is prevented by sequences in its 3′UTR that appear to interact with Smaug, although the role of that protein has not been shown directly. Activation of *nanos* mRNA translation requires Vasa. Posteriorly localized Nanos, in concert with Pumilio, represses the translation of uniformly distributed *hunchback* mRNA in the posterior. Repression occurs in the posterior because there Nanos is present at its highest concentration. In this figure we include genes and proteins that are discussed in the text; many other genes such as *cappuccino*, *spire*, and *egalitarian* contribute to these processes but have not been included. In particular, proteins that participate in localization but not explicitly in translational regulation are not depicted. A similar figure appears in Macdonald & Smibert 1996.

1994) and translational regulation in plants (Browning 1996) and prokaryotes (McCarthy & Brimaconbe 1994, Voorma et al 1994, Jackson 1996).

We begin with a brief summary of the roles of the core components in translation initiation in eukaryotes. Our intent is to provide an outline of the pathway sufficient for discussion of its regulation. More detailed information is available elsewhere (Jackson 1996, Merrick & Hershey 1996, Pain 1996, Sachs et al 1997).

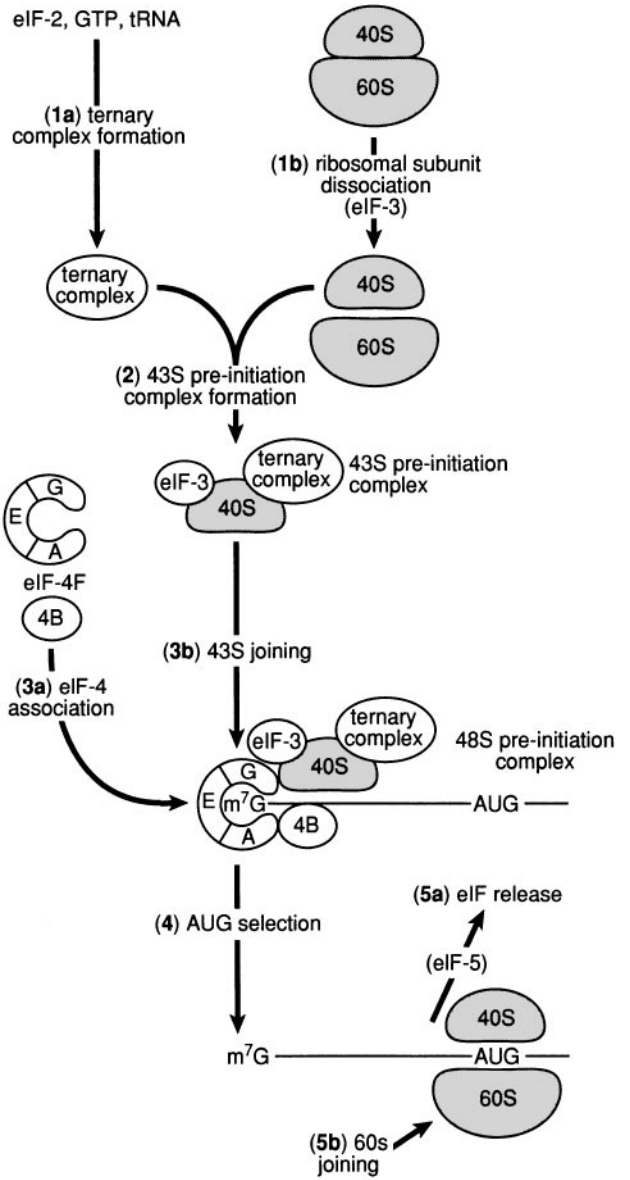
MECHANISMS OF INITIATION: OVERVIEW

Initiation is a complex multi-step process involving a large number of protein factors and multi-protein complexes, in addition to ribosomes. At least 25 proteins are involved in the initiation process per se, excluding ribosomal proteins and tRNA synthetases (Merrick & Hershey 1996). Additional proteins modulate the activities of the core translational components. Given this complexity, it is not surprising that cells and viruses regulate initiation through a diverse array of mechanisms.

A single, cap-dependent mechanism accounts for the translation of the vast majority of cellular mRNAs. A collection of alternative initiation mechanisms, one of which is cap-independent, is responsible for translation of a small number of mRNAs.

Cap-Dependent

A working model for cap-dependent initiation, consistent with most of the current data, is depicted in Figure 2. Although this model represents a general consensus, it is not unambiguously established (discussed in Jackson 1996, Merrick & Hershey 1996, Pain 1996), and we refer only to those factors that are relevant to later discussions. Following ribosomal subunit dissociation, which is assisted by a number of initiation factors (eIFs), including eIF-3, the small (40S) ribosomal subunit (carrying eIF-3) associates with a ternary complex to generate a 43S pre-initiation complex. The ternary complex contains the methionine-charged initiator tRNA, initiation factor eIF-2, and GTP. This 43S complex is then recruited, with the aid of the eIF-4 group of initiation factors, to the 5′ end of the mRNA. Mammalian eIF-4F is normally considered to be composed of three subunits: eIF-4E (which binds to the m7GpppG cap, an interaction crucial to recruiting eIF-4F), eIF-4A (which has ATPase-dependent RNA helicase activity), and eIF-4G (which through its interaction with eIF-3 aids the binding of the 43S pre-initiation complex). In plants and yeast only, eIF-4E and eIF-4G [and eIF-iso4E and eIF-iso4G in plants (Browning 1996)] can be isolated as a complex. It is generally accepted that the helicase activity of eIF-4F unwinds secondary structures from the 5′ untranslated region (UTR),


eIF-2, GTP, tRNA
(1a) ternary complex formation
40S
60S
(1b) ribosomal subunit dissociation (eIF-3)
ternary complex
40S
60S
(2) 43S pre-initiation complex formation
eIF-3
ternary complex
40S
43S pre-initiation complex
G
E
A
eIF-4F
4B
(3a) eIF-4 association
(3b) 43S joining
eIF-3
ternary complex
40S
48S pre-initiation complex
G
E
m7G
A
4B
AUG
(4) AUG selection
(5a) eIF release
(eIF-5)
40S
m7G
AUG
60S
(5b) 60s joining

which would otherwise impede the initiation apparatus. This helicase activity is stimulated by eIF-4B, which binds simultaneously with, or very closely after, eIF-4F. The interaction of eIF-4B with eIF-3 may also aid in the binding of the 43S complex. Once bound, the 43S complex migrates along the 5′UTR in an ATP-dependent process known as scanning, until it encounters an initiator AUG codon, normally the first AUG. The exact nature of the scanning process remains unclear. For example, it is unclear whether ATP hydrolysis is required purely for helicase activity or whether movement of the 43S complex also requires ATP (discussed in Jackson 1996). Following AUG recognition, eIF-5 triggers hydrolysis of the GTP in the ternary complex, initiation factor release occurs, and the large (60S) ribosomal subunit enters. Elongation now begins.

The minimal features of an mRNA essential for cap-dependent initiation are deceptively simple: a m7GpppG cap structure and an AUG in a favorable context (see LEAKY SCANNING). Non-AUG codons, such as a GUG or CUG, occasionally serve as initiators but are inherently inefficient (Kozak 1989b). However, an AUG in an optimal context does not ensure efficient initiation. Rather, the frequency of subunit loading is set by the accessibility of the 5′ end of the mRNA to initiation factors and small ribosomal subunits. Subunit migration to the initiator is limited by features of the 5′UTR, which can impede or perhaps even derail the scanning complex. Thus in simplest form, although the context surrounding the initiation codon influences which initiation site is used, it may not be the primary determinant of the total number of initiation events per mRNA. The translational efficiency of an mRNA can also be influenced by sequences at or near the 3′ end of the mRNA. Thus although its essential features may be simple, initiation can be regulated through a wide diversity of mechanisms (see Figure 3).

Variations of the Cap-Dependent Scanning Model

LEAKY SCANNING This variation of the scanning model relates to the choice of initiation codon. In general, the first AUG encountered by a scanning ribosome

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Figure 2 Cap-dependent translation initiation pathway. Step (1a): A ternary complex is formed between eIF-2, GTP, and the initiator tRNA. Step (1b): Dissociation of ribosomal subunits is aided by initiation factors including eIF-3. Step (2): The ternary complex is recruited by the small ribosomal subunit to form a 43S pre-initiation complex. Step (3a): The eIF-4 group of the initiation factors interacts with the 5′ end of the mRNA and aids in Step (3b), the binding of the 43S pre-initiation complex to form a 48S pre-initiation complex. Step (4): The 43S pre-initiation complex, aided by associated factors, migrates to the initiator AUG. This process is termed scanning. Step (5a): eIF-5 hydrolyzes the GTP in the ternary complex, initiation factor release occurs, and in Step (5b), the 60S ribosomal subunit joins. The role of a number of initiation factors is not depicted. See Merrick & Hershey (1996) for a more detailed description of initiation factors. The figure is schematic and is not meant to indicate the spatial arrangement of proteins within the various complexes nor the full extent of RNA-protein interactions.

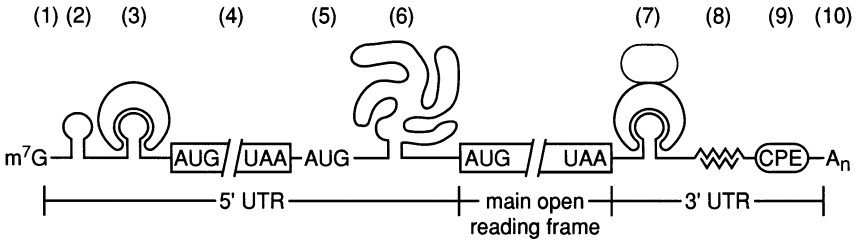

Figure 3 Forms of translational regulation by the 5′ and 3′ ends of the mRNA. Regulation via the 5′ UTR can be mediated by (1) modification of the cap structure, (2) secondary structure, (3) RNA-protein interactions, (4) upstream open reading frames, (5) upstream AUGs, and (6) IRES elements. 3′UTR-mediated regulation can occur through (7) RNA-protein interactions, which may involve multi-protein complexes, (8) RNA-RNA interactions, (9) cytoplasmic polyadenylation elements, and (10) changes in poly(A) tail length. The figure is diagrammatic and does not infer any organization of these elements within the 5′ and 3′ UTRs.

is selected as the site of initiation (Kozak 1983, 1987a). However, an AUG or non-AUG initiator codon may be inefficiently recognized or ignored if it is located very close to the cap (Sedman et al 1990, Kozak 1991c), or if it lies in a poor context (Kozak 1984a,b, 1986b, 1989b). A consensus sequence GCCGCC$^{A/G}$CCAUGG for initiator codons has been defined by a survey of vertebrate mRNAs (Kozak 1987a). The most important positions for efficient translation are a purine at position -3 and a G at position $+4$, where A of the AUG codon is position $+1$ (reviewed in Kozak 1991a, 1997). The effects of other positions become apparent when either of the main features is unfavorable. Positions $+5$ and $+6$ can also influence start site efficiency, with a A or C being preferred at position 5 and a U at position 6 (Boeck & Kolakofsky 1994, Grünert & Jackson 1994). Inefficient recognition of an initiator codon results in a portion of 43S pre-initiation complexes continuing to scan and initiating at a downstream site, in a process known as leaky scanning.

RE-INITIATION Re-initiation is a relatively rare and inefficient initiation mechanism in which a second initiation event occurs following the translation of an upstream open reading frame (uORF) (Liu et al 1984, Kozak 1984b, 1987b). By definition re-initiation can occur only on mRNAs that are bi- or polycistronic. A genuinely bi-cistronic mRNA, containing two extensive ORFs, has recently been reported in *Drosophila* (Brogna & Ashburner 1997). However, it is not clear whether translation of the downstream ORF in this mRNA involves re-initiation or internal initiation (Brogna & Ashburner 1997). Regardless, this rare, functionally bi-cistronic eukaryotic mRNA is exceptional: uORFs are mostly short, a property that is generally thought to enhance re-initiation. After

translation of a short uORF is complete, it is postulated that the 60S ribosome dissociates from the mRNA while the 40S ribosomal subunit resumes scanning. However, it remains to be directly determined which, if any, of the components of the translation initiation machinery remain associated with the mRNA after translation of short uORFs; at a minimum, a new ternary complex (Figure 2) must be acquired to allow re-initiation at downstream sites.

DISCONTINUOUS SCANNING OR HOPPING A small number of viral mRNAs have been described containing structures within their 5′UTRs that are unfavorable to progression of scanning complexes but yet do not promote internal initiation (Fütterer et al 1993, Yeuh & Schneider 1996, Dominguez et al 1998). These mRNAs, exemplified by the cauliflower mosaic virus (CMV) 35S mRNA, are translated by a discontinuous scanning mechanism (Fütterer et al 1993). The pre-initiation complex begins scanning at the 5′ end of the mRNA but bypasses this structure by hopping (also known as shunting and jumping). The mechanism of the hop is unclear, but requires specialized sequence elements (Fütterer et al 1993, Yeuh & Schneider 1996, Dominguez et al 1998, Hemmings-Mieszczak et al 1998).

Alternative Modes of Initiation

INTERNAL INITIATION Internal initiation, sometimes referred to as cap-independent initiation, is mediated by a secondary structure within the 5′UTR known as an internal ribosome entry site (IRES) and was first discovered in picornaviruses (Jackson & Kaminski 1995). IRES-mediated translation does not require a free 5′ end, as demonstrated by the translation of circular IRES-containing RNAs (Chen & Sarnow 1995). Viral IRESes can be divided into several functional groups based on their primary sequence and structure (Jackson & Kaminski 1995). IRESes can be functionally discriminated from other 5′ UTR secondary structures by their ability to mediate translation of the downstream ORF of a bi-cistronic reporter mRNA, independent of the translational status of the first ORF (Jang et al 1988, Pelletier & Sonenberg 1988).

The majority of general initiation factors, including eIF-4F, appear to be required for IRES-mediated translation (reviewed in Jackson 1995). Exceptionally, the IRESes from hepatitis C and classical swine fever viruses appear to bind the small ribosomal subunit and position it properly at the AUG, without the need for initiation factors (Pestova et al 1998). This mechanism of initiation may be most analogous to prokaryotic translation, in that the IRES may be functionally equivalent to Shine-Dalgarno sequences (Pestova et al 1998). Translation from other IRESes requires additional *trans*-acting factors, such as polypyrimidine tract-binding protein (PTB) (Hellen et al 1993, Kaminski et al 1995) and the La autoantigen (Meerovitch et al 1993, Svitkin et al 1994a).

The function of some of these proteins may be to maintain the structure of the IRES in a favorable conformation (Svitkin et al 1994b, Kaminski et al 1995). Different cellular factors appear to be required to mediate internal initiation from the different viral classes (discussed in Jackson & Kaminski 1995).

Internal initiation allows picornaviruses, which do not possess a cap structure, to escape the shut down of host translation machinery that occurs during picornaviral infection. Translation of host mRNA is shut off, in part, by the cleavage of eIF-4G. The cleavage separates the eIF-4E binding domain of eIF-4G (in the N-terminal portion of the protein) from those that interact with eIF-4A and eIF-3, located nearer the C terminus (Lamphear et al 1995). This separates the cap-binding function of eIF-4G from its RNA-helicase and ribosome-binding activities and thus inactivates translation of most cellular mRNAs. However, the C-terminal fragment of eIF-4G can substitute for intact eIF-4G in IRES-mediated translation and in fact may be more efficient (Buckley & Ehrenfeld 1987, Liebig et al 1993, Ziegler et al 1995, Ohlmann et al 1996, Pestova et al 1996).

REGULATION VIA 5′UTRs

The majority of eukaryotic mRNAs have 5′UTRs of 20–100 nucleotides (Kozak 1987a). Shortening the 5′UTR of reporter mRNAs to less than 12 nucleotides impairs the efficiency of translation from the first AUG (Sedman et al 1990, Kozak 1991c). Increasing the length of a 5′UTR can increase the efficiency of translation, as additional 43S pre-initiation complexes can be loaded. This is sometimes described as pre-loading (Kozak 1991b). However, many cellular mRNAs with unusually long 5′UTRs are poorly translated owing to the presence of upstream AUGs, uORFs, and/or secondary structure (Kozak 1987a, 1991a; summarized in Figure 3). This appears to be especially common in mRNAs encoding proto-oncogenes, transcription factors, growth factors, and their receptors (Kozak 1987a, 1991a), which suggests that their translation is tightly controlled.

Short uORFs

MECHANISMS THAT ARE INDEPENDENT OF uORF PEPTIDE SEQUENCE uORFs in the 5′UTR can modulate translation of the main ORF. Much of the current information about the function of uORFs has been obtained through elegant studies of the yeast *GCN4* mRNA, whose 5′UTR contains four short uORFs. These uORFs function together to regulate the translation of *GCN4* mRNA in response to amino acid starvation. This has been thoroughly reviewed elsewhere (Hinnebusch 1996).

In general, the first AUG encountered by a scanning ribosome is selected as the site of initiation (Kozak 1983, 1987a). uORFs and upstream AUGs can

modulate initiation from the main ORF simply by exploiting this polarity. uORFs also provide a termination site before the main ORF, allowing a degree of re-initiation and thus are less inhibitory than uAUGs (Kozak 1984b, Liu et al 1984). The sequences of the uORF are typically unimportant.

Translation of the main ORF of uORF-containing mRNAs can occur by two mechanisms: leaky scanning and re-initiation. Factors that influence the efficiency of these processes determine the magnitude of inhibition. For example, uORFs with less efficient start sites are less inhibitory (e.g. Cao & Geballe 1994). Surprisingly, the frequency with which scanning is resumed can be modulated by the uORF stop codon and downstream sequences, at least in yeast (Miller & Hinnebusch 1989). The resumption of scanning may also be affected by the length of the uORF, although this has never been systematically tested. In addition, uORFs in close proximity to the main ORF are more inhibitory, possibly because insufficient time is available for a complete initiation complex to re-assemble (Kozak 1987b).

uORF PEPTIDES In an expanding number of cases, the peptide encoded by a uORF contributes to translational inhibition of the downstream ORF. Translational control of S-adenosylmethionine decarboxylase (AdoMet) mRNA in response to polyamine levels is regulated in this manner. Translation of the AdoMet uORF is predicted to produce a hexapeptide, MAGDIS (Hill & Morris 1993). A number of mutations that alter the sequence of the peptide enhance translation of the main ORF, whereas those that preserve the peptide do not. Surprisingly, the uORF peptide does not inhibit translation in *trans*, leading to a model in which the nascent peptide stalls the ribosome, preventing or delaying termination (Hill & Morris 1993, Geballe & Morris 1994). A decrease in intracellular polyamine concentration may disrupt interactions between the peptide and translational machinery, allowing translation of the main ORF to proceed (Ruan et al 1996). However, the mechanisms of repression and de-repression have not been elucidated. Other examples of this regulatory strategy include yeast *CPA1* (Werner et al 1987, Delbecq et al 1994) and *Neurospora* arg-2 (Wang & Sachs 1997), mammalian $\beta 2$ adrenergic receptor (Parola & Kobilka 1994), mouse retinoic acid receptor $\beta 2$ mRNA (Reynolds et al 1996), and the human cytomegalovirus gp48 transcript (Schleiss et al 1991, Degnin et al 1993).

It is unclear how frequently the peptide sequences of uORFs are important because detailed mutational studies are required to differentiate between uORFs that produce inhibitory peptides and uORFs that function simply by providing upstream initiation and termination sites. Nor does the presence of uORFs in a 5′UTR necessarily indicate that they account for the inefficient translation of an mRNA. Human PDGF2 mRNA contains three uORFs (Rao et al 1988), but it is the presence of extensive secondary structure within its 5′UTR that causes its poor translation, at least under the conditions tested.

uORFs can occasionally have a stimulatory effect on translation, as in the case of the first of four uORFs in yeast GCN4 mRNA. uORF1 stimulates translation from the main ORF by decreasing the frequency of re-initiation from downstream uORFs (reviewed in Hinnebusch 1996). uORFs may also play a role in overcoming secondary structure by facilitating formation of 80S ribosomes upstream of the structured area and appear to enhance shunting on CMV 35S mRNA (Dominguez et al 1998).

Regulation via Selective Use of Alternative Initiation Sites

Many mRNAs contain AUGs within their 5′UTR that are not followed by in-frame stop codons prior to the start of the main ORF. Initiation at the downstream AUGs is the result of leaky scanning. Interestingly, several mRNAs produce full-length proteins from both upstream and downstream AUGs.

LIP (liver-enriched transcriptional inhibitor protein) and LAP (liver-enriched transcriptional activator protein) provide an elegant example of this type of translational control (Descombes & Schibler 1991). LAP is a transcriptional activator most abundant in liver. The mRNA that encodes LAP also gives rise to a shorter protein product, LIP, owing to leaky scanning at the LAP initiation codon. LIP does not contain the transcriptional activation domain and is therefore thought to impede the activity of LAP by occlusion of the promoter (Descombes & Schibler 1991). Interestingly, during postnatal development, a shift in the LAP/LIP ratio coincides with the function of LAP in terminal liver differentiation.

The use of leaky scanning to produce more than one protein from a single mRNA is not restricted to the use of alternative AUGs. For example, the mRNA encoding Int-2, an FGF related protein that functions in mouse embryonic development, uses both an initiator AUG and an upstream initiator CUG to synthesize an N-terminal extended protein (Acland et al 1990). The protein produced from the CUG is targeted to the nucleus while the AUG-initiated protein is shuttled through the endoplasmic reticulum and Golgi (Acland et al 1990). Other examples of this type of bifunctional mRNA exist including human bFGF (Florkiewicz & Sommer 1989, Prats et al 1989), murine *pim-1* (Saris et al 1991), *Drosophila oskar* (Markussen et al 1995), and human c-*myc* (Hann et al 1988, 1992).

The effects of uORFs and upstream AUGs can vary with cell type and during differentiation (e.g. Descombes & Schibler 1991, Lin et al 1993, Imataka et al 1994, Zimmer et al 1994). Regulation of initiation factor activity may play a role by influencing the recognition of AUGs and/or the rate of re-initiation. Indeed, preferential selection of cap-proximal, rather than subsequent AUGs, can be influenced by the phosphorylation state of initiation factor eIF-2 (Dasso et al 1990), as can the rate of reinitiation.

Secondary Structure

The inhibitory effect of introducing secondary structures into the 5′UTR depends on their stability and position and can be elicited by at least two mechanisms (Pelletier & Sonenberg 1985a, Kozak 1986a, 1998, 1989a). First, cap-proximal, moderately stable structures block the access of 43S pre-initiation complexes (Kozak 1989a) and initiation factors eIF-4A and eIF-4B to reporter mRNAs (Pelletier & Sonenberg 1985b, Lawson et al 1986). However, when the distance between the cap and the structure is sufficient to allow 43S entry, a moderately stable stem-loop is insufficient to inhibit translation (Kozak 1989a). The translational machinery appears to progress linearly through the stem-loop, rather than by hopping, since an AUG introduced into the distal side of the stem can be utilized as an initiation site (Kozak 1986a).

Second, insertion of a more stable stem-loop structure (−50/61 kcal/mol) blocks translation even when located downstream of the cap (Kozak 1986a, 1989b). RNAse protection experiments identified a stalled 43S pre-initiation complex 5′ to the inhibitory structure, suggesting that the stem-loop was an impenetrable barrier to the migration of this complex (Kozak 1989a).

As implied by these examples using artificial mRNAs, secondary structure in the 5′UTR can also regulate cellular mRNAs. Ornithine decarboxylase (ODC) is involved in the synthesis of polyamines required for cell proliferation. Translation of its mRNA is normally inefficient but can be stimulated by growth factors and mitogens such as insulin. An approximately 140 nucleotide region close to the cap of rat and hamster ODC mRNAs is responsible for its inefficient translation (Grens & Scheffler 1990, Manzella & Blackshear 1990). This G-C–rich region forms an inhibitory secondary structure and does not appear to be a protein-binding site because inverting the region does not diminish repression (Grens & Scheffler 1990). Interestingly, the translation of ODC mRNA in renal tissues of two murine species, *Mus domesticus* and *Mus pahari*, differs significantly (Johannes & Berger 1992) and may be caused by small differences in their 5′UTRs that alter their predicted structures. Because the translation of reporter mRNAs with 5′ secondary structure is increased in response to eIF-4E overexpression (Koromilas et al 1992a), it is possible that the stimulation of ODC mRNA translation by mitogens is achieved through elevated eIF-4E activity. Consistent with this, eIF-4E (and eIF-4B) are phosphorylated in response to insulin (Manzella et al 1991), and overexpression of eIF-4E increases ODC translation (Shantz & Pegg 1994). Other well-characterized examples of mRNAs that are regulated through secondary structure include human PDGF2 mRNA (Rao et al 1988).

In experimentally manipulated mRNAs, secondary structure can also increase the use of a particular initiation site when located downstream of that site. This effect requires the presence of an inefficient initiation site, due to

either a poor context, a non-AUG initiator, or a very short 5′UTR (Kozak 1989b, 1990, 1991c). The optimal placement for the structure is 14 nucleotides downstream from the initiation codon, which corresponds well with the distance between the leading edge of the ribosome and the initiation codon, as measured by RNAse protection (Kozak 1990). Thus it seems that the structure pauses the 43S pre-initiation complex at or near the initiation codon, presumably allowing more time for its recognition (Kozak 1990). The resulting 80S ribosome is not impeded by the secondary structure and initiates elongation.

RNA-Protein Interactions

Proteins can regulate translation by interacting with target sequences within the 5′ UTR. The best characterized of these is iron regulatory protein (IRP)-mediated regulation, first identified in mammalian cells. IRP-1 and IRP-2 regulate the translation of a number of mRNAs, including ferritin (reviewed in Hentze & Kuhn 1996), erythroid 5-aminolevulinate synthase (eALAS) (Cox et al 1991, Dandekar et al 1991, Bhasker et al 1993, Melefors et al 1993), mitochondrial aconitase (Gray et al 1996, Schalinske et al 1998), and succinate dehydrogenase-iron protein (Kohler et al 1995, Gray et al 1996, Melefors 1996), in response to a number of physiological stimuli. The binding site for IRP, the iron responsive element (IRE) (Aziz & Munro 1987, Hentze et al 1987), is generally located close to the 5′ cap in these messages. In transfection studies, when the IRE is moved to a more cap-distal position, IRP-mediated regulation is diminished; this is described as the position effect (Goossen et al 1990, Goossen & Hentze 1992). This suggests that IRP may prevent a relatively early mRNA-dependent step in translational initiation. Indeed, binding of the 43S pre-initiation complex is prevented by the presence of IRP-1 (Figure 2, step 3b; Gray & Hentze 1994a). Moving the IRE to a more cap-distal position allows free access to this complex: Residual regulation by cap-distal IRE/IRP complexes results from their ability to pause scanning (Figure 2, step 4; E Paraskeva, NK Gray, B Schlaeger, K Wehr, MW Hentze, submitted). These stalled scanning complexes appear to overcome cap-distal IRE/IRP complexes by active displacement of IRP-1, rather than by hopping or waiting for passive dissociation of IRP (E Paraskeva, NK Gray, B Schlaeger, K Wehr, MW Hentze, submitted). It remains to be determined whether displacement of cap-distal regulatory proteins is achieved by a novel protein-removal activity or via disruption of protein binding sites, which tend to be structured, by RNA helicases. Interestingly, reporter mRNAs in yeast and plants, which do not contain endogenous IRP, are equally repressed by cap-distal and cap-proximal IREs (Koloteva et al 1997; E Paraskeva, NK Gray, B Schlaeger, K Wehr, MW Hentze, submitted).

Although IRP-1 prevents the association of the 43S complex with the mRNA, previous mRNA-dependent steps in initiation (Figure 2, step 3a) can occur

(Muckenthaler et al 1998). Thus the cap-binding complex is present on IRP-1–repressed mRNAs. The function of this complex may be to stabilize the mRNA by preventing decapping or to allow rapid translation once cellular conditions change.

Interestingly, IREs can be replaced by binding sites for proteins such as U1A or MS2 coat protein that have no function in eukaryotic translation (Stripecke & Hentze 1992, Stripecke et al 1994). Translational repression by at least one of these proteins occurs by the same mechanism as IRP-1 and displays a position effect (Stripecke & Hentze 1992, Gray & Hentze 1994a). These findings establish that the presence of an RNA-protein complex in the 5′ untranslated region of mRNAs can modulate both ribosomal subunit entry and migration, depending on the position of the complex (Gray & Hentze 1994a; E Paraskeva, NK Gray, B Schlaeger, K Wehr, MW Hentze, submitted). Species vary in whether they are more strongly inhibited by cap-proximal or cap-distal RNA/protein complexes. Thus some species (e.g. yeast and wheat germ) can strongly repress translation via RNA/protein complexes located at various positions in the 5′ UTR; others (e.g. mammalian cells) can modulate the degree of repression by altering the position of the complex.

The number of mRNAs for which good evidence for the involvement of 5′UTR-bound repressor proteins exists is surprisingly small (reviewed by Gray & Hentze 1994b). It includes Mst87F and related genes in *Drosophila* spermatogenesis (Schäfer et al 1990, Kempe et al 1993), mouse superoxide dismutase mRNA (Gu & Hecht 1996), and the autoregulation of poly(A) binding protein mRNA (de Melo Neto et al 1995). Proteins also have been implicated in the 5′UTR-mediated control of TOP mRNAs (see *Ribosomal Protein S6*).

Internal Initiation of Cellular mRNAs

Certain cellular mRNAs possess IRES elements, as defined by bi-cistronic assays (Jang et al 1988, Pelletier & Sonenberg 1988). IRESes in cellular mRNAs appear similar but less complex than those of viruses, consisting of as little as 55 nucleotides (OH & Sarnow 1993). The specialized *trans*-acting factors involved in cellular IRES-mediated translation may differ from those utilized by their viral counterparts (Vagner et al 1996, Yang & Sarnow 1997).

From the biological standpoint, IRES-mediated translation of cellular mRNAs provides a simple way to allow translation of a specific mRNA in circumstances in which the cap-dependent mechanism is impaired. The rich potential of such a regulatory device has been realized in several biological contexts with mRNAs that include human immunoglobulin heavy-chain-binding protein (BiP) (Macejak & Sarnow 1991), human IGF-II (Teerink et al 1995), human FGF-2 (Vagner et al 1995), human PDGF2 (Bernstein et al 1997), *Drosophila antennapedia* (OH et al 1992, OH & Sarnow 1993), and the proto-oncogene c-*myc* (Stoneley et al 1998).

Platelet-derived growth factor-2 (PDGF2/c-*sis*) mRNA is exemplary. It encodes one of two proteins that form PDGF, a powerful mitogen, important in wound healing, embryogenesis, and development. Its role in wound healing involves its expression in bone marrow. Prior to megakaryocytic differentiation, PDGF2 translation is thought to be repressed by extensive secondary structure. Differentiation appears to activate IRES-mediated translation of PDGF2 mRNA, circumventing the impediment to cap-dependent initiation (Bernstein et al 1995, 1997). Thus several features of the mRNA act in concert to achieve its correct expression.

5′ UTRs: Problems and Perspectives

In light of the diversity of mechanisms through which 5′UTRs can modulate translation, it is difficult to predict the biological effects of a 5′UTR if one is armed with only its sequence. The presence of a long 5′UTR seems to be good a priori evidence that some form of control lies within it.

Yet many mRNAs, particularly those that encode growth regulators and transcription factors, seem to have adopted 5′UTR regulation in excess and appear not very different from the absurdly crowded mRNA depicted in Figure 3. They have long 5′UTRs, laden with potential structure and upstream AUGs. The speculation that these multiple control elements allow complex responses to different cellular requirements, permit regulation among cell types, or modulate translation during differentiation is appealing. This complex responsiveness appears to be true for PDGF2 mRNA. Multiple transcription start sites and alternative splicing patterns offer yet more versatility: They can enhance, reduce, or eliminate the effect of a regulatory element by altering its position with respect to the cap or the initiation codon, or by eliminating it altogether.

From one perspective, the pressing questions of 5′UTR-mediated and 3′UTR-mediated control are at present mirror images. In the 5′UTR, our understanding of the mechanisms of regulation and their diversity is relatively advanced, although key mechanistic questions remain. Another priority is to uncover how the mechanisms revealed by studying reporter mRNAs are exploited to control specific cellular mRNAs, with interpretable physiological consequences. To date, this has been achieved with only a modest number of mRNAs, some of which we have chosen as examples. It may ultimately be necessary to analyze the expression of genes bearing manipulated 5′UTRs in a variety of physiological circumstances, and perhaps ideally, in transgenic animals. If the correlation between long 5′UTRs and growth regulators is any indication, it seems inescapable that a wealth of biological information embedded in the 5′UTR will emerge from such analyses. For 3′UTRs, biological significance often is demonstrable; it is molecular mechanisms that are elusive.

REGULATION BY 3′UTRS

Surprisingly, many mRNAs are translationally controlled via sequences in their 3′UTR (Figure 3; reviewed in Sonenberg 1994, Curtis et al 1995, Macdonald & Smibert 1996, Wickens et al 1996). The challenges of understanding how such elements work are intimately linked to understanding the role of poly(A) in translation. In part, this is because both reside far from the cap and initiation codon: Action at an apparent distance raises questions of how both 3′UTR- or poly(A)-bound factors communicate with the initiation apparatus, and with the 5′ end of the mRNA.

Yet the link between 3′UTRs and poly(A) is more intimate than this apparent spatial problem. During early development, specific mRNAs undergo changes in poly(A) tail length that are often accompanied by changes in translational activity: increases in poly(A) length generally correlate with increases in translation, and decreases correlate with repression (Richter 1996, Wickens et al 1997). Moreover, sequences in the 3′UTR identified genetically by their effects on translation often also govern poly(A) length, e.g. elimination of a negative regulatory element may cause both translational activation and a longer poly(A) tail. Similarly, elements identified through their effects on poly(A) length can also regulate translation. Although most of the relevant studies have been performed in oocytes and embryos, the same phenomena exist in somatic cells.

For simplicity, we have divided our consideration of 3′UTRs and poly(A) into two separate sections, followed by a single speculative discussion of how they may exert their effects.

Regulatory Elements and Cascades

Developmental genetics in *Drosophila* (Figure 1) and *C. elegans* has revealed cascades of 3′ UTR-based translational control. In *C. elegans*, the first concerns the determination of cell fate and patterning in its germ line (reviewed in Puoti et al 1997). *C. elegans* hermaphrodites produce sperm first and then switch to producing oocytes. The *tra-2* gene is required for the female phase of development, including oogenesis, whereas the *fem-3* gene is required to promote male development, including spermatogenesis. Early in gametogenesis, *tra-2* must be repressed to make sperm, and later *fem-3* must be repressed to make oocytes. Regulation of both of these mRNAs is achieved through their 3′UTRs; however, although *fem-3* is post-transcriptionally regulated, it has not been conclusively shown to be at the level of translation.

A second cascade of 3′UTR-mediated translational controls in *C. elegans* contributes to cell fate determination in the early embryo (Schnabel & Priess 1997). *glp-1* mRNA encodes a transmembrane receptor required to specify anterior cell fates. Although its mRNA is uniformly distributed in the early embryo,

it is translated in only presumptive anterior blastomeres beginning at the 2-cell stage (Evans et al 1994). Reciprocally, *pal-1* mRNA is translated only in posterior blastomeres (Hunter & Kenyon 1997); its repression in anterior cells may require *mex-3*, a putative RNA-binding protein (Draper et al 1996). The cascade of spatial and temporal control likely encompasses several other mRNAs that play key roles in cell fate decisions, including *skn-1*, *pie-1*, and *apx-1*, each of which produces maternal mRNAs that are distributed throughout the early embryo but translated only in specific blastomeres (Schnabel & Priess 1997).

Positive Elements that Enhance Translation of Uncapped mRNA

The role of positive elements in translational control is emphasized by work with certain plant viruses in which the 3′UTR enhances translation. Several of these elements can also function in animal cells (Gallie & Walbot 1990, Hann et al 1997). Often, an interaction between the 3′ and 5′UTR is required (Gallie & Walbot 1990, Danthinne et al 1993, Timmer et al 1993, Wang & Miller 1995). For example, an element in the 3′UTR of the barley yellow dwarf virus (BYDV-PAV) genome can act when separated from the stimulated AUG by several ORFs and kilobases of sequence; in this situation, stimulation requires the presence of the natural 5′UTR (Wang & Miller 1995, Wang et al 1997). When placed at the 5′ end of the mRNA, the element can function on its own (Wang et al 1997). This suggests that the natural 5′ UTR is required to mediate long-range interactions (Wang et al 1997). Such interactions may be mediated by base-pairing or, potentially, by protein-protein interactions.

These positive elements are often suggested to be the functional equivalents of the cap or poly(A) tail in cognito and may bind basal initiation factors. A poly(A) tail can functionally replace a positive element in the 3′UTR of tobacco mosaic virus (Gallie & Walbot 1990), whereas a cap can substitute for the elements in BYDV-PAV and satellite tobacco necrosis virus (STNV) (Timmer et al 1993, Wang & Miller 1995). Moreover, addition of eIF-4F can overcome translational inhibition by excess BYDV-PAV element in *trans* (Wang et al 1997) and is also implicated in the enhancement by the STNV element (Timmer et al 1993). However they act, these positive elements emphasize the diversity of modes through which 3′UTRs can modulate translation.

Regulatory Proteins: Negative and Positive Factors

Relatively few of the cognate repressors that bind to repressive elements in the 3′UTR have been identified. In part, this may be due to the involvement of multiprotein complexes and to the fact that the proteins providing the RNA-binding activity do not necessarily directly affect repression.

The control of *Drosophila hunchback*, which encodes a transcription factor, exemplifies this situation (reviewed by St Johnston & Nüsslein-Volhard 1992,

Wharton 1992, Curtis et al 1995, Macdonald & Smibert 1996). *hunchback* mRNA, which is present throughout the early syncitial embryo (Tautz & Pfeifle 1989), is initially repressed by Nanos protein in the posterior, where it is later degraded. Nanos-dependent repression is mediated via nanos response elements (NREs) in the 3′UTR (Wharton & Struhl 1991). Yet Nanos does not specifically bind to the NREs (Curtis et al 1997). Rather, another protein, Pumilio, does so: Pumilio is not localized nor does its mere presence lead to repression (Murata & Wharton 1995). In one simple model, Pumilio binds to NREs throughout the embryo and Nanos, which is restricted to the posterior, either binds to or modifies the Pumilio/*hunchback* mRNA complex. Additional proteins may also be needed, as no direct interaction between Nanos and Pumilio has been demonstrated. Interestingly, FBF, recently identified as a factor required for repression of *C. elegans fem-3* mRNA, shares sequence similarity with *Drosophila* Pumilio (Zhang et al 1997). Moreover, like Pumilio, FBF represses by binding to a site within the 3′UTR and causes a switch in cells fates.

Regulation of 15-lipoxygenase (LOX) mRNA via its 3′UTR involves more than one protein but differs conspicuously from regulation of *hunchback* mRNA. The LOX enzyme participates in internal membrane breakdown during the later stages of reticulocyte maturation (Rapoport & Schewe 1986). Its mRNA is controlled via CU-rich sequences known as DICE within its 3′UTR (Ostareck-Lederer et al 1994). Although rabbit LOX mRNA contains ten tandem repeats, two are sufficient for repression (Ostareck et al 1997). A complex of two proteins, hnRNP K and hnRNP E1 (αCP-1), interact with each DICE to mediate repression (Ostareck et al 1997). In contrast to Nanos, both proteins have specific DICE-binding activity and can repress translation in vitro to a lesser degree alone (Ostareck et al 1997). Additional components of this complex, if any, must be neither tissue nor species specific (Ostareck et al 1997).

Translational repression of *tra-2* in *C. elegans* requires GLD-1 protein, which binds specifically to the regulatory elements in the mRNA's 3′ UTR (E Jan et al, submitted). GLD-1 is necessary for repression in vivo and sufficient for repression in a cell-free yeast extract. GLD-1 is a member of the STAR subfamily of KH-domain RNA-binding proteins (Jones & Schedl 1995, Vernet & Artzt 1997). Other members of this subfamily may also be translational repressors (E Jan et al, submitted).

Regulation via 3′UTRs involves not only repressors but activators. In *Drosophila*, many of the proteins required to activate mRNA expression act indirectly through their role in mRNA localization. These include gene products that affect cytoskeletal organization and function (e.g. *cappuccino* and *spire*) (Ephrussi et al 1991, Kim-Ha et al 1991, Theurkauf 1994), as well as proteins that interact with specific mRNAs (e.g. Staufen) (St Johnston et al 1991). Staufen appears to contribute to the expression of *oskar* mRNA by establishing and maintaining its localization and is a double-stranded RNA-binding protein (St Johnston et al

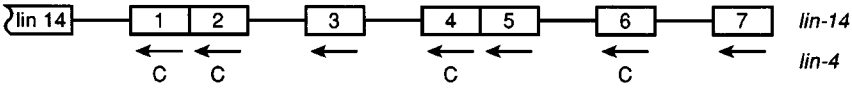

Figure 4 Regulation of *lin-14* mRNA by *lin-4*. The 3′UTR of *lin-14* mRNA is depicted. Seven sequence elements within the 3′UTR have the capacity to form anti-sense duplexes with *lin-4* RNA. The elements predicted to form duplexes with a bulged C residue are indicted by the letter C.

1992). Vasa protein (Hay et al 1988, Lasko & Ashburner 1988) is also required for complete activation of *oskar* mRNA translation (Rongo et al 1995) through an unknown mechanism. However, since it is an ATP-dependent RNA helicase, unwinding of an RNA structure may be important (Liang et al 1994).

Proteins that promote cytoplasmic polyadenylation of specific mRNAs constitute another group of positive-acting factors in translational control. These are discussed in the context of changes in poly(A) length.

RNAs as Regulators

3′UTR repressors can be RNA, as well as protein (see Figures 3 and 4). *lin-14* is required for the proper timing of a range of developmental events in *C. elegans* (Ambros & Horvitz 1987). Temporal repression of *lin-14* requires sequences in its 3′UTR, and the *lin-4* gene product (Wightman et al 1991, 1993; Lee et al 1993). Repression likely involves regulation of translation, although effects on mRNA transport have not been eliminated. Surprisingly, *lin-4* encodes two short mRNAs of 22 and 61 nucleotides, not a protein (Lee et al 1993). *lin-4* RNA can potentially base-pair with seven sequence elements of 14–19 nucleotides within the *lin-14* 3′UTR, prompting the proposal that *lin-4/lin-14* RNA duplexes cause translational repression (Lee et al 1993, Wightman et al 1993) (Figure 4). Four of the predicted duplexes contain a bulged C residue, whereas the other three do not. The bulged C appears to be critical for repression: Only those reporter mRNAs containing multiple copies of the elements that form putative bulged C duplexes were subject to *lin-4* regulation, and the bulged C could not be substituted by another nucleotide (Ha et al 1996). It appears likely that the bulged duplexes may bind an as yet unidentified repressor protein (Ha et al 1996). *lin-28*, another gene that regulates timing of developmental decisions, is also controlled by *lin-4* and contains only a single sequence complementary to *lin-4* in its 3′UTR that does not form the bulged duplex (Moss et al 1997).

POLY(A)

The effects of poly(A) on translation have two distinct facets. First, the mere presence of a poly(A) tail can stimulate translation, as demonstrated by

comparing the translation of reporter mRNAs with and without a poly(A) tail. The magnitude of the effects varies between assay systems but is very substantial in yeast extracts (Iizuka et al 1994), electroporated cells (Gallie 1991), and oocytes and embryos (e.g. Sheets et al 1994).

A second facet of poly(A)'s effects on translation concerns regulated changes in the length of poly(A) on specific mRNAs (Richter 1996, Wickens et al 1997). For example, c-*mos* mRNA receives poly(A) in the cytoplasm as its translation increases (Sheets et al 1994). However, such mRNAs typically have respectably long poly(A) tails when they are repressed, longer than would be required to enhance translation when appended to a reporter mRNA (Richter 1996, Wickens et al 1997). This apparent contradiction emphasizes the importance of dissecting the mechanism by which regulated changes in poly(A) length regulate translation and raises the possibility that the mechanism may differ from that observed by comparing mRNAs with and without poly(A).

Cytoplasmic Poly(A) Addition and Removal

mRNAs initially receive poly(A) tails of approximately 250 nucleotides in the nucleus of vertebrate cells. Upon entering the cytoplasm, poly(A) is slowly removed in most cells. Abrupt changes in poly(A) length can occur at specific times and have been extensively characterized during oocyte maturation and early development in frogs and mice (Richter 1996, Wickens et al 1996). During oocyte maturation, frog and mouse oocytes advance from first to second meiosis and can then be fertilized. During maturation, specific mRNAs gain poly(A) in the cytoplasm and become active or lose poly(A) and become inactive. Other mRNAs undergo these reactions after fertilization. The apparatus that adds poly(A) appears to be quiescent during oogenesis, and becomes active in *Xenopus* at the onset of maturation. The mechanism of its activation is unclear.

Signals that control poly(A) length during oocyte maturation and after fertilization have been identified in frogs and mice (Richter 1996, Wickens et al 1996). These signals are bipartite and lie within the 3′UTR: They are AAUAAA, a highly conserved sequence that is required for nuclear polyadenylation and present in every mRNA, and a separate sequence that is typically U-rich. Because the U-rich sequence distinguishes those mRNAs that receive poly(A) from those that do not, it has been termed a cytoplasmic polyadenylation element (CPE) or an adenylation control element (ACE). The precise sequence of the CPE can control both the timing and extent of polyadenylation, determining, for example, whether the mRNA will receive poly(A) early or late in maturation or 50 versus 300 adenosine monophosphates (e.g. Simon et al 1992, Sheets et al 1994). Insertion of a CPE into the 3′UTR of a reporter mRNA is sufficient to cause both polyadenylation and translational stimulation during

oocyte maturation or early development (Fox et al 1989, McGrew et al 1989, Huarte et al 1992).

Changes in Poly(A) Tail Length and Translation

CHANGES IN POLY(A) TAIL LENGTH AS A CAUSE OF TRANSLATIONAL CONTROL Cytoplasmic polyadenylation has been shown to be required for the translational activation of a number of mRNAs. In *Drosophila*, translational activation of *bicoid* mRNA is required for the determination of anterior structures in the embryo and is accompanied by polyadenylation. Injection of wild-type *bicoid* mRNA but not a mutant mRNA, which lacks polyadenylation signals, can rescue the lethal phenotype of a *bicoid* mutant embryo (Salles et al 1994). A mutant mRNA that lacks the polyadenylation signals but has an artificial poly(A) tail also rescued (Salles et al 1994), strongly suggesting that *bicoid* polyadenylation is critical to its translational activation and hence pattern formation. Similarly, the over-expression phenotype of injected *Xenopus* activin receptor mRNA requires its polyadenylation (Simon et al 1996).

The role of polyadenylation in translation activation has also been examined in *Xenopus* by manipulating an endogenous mRNA, c-*mos*. It encodes a serine-threonine kinase and is required for the resumption of meiosis and initiation of oocyte maturation (Gebauer & Richter 1997, Sagata 1997). Removal of the polyadenylation signals from endogenous c-*mos* mRNA by targeted RNAse H cleavage blocks its translational activation and oocyte maturation (Sheets et al 1995). c-*mos* translation and maturation can be rescued by the use of prosthetic mRNAs, which bind to sequences in the truncated 3′ UTR and contain polyadenylation signals (Sheets et al 1995) or a long synthetic poly(A) tail (Barkoff et al 1998). Thus polyadenylation is critical for the translation of c-*mos* and maturation of *Xenopus* oocytes. Similarly, polyadenylation of c-*mos* mRNA in the mouse is required for its translational activation (Gebauer et al 1994).

While cytoplasmic polyadenylation can activate translation, so can regulated decreases in poly(A) length cause repression. For example, in *Drosophila*, translational repression of *hunchback* mRNA by Nanos and Pumilio involves rapid deadenylation mediated by regulatory elements in the *hunchback* 3′UTR (Wreden et al 1997). In mice, mutations in the CPE (or ACE) of mouse tPA mRNA cause a failure to remove the poly(A) tail when the mRNA emerges from the nucleus and prevent silencing of the mRNA prior to meiotic maturation (Huarte et al 1992). In *Xenopus*, overexpression of poly(A) binding-protein, the main protein bound to cytoplasmic poly(A) tails, prevents deadenylation and silencing of mRNAs that normally would undergo such regulation during maturation, strongly suggesting that removal of the tail is required for repression (Wormington et al 1996).

Changes in polyadenylation may also play a role in the regulation of some mRNAs controlled via their 5′UTRs. For example, the mRNAs encoded by Mst87F and related genes in *Drosophila* undergo polyadenylation when they are activated during spermatogenesis (Kuhn et al 1991). It remains to be determined whether these poly(A) tail length changes are casual in the translational activation of these mRNAs.

CHANGES IN POLY(A) TAIL LENGTH AS AN EFFECT OF TRANSLATIONAL CONTROL Studies of 5′UTR-mediated repression in somatic cells have revealed that deadenylation can also be a consequence of translational control, rather than a cause. IRP-mediated repression can cause partial deadenylation in mammalian somatic cells (Muckenthaler et al 1997), as can repression by proteins targeted to the 5′UTR that do not normally function in eukaryotic translational control. Deadenylation can also be caused by translational inhibitors that promote ribosomal release but not by those that maintain the mRNA on polyribosomes. Since IRP-mediated repression prevents ribosomal association (Gray & Hentze 1994a), and because changes in polyadenylation are not essential to this repression either in vitro (Walden et al 1988, Brown et al 1989, Gray et al 1993) or for one of the two forms of ferritin mRNA in cells (Muckenthaler et al 1997), deadenylation appears to be a consequence, rather than a cause, of the release of repressed mRNAs from ribosomes (Muckenthaler et al 1997). However, the absence of measurable poly(A) shortening of one form of ferritin mRNA raises the possibility that deadenylation is not an inevitable consequence of repression.

CHANGES IN POLY(A) TAIL LENGTH UNCOUPLED FROM TRANSLATIONAL CONTROL Translational activation of clam ribonucleotide reductase in vitro is not accompanied by changes in polyadenylation (Standart et al 1990). This suggests that the polyadenylation of this mRNA observed during its activation following fertilization in *Spisula solidissima* may not be causal to its activation. Similarly, in *Xenopus*, translation of FGF receptor mRNA can be activated even in circumstances in which its normal extensive polyadenylation does not occur (Culp & Musci 1998).

CONTROLS THAT ARE INDEPENDENT OF CHANGES IN POLY(A) TAIL LENGTH Changes in poly(A) tail length are not an inevitable corollary of 3′UTR-mediated translational control. For example, in the *Drosophila* embryo, poly(A) tail changes accompany the regulation of *toll, torso, hunchback,* and *bicoid* mRNAs, but not the activation of *nanos* and *oskar* mRNAs (Salles et al 1994, Webster et al 1997, Wreden et al 1997). Furthermore, *nanos* mRNA activation is unimpaired in *cortex* and *grauzone* mutant embryos, which are defective in polyadenylation and activation of *bicoid* mRNA (Lieberfarb et al 1996). Repression of LOX mRNA in somatic cells can be reconstituted in cell-free systems using

non-adenylated mRNAs (Ostareck-Lederer et al 1994). However the poly(A) status of LOX mRNA has not been examined in cells.

In summary, while changes in poly(A) tail length can cause changes in translational activity, as in the case of *bicoid* and c-*mos*, it is also clear that changes in poly(A) tail length can be secondary effects of repression. In some cases, changes in poly(A) tail length may sustain the change in translational activity achieved by independent mechanisms, including relief of sequence-specific repression (reviewed by Standart & Jackson 1994, Wickens et al 1996). For example, de-repression may be perpetuated or enhanced by elongation of the poly(A) tail.

Variations in the Magnitude of Poly(A) Stimulation

The magnitude of translational stimulation of mRNAs that are activated in a poly(A)-dependent manner varies. This may relate in part to their basal translational efficiency, such that mRNAs which are inefficiently translated may be stimulated the most. The relief of repression of regulated mRNAs may be accompanied by polyadenylation, both effects stimulating translation independently or coordinately.

The effects of poly(A) during development may be especially profound owing to strong competition between mRNAs for the translational machinery, as exists in *Xenopus* oocytes (Laskey et al 1977). This is supported by studies showing that the effects of poly(A) are greatest under competitive conditions (Proweller & Butler 1994, 1997; Preiss & Hentze 1998). Competition, and hence the effects of poly(A), may also be modulated by modification of the translational apparatus.

Poly(A) Length Changes in Somatic Cells

As alluded to above, changes in poly(A) length also occur in somatic cells, although in most cases it is unclear whether these are a consequence of the modifications that occur in the cytoplasm. Examples include human and rat growth hormone mRNA (Paek & Axel 1987, Jones et al 1990, Murphy et al 1992) and rat insulin mRNA (Muschel et al 1986). Poly(A) tail length changes have also be observed when the translation of *Chlamydomonas* α-tubulin is perturbed (Baker et al 1989), or when β-interferon-expressing cells are infected with Sendai virus (Dehlin et al 1996). Heat shock causes a dramatic increase in the proportion of poly(A)-deficient mRNAs in *Drosophila* (Spradling et al 1975, Storti et al 1980). Although these examples are consistent with direct effects of poly(A) on translation in somatic cells, they should interpreted with caution. For example, the change in the length of an mRNA's poly(A) tail can be an effect rather than a cause of translational control (Muckenthaler et al 1997). Future work examining the polyadenylation of specific somatic mRNAs

in detail, including the identification of their repressors and activators, will be needed to clarify the issue.

End-to-End Interactions and the Role of Poly(A) Binding Protein

The finding that poly(A) and 3′UTRs can affect initiation suggests that the two ends of the mRNA may interact or be in close proximity (Jacobson 1996). Consistent with this view, electron micrographs show polysomes with nearby 5′ and 3′ ends (e.g. Christensen et al 1987), and the translational effects of the cap structure and poly(A) tails are synergistic rather than additive (e.g. Gallie 1991). The finding that loss of poly(A) triggers enzymatic cleavage of the cap in vivo also provides strong circumstantial evidence for an end-to-end interaction (Muhlrad et al 1994).

In spite of apparent 5′-3′ interactions, the poly(A) tail can also stimulate translation of uncapped reporter mRNAs (e.g. Munroe & Jacobson 1990, Gallie 1991, Iizuka et al 1994). It is unclear how to interpret these effects in terms of cellular capped mRNAs. Recent studies underline the importance of a cap in poly(A)-mediated stimulation: The cap tethers the stimulatory effect of the poly(A) tail to the 5′ end of the mRNA and prevents spurious initiation at downstream initiator codons (Preiss & Hentze 1998).

One likely participant in poly(A)'s effects is poly(A)-binding protein (PAB), the most abundant protein associated with cytoplasmic poly(A) tails. A single molecule of PAB requires 10–12 nucleotides to bind tightly (Sachs et al 1987) and occupies roughly 25 adenosine monophosphates, such that multiple PAB molecules can reside on a single poly(A) tail (Baer & Kornberg 1983). The gene encoding PAB, *pab-1*, is essential for viability in yeast (Sachs et al 1987).

Two different models have been proposed for the mechanism by which PAB enhances translation. The first proposes that poly(A)/PAB complexes increase the efficiency of 60S subunit joining (step 5b in Figure 2). Consistent with this hypothesis, a number of mutations in yeast that increase the 40S/60S ribosomal subunit ratio (Sachs & Davis 1989, 1990) suppress the lethality of PAB deletions. Additionally, poly(A) mildly stimulates the formation of 80S ribosomes on mRNAs bound by 40S ribosomal subunits in a rabbit reticulocyte lysate (Munroe & Jacobson 1990). The second model has been derived from experiments conducted in a poly(A)-dependent yeast cell-free translation system. In this model, 40S ribosomal subunit joining is stimulated by the PAB/poly(A) tail complex (step 3b in Figure 2; Tarun & Sachs 1995). However, the inhibitory effect of PAB immunodepletion in this system on translation and ribosomal association of capped-and-polyadenylated mRNAs varies and can be modest. The two models are not mutually exclusive, and poly(A)/PAB may stimulate both events.

THE EIF-4G CONNECTION Recently, it was proposed that the stimulation of small subunit joining in *S. cerevisiae* is facilitated by an interaction between PAB and yeast eIF-4G (Tarun & Sachs 1996, Tarun et al 1997). Since eIF-4G is associated with eIF-4E, this could bring the two ends of the mRNA together (Figure 5*A*). An interaction between eIF-iso4G and PAB has also been detected in wheat germ extracts (Le et al 1997, Wei et al 1998). The yeast eIF-4G/PAB interaction in vitro is RNA dependent, a finding that could reflect a need for a PAB/poly(A) complex for eIF-4G interactions. (Tarun & Sachs 1996, Tarun et al 1997). However, point mutations in the site in eIF-4G required for this RNA-dependent interaction do not significantly affect the translation of capped, or capped-and-polyadenylated, mRNAs, nor lead to a loss of synergistic translation in vitro, nor

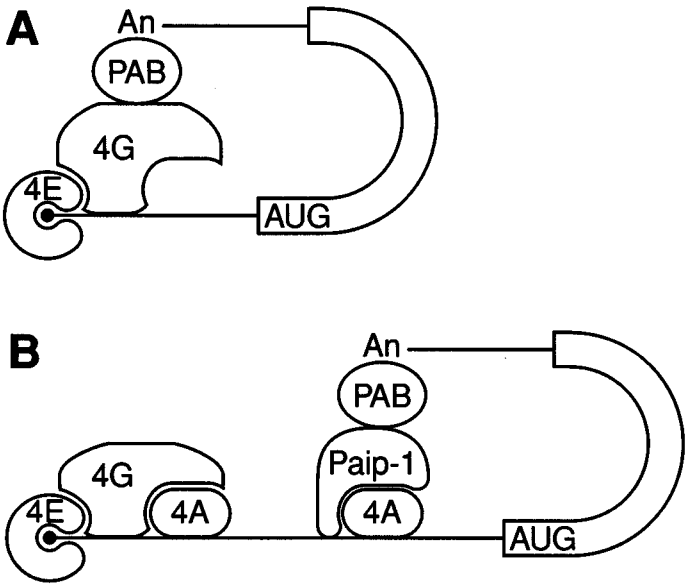

Figure 5 Models for 5′-3′ interactions mediated by PAB. *A*. The direct interaction between the PAB and eIF-4G mediates an indirect interaction between the cap-binding complex and the poly(A) tail. Although this model depicts PAB interacting through the 4E component of the cap-associated eIF-4F, point mutations that disrupt PAB-eIF-4G interactions in vitro do not strongly affect translation of capped mRNAs (see text). A similar interaction has been proposed based on experiments in wheat germ extract. In both yeast and plants, eIF-4F contains only eIF-4E and eIF-4G when isolated. The positions of eIF-4A and eIF-4B are not indicated. Figure adapted from Sachs & Buratowski (1997). *B*. The interaction of Paip-1 with eIF-4A and PAB forms a bridging interaction between the 5′ and 3′ ends of the mRNA. This interaction does not include the cap-binding complex. The position of eIF-4B is not indicated. Figure adapted from Craig et al (1998). Both models predict that the interactions depicted lead to a stimulation of small ribosomal recruitment; the small ribosomal subunit is not depicted.

to inviability in vivo (Tarun & Sachs 1996, Tarun et al 1997). Thus although interactions mediated by this site in eIF-4G contribute to the translation of uncapped-polyadenylated mRNAs, they are not required for protein synthesis in the cell under standard laboratory conditions (Tarun et al 1997).

AN ALTERNATIVE ROUTE: PAIP-1 In mammalian cells, a PAB-interacting protein (Paip-1), with some homology to eIF-4G, has been identified (Craig et al 1998). It mildly stimulates both cap-dependent and IRES-mediated translation in transfected COS cells. Unlike eIF-4G, Paip-1 does not contain a eIF-4E-binding site, but has homology through the region that contains eIF-3 and eIF-4A binding sites. However, no interaction with eIF-3 has been detected, suggesting that Paip-1 may not directly link PAB with the 40S ribosomal subunit, as suggested in the model depicted for yeast in Figure 5*A*. Paip-1 does interact with eIF-4A, and this could link the two ends of the mRNA (Figure 5*B*). Several lines of evidence suggest that eIF-4A cycles in and out of eIF-4F complexes. This is proposed to facilitate the delivery of multiple molecules of eIF-4A to the mRNA, which migrate through the 5′UTR to achieve unwinding (Pause et al 1994b). Paip-1 may interact with free eIF-4A rather than with eIF-4A as part of the eIF-4F complex (Craig et al 1998). Despite the lack of a direct interaction with the small ribosomal subunit, Paip-1/eIF-4A interactions are proposed to cause more efficient recruitment or reinitiation of small ribosomal subunits (Craig et al 1998).

POSSIBLE MEDIATORS OF POLY(A)-STIMULATED TRANSLATION IN EARLY DEVELOPMENT In oocytes and embryos, where the effect of poly(A) tails can be profound, it is unclear whether eIF-4G, Paip-1, or even PAB is involved. *Xenopus* oocytes apparently contain very little PAB protein (Zelus et al 1989), and no role for PAB in translation stimulation in *Xenopus* or other oocytes or embryos has been reported. Furthermore, mRNAs that are repressed often contain poly(A) tails of respectable, though short lengths; it is unclear why doubling the length of the tail should make a dramatic difference in translational activity if it is solely mediated through PAB (Wickens et al 1996). Finally, in *Xenopus* oocytes and embryos, translational activation of some mRNAs requires the presence of a long poly(A) tail, whereas for others the act of poly(A) addition appears to be critical (McGrew et al 1989, Simon et al 1992). For such mRNAs, ribose methylation of the cap structure has been linked to polyadenylation (Kuge & Richter 1995). The recent discovery of Paip-1 raises the possibility of variations of the recruitment theme, including the possibility that multiple protein factors may function in ribosomal recruitment in oocytes and embryos.

CPEB: Activator, Repressor, or Both?

mRNAs that undergo cytoplasmic polyadenylation contain specific sequences (CPEs) that promote the reaction. CPEs are thereby implicated in translational

activation. However, earlier in oogenesis, the CPE (or ACE) of mouse tPA mRNA, which receives poly(A) during maturation, is required for poly(A) tail shortening and translational repression (Huarte et al 1992). Thus CPE-mediated activation could include loss of a repressor, accumulation of an activator that might attract the polyadenylation machinery, or both.

Cytoplasmic polyadenylation element-binding protein (CPEB) binds the CPEs of a variety of mRNAs (Paris et al 1991, Stebbins-Boaz et al 1996) and has been isolated and cloned from *Xenopus* and mouse (Hake & Richter 1994, Gebauer & Richter 1996). It is a positive-acting factor in that CPEB-immunodepletion prevents polyadenylation in frog egg extracts (Hake & Richter 1994), and injection of anti-CPEB into intact oocytes prevents oocyte maturation and polyadenylation of c-*mos* mRNA (Stebbins-Boaz et al 1996). The protein complex that binds to the AAUAAA element, called the cleavage and polyadenylation specificity factor (CPSF), is likely a second positive-acting factor. Subunits of CPSF are detected in the oocyte cytoplasm, and their immunodepletion reduces polyadenylation efficiency in egg extracts (A Bilger, K Dickson, S Ballantyne, A Jenny, M Wickens, in preparation). Interactions between CPEB and CPSF could underlie the problematic reconstitution of polyadenylation activity by recombinant CPEB in depleted extracts (Stebbins-Boaz et al 1996).

Two provocative CPEB orthologues have been identified. The first, Orb, is a *Drosophila* protein involved in mRNA localization. *orb* mutants are defective in several aspects of oogenesis and in the establishment of polarity in the oocyte and embryo (Lantz et al 1992, Christerson & Mckearin 1994, Lantz et al 1994). The second orthologue, p82, is a protein isolated from the surf clam *S. solidissima* that is 41% identical to CPEB overall (N Standart, personal communication). Interestingly, p82 was first identified via its role in maintaining the repression of ribonucleotide reductase mRNA in oocytes (Walker et al 1996). After fertilization the same protein appears to be required for polyadenylation, suggesting that it may be a functional homologue of CPEB (N Standart, unpublished observation). The conservation between p82 and CPEB is most striking in the region that confers RNA binding, containing two RRM motifs and a zinc finger (Hake et al 1998; N Standart, unpublished observation). During maturation, *Xenopus* CPEB decreases dramatically in abundance, consistent with a function as a repressor. Both CPEB and p82 are phosphorylated during translational de-repression, but remain mRNA associated (Paris et al 1991, Standart 1992). Thus phosphorylation may modulate translation through effects on interactions with other proteins, rather than by affecting mRNA binding.

If CPEB and p82 are indeed functional homologues, then this protein is likely to be both a translational activator and repressor. Although the mechanism of repression is unclear, activation might be the result of recruitment of the polyadenylation apparatus, including CPSF and poly(A) polymerase.

PLAUSIBLE MODELS OF 3′ END–MEDIATED REGULATION

Mechanisms of Repression

The mechanism by which repressors inhibit translation through the 3′UTR is not understood and is likely more complex than repression mediated by the 5′UTR. In the 5′UTR, a single protein is often sufficient for repression; in the 3′UTR, multi-protein complexes may be the norm. Moreover, repression in the 5′UTR can be achieved by simple steric inhibition of the translational machinery (Stripecke & Hentze 1992, Gray & Hentze 1994a); this is not possible, at least in a simple fashion, in the 3′UTR.

Models have been proposed for the mechanism by which negative elements in the 3′UTR repress translation (reviewed by Sonenberg 1994, Standart & Jackson 1994, Wickens et al 1996). We briefly present several such speculations below, recognizing that a diversity of mechanisms may be employed and that those we list are not mutually exclusive. We draw particularly on those examples discussed in the preceding sections.

NUCLEATION SITES Negative elements may form a nucleation site for large protein complexes that make the mRNA inaccessible to the translational machinery. For example, a sequence-specific RNA-binding protein could recruit other factors that sequester the mRNA, much as higher order chromatin structure can sequester DNA from the transcriptional machinery. Such repressed structures could include the FRGY proteins since FRGY2 is found associated with many repressed mRNAs in the oocyte cytoplasm and can inhibit their translation in vitro (reviewed by Sommerville & Ladomery 1996, Wolffe & Meric 1996). The role of FRGY proteins is unclear because of their ubiquitous association with mRNAs, including both translationally competent and repressed mRNAs (Tafuri & Wolffe 1993). Their role may be to prevent spurious initiation at internal sites within the mRNA rather than to mediate mRNA-specific regulation. A requirement for non-specific RNA-binding proteins to ensure faithful initiation has been described in vitro (Svitkin et al 1996).

DISRUPTION OF END-TO-END CONTACTS The growing body of evidence that the two ends of the mRNA interact has led to many models in which 3′UTR-binding proteins either directly interrupt that interaction or prevent its activity in initiation. 3′UTR-binding proteins might hide the cap from initiation factors or interfere with any of the protein-protein contacts necessary for initiation. The ability of a repressor to mediate repression of uncapped mRNAs or of IRES-mediated translation would strongly argue against cap-occlusion models because neither the cap nor eIF-4E are required: Repression of LOX mRNA satisfies these criteria (Ostareck-Lederer et al 1994, Ostareck et al 1997).

A specific form of this model posits that repressor proteins sequester PAB, prevent its binding to poly(A), or sterically occlude its interactions with initiation factors. In those cases, mRNAs lacking a poly(A) tail should not be subject to regulation. For that reason, this model is unlikely to apply to LOX mRNA because its repression can be reconstituted in cell-free systems using unadenylated mRNAs (Ostareck-Lederer et al 1994).

One provocative model suggests that ribosomes or ribosomal subunits enter the 3′UTR and commonly reinitiate translation on the same mRNA. Circumstantial evidence that ribosomes or subunits may continue past a termination codon exist (Peabody & Berg 1986, Kaufman et al 1987, Hinnebusch 1996), as do indications that ribosomes may preferentially reinitiate (Nelson & Winkler 1987, Galili et al 1988). In this view, factors bound to the 3′UTR might repress or enhance reinitiation.

MICROLOCALIZATION Repressors bound to the 3′UTR might place an mRNA in a micro-environment in which translation is inefficient. Upon their activation, an mRNA might, for example, associate with the cytoskeleton where translation is more efficient, without any gross movement within the cell (Decker & Parker 1995, Bassell & Singer 1997). The identification of specific mutant alleles of cytoskeletal components, such as actin and tubulin, that are specifically defective in translation, would strongly support this model. It is unlikely that mRNAs whose repression can be reconstituted in cell-free translation systems, including LOX (Ostareck-Lederer et al 1994) and clam ribonucleotide reductase (Standart et al 1990), are regulated in this manner.

Regulation linked to the large scale movement of mRNAs within a cell (e.g. *oskar* mRNA) may differ from that of micro-localization. Regulation of *oskar* and *nanos* translation is intimately linked to their being positioned in the correct place within the oocyte (see LOCALIZATION AND TRANSLATION). One might expect that this form of control requires localized activators or repressors; microlocalization does not, as it modulates interactions with the cytoskeleton that are not necessarily involved in trafficking. The growing collection of putative localization proteins may provide an entree into this important problem. It will be of considerable interest to determine whether the same proteins that mediate an mRNA's association with microtubules or microfilaments are also required for that mRNA's proper translation, as with Staufen and *oskar* mRNA.

CHANGES IN POLY(A) LENGTH For those mRNAs whose translational control requires changes in poly(A) length, repressor proteins bound to the 3′UTR may directly modulate accessibility of the mRNA to the cytoplasmic polyadenylation or deadenylation apparatus (Standart & Jackson 1994, Wickens et al 1996). It remains unclear why poly(A) tail lengths sufficient to mediate translational

stimulation of reporter mRNAs are shorter than the tails of many repressed messages. Perhaps repression of these mRNAs involves both components that modulate poly(A) tail length and repressors that act independently, modulating end-to-end contacts for example. The specific contribution of polyadenylation or deadenylation might be assessed using mutants in the enzymes or sequence-specific factors involved. To date, no such studies have been reported.

Regardless, these models leave open the question of how changes in poly(A) length facilitate translation. In brief, current models are divided into two categories: those that posit an effect of poly(A) and PAB on the translation apparatus, and those that suggest that the mRNA is covalently modified by N-7 or ribose methylation of the cap to facilitate initiation. Tests of the involvement of specific initiation factors in poly(A)-mediated enhancement in vivo are needed, especially in oocytes and embryos. In simple form, the ribose methylation model predicts an enhanced affinity of an initiation component for a ribose-methylated cap and raises the issue of whether removal of the modification is required for the repression owing to deadenylation.

Mechanisms of De-Repression

Whatever the mechanism by which a 3′UTR-bound protein represses translation, that effect must be relieved at a specific time or place. Potential mechanisms of de-repression include loss or modification of the repressor and recruitment of an activator. In several cases, phosphorylation of 3′UTR-bound factors correlates with activation, e.g. clam p82 and CPEB (Paris et al 1991, Standart 1992). The functional significance of the modifications is not clear however.

ROLE OF 5′ END MODIFICATIONS DURING DEVELOPMENT

The majority of nuclear encoded mRNAs receive a 5′m^7GpppG co-transcriptionally. This cap promotes translation initiation via interaction with eIF-4F (Banerjee 1980, Sonenberg 1996). Changes in its structure may regulate translation. Developmentally regulated changes in cap structures were first reported over two decades ago. N-7 methylation of the cap structure occurs following fertilization in the hornworm *Manduca sexta* (Kastern & Berry 1976, Kastern et al 1982) and the sea urchin, *Stronglyocentrotus purpuratus* (Caldwell & Emerson 1985). Cytoplasmic N-7 methyltransferase activity is present in frog oocytes and increases during oocyte maturation and could contribute to such control (Gillian-Daniel et al 1998). However, to date, the only specific mRNA thought to be selectively N-7-methylated is histone mRNA in *S. purpuratus* (Caldwell & Emerson 1985). Clearly, the absence of the N-7 methyl group would require that it was either removed or never put on in the nucleus.

Methylation of the 2′ position of the second and third ribose moieties of the mRNA (i.e. 7mGpppGmGm) may be linked to polyadenylation and hence to translational control of certain mRNAs. Polyadenylation-dependent ribose methylation has been reported using synthetic B4 RNA injected into *Xenopus* oocytes (Kuge & Richter 1995). Methylation inhibitors prevent both the modification and translational stimulation (Kuge & Richter 1995). A precedent for a functional link between polyadenylation and cap ribose methylation comes from vaccinia virus, in which the poly(A) polymerase has ribose methylation activity (Schnierle et al 1992). Early studies of the effects of ribose methylation indicated only small differences in translational efficiency (Muthukrishnan et al 1978). Moreover, ribose methylation cannot be the universal cause of the effects of poly(A) on translation because translation of injected reporter RNAs that do not undergo efficient ribose methylation nonetheless can be dramatically enhanced by polyadenylation (Gillian-Daniel et al 1998). Similarly, yeast mRNAs lack ribose methylation, yet poly(A) enhances their translation (Banerjee 1980). Nevertheless, a model in which polyadenylation in situ causes activation of N-7 or ribose methylation can accommodate repression of mRNAs with respectable tail lengths simply by inferring that they lack the methyl or ribose group prior to polyadenylation. However, this applies equally to any event that may occur in response to polyadenylation in situ, and not to pre-existing poly(A) tails.

Deadenylation leads to enzymatic cleavage of the cap structure and hence to mRNA decay in yeast (reviewed by Beelman & Parker 1995). A comparable deadenylation-dependent decapping reaction could, in principle, provide a simple mechanism by which poly(A) removal results in translational repression. However, this does not appear to be the case in *Xenopus* oocytes: RNAs that are completely deadenylated during maturation retain their caps in a methylated form (Gillian-Daniel et al 1998).

LOCALIZATION AND TRANSLATION

Macro-Localization and Translational Activity

mRNAs are sometimes localized in order to produce protein in only one region of the cell. Mechanisms exist to repress mRNAs that have not yet reached their proper destination or are not properly anchored there. Although this form of repression has been conclusively demonstrated only in the fly embryo, circumstantial evidence suggests it may be more general.

oskar and *nanos* mRNAs are required for formation of the posterior region of *Drosophila*. Both are localized to the presumptive posterior of the oocyte and early embryo (St Johnston 1995, Macdonald & Smibert 1996). To reach

that destination, the mRNAs must move across the oocyte, since they enter the anterior end of the oocyte from nurse cells. Translational repression of *oskar* mRNA during its transit is mediated by a protein, Bruno, which binds to Bruno responsive elements (BRE) in the *oskar* 3′UTR (Kim-Ha et al 1995, Webster et al 1997). Repression by Bruno may involve deadenylation, as a rapid deadenylation signal in Eg2 mRNA of *Xenopus* contains consensus BRE sequence that may interact with a *Xenopus* orthologue of Bruno Etr, (Bouvet et al 1994, Webster et al 1997). However, regulation of *oskar* mRNA does not appear to be accompanied by changes in its poly(A) length (Webster et al 1997). Bruno may have other mRNA targets, as suggested by the *bruno* mutant phenotype (Schupbach & Wieschaus 1991, Webster et al 1997).

nanos mRNA also contains signals within its 3′UTR that direct it to the posterior and control its translation (Gavis & Lehmann 1994). Unlike the BREs in *oskar*, the regulatory elements in *nanos* do not inhibit translation during transit of the mRNA; rather ectopic Nanos protein in the developing oocyte may be rapidly degraded (Smibert et al 1996, Wang et al 1994). The control elements do, however, prevent translation of mRNAs that remain unlocalized in the early embryo (Dahanukar & Wharton 1996, Gavis et al 1996, Smibert et al 1996). A 135-kDa protein, Smaug, has been identified that likely binds to these elements (Smibert et al 1996).

As the numbers of examples of localized mRNAs increases, it should become clear whether mRNAs that are mis-localized or still in transit are commonly less active. At this early stage, it appears this may be the case. For example, expression of *ASH1* mRNA appears to be more efficient once it is localized in budding yeast (Long et al 1997). The mechanisms responsible for such regulation are not known, but may include the formation of transport particles in which the mRNAs are trafficked but translation does not occur (reviewed by Bassell & Singer 1997).

Micro-Localization: The Role of the Cytoskeleton in Translational Control

The cytoskeleton may have several functions in the regulation of mRNA expression (reviewed in Bassell & Singer 1997). It may provide a surface for the interaction of cellular components, allow mRNAs to be spatially organized where their products are to be utilized, provide an opportunity for feedback regulation, and/or sequester mRNAs from the translational machinery until they reach their destination.

Links between translation and cytoskeletal association have been suggested from at least four lines of evidence. First, the majority of mRNAs and polyribosomes are found to be associated with the cytoskeleton after extraction with certain detergents (Lenk et al 1977, Fulton et al 1980, Zambetti et al 1985,

Ornelles et al 1986). Second, drugs that depolymerize the cytoskeleton result in a release of mRNAs and a decrease in general protein synthesis (Lenk et al 1977, Ornelles et al 1986, Taneja et al 1992). Moreover, efficient translation of *oskar* mRNA requires mRNA localization, which can be disrupted by mutations in the mRNA and by chemical or genetic disruptions of the cytoskeleton (Theurkauf et al 1993, Clark et al 1994, Theurkauf 1994, Erdelyl et al 1995, Kim-Ha et al 1995, Markussen et al 1995, Rongo et al 1995, Tetzlaff et al 1996). Third, in granules that contain cytoskeletally associated mRNAs, ribosomes, at least one tRNA and elongation factor eEF-1α (Yang et al 1990, Barabese et al 1995) have been described. Fourth, by electron micrographic in situ hybridization, the majority of polyadenylated RNA in fibroblasts is found in actin filament intersections that also contain eEF-1α and ribosomes (Bassell et al 1994).

Recently, proteins that may mediate the interaction of mRNAs with the cytoskeleton have been identified. For example, *Drosophila* Staufen appears to form a cytoskeletally associated complex with *oskar* mRNA that leads to its localization and translation (Ephrussi et al 1991, Kim-Ha et al 1991, St Johnston et al 1991, Ferrandon et al 1994, Manseau et al 1996). It appears to be involved in the maintenance (anchoring) of *oskar* mRNA at the posterior pole, a function that requires oskar protein itself (Ephrussi et al 1991, Kim-Ha et al 1991, Markussen et al 1995, Rongo et al 1995). Staufen is also required for the localization of *bicoid* and perhaps other mRNAs (St Johnston 1989, St Johnston et al 1991, Ferrandon et al 1994). A number of putative localization proteins in other species have also been identified. These include Spnr, a 71-kDa protein, located on cytoplasmic microtubules, which interacts with the 3′UTR of protamine-1 mRNA in mouse spermatids (Schumacher et al 1995), and a 70-kDa protein (ZBP-1) that binds to β-actin zipcodes (Ross et al 1997). A protein with homology to ZBP-1 has been implicated in Vg1 mRNA localization in *Xenopus* oocytes (L Havin, submitted).

The biochemical mechanism(s) by which the cytosketeton contributes to translation regulation is not understood. In one simple model, the role of the cytoskeleton is restricted to positioning the mRNA correctly. For example, Staufen, contributes to both the localization and translational activation of *oskar*: It may activate *oskar* translation by bringing and maintaining the mRNA in a region conducive to its translation.

NUCLEAR AND CYTOPLASMIC CROSS-TALK

Because translation occurs in the cytoplasm, nuclear events are often thought to be entirely unrelated to its control. Recently, a growing body of evidence has begun to suggest otherwise.

Two key features of mRNAs, the cap and poly(A) tail, are recognized in both the nucleus and cytoplasm. In the nucleus, the cap is recognized by CBC α complex of two proteins involved in pre-mRNA processing (Izaurralde et al 1994), while in the cytoplasm, a different cap binding complex, eIF-4F, is bound (Sonenberg 1996). The exchange between the two complexes, perhaps soon after or coincident with emergence of the mRNA from the nucleus, is a critical event in translation and could, in principle, be modulated. A significant fraction of eIF-4E is located in the nucleus, in both yeast and mammalian cells (Lejbkowicz et al 1992, Lang et al 1994). The function of this nuclear subpopulation is unclear. Similarly, PAB, which participates in cytoplasmic events including translation, also modulates the length of poly(A) added in the nucleus (Amrani et al 1997, Minvielle-Sebastia et al 1997). A second protein, PABII, is exclusively nuclear and participates in nuclear polyadenylation (Wahle 1991).

In some cases, sequence-specific translational repression may be established in the nucleus and carried to the cytoplasm. For example, hnRNP K, an abundant nuclear protein that can shuttle to and from the cytoplasm (Michael et al 1997), represses translation of LOX mRNA. Several other proteins that regulate translation may also have nuclear functions. These include *Drosophila* Sex Lethal (Green 1991) and Bicoid (Driever 1992), and the yeast ribosomal protein L32 (Dabeva & Warner 1993).

In Xenopus oocytes the translation of mRNAs originating from the nucleus can be repressed relative to that of the same mRNAs injected into the cytoplasm. The repressive effect of the "nuclear experience" may be from the binding of so-called FRGY proteins (Sommerville & Ladomery 1996, Wolffe & Meric 1996), which also are transcription factors.

NETWORKS: REGULATORS WITH MULTIPLE ROLES

An increasing number of complex regulatory networks involving translational control have appeared in the literature. In some, regulatory proteins respond to a variety of regulatory signals and/or control a number of mRNA targets. In others, the same proteins are used to control gene expression at different levels.

hnRNP E1 (αCP1) and hnRNP K

hnRNP E1 (αCP-1) and hnRNP K can repress the translation of LOX mRNA (Ostareck et al 1997). hnRNP E1 is also part of a complex (α-complex) that controls the stability of α-globin mRNA by binding to CU-rich sequences in its 3′UTR (Kiledjian et al 1995, Wang et al 1995). Interestingly, the CU-rich sequence of α-globin mRNA cannot substitute for the DICE element of LOX mRNA in mediating translational repression (Ostareck et al 1997). However, a

second protein, E2 (αCP2), which is a close relative of E1, is also involved in globin stability (Kiledjian et al 1995) and may be able to mediate translational repression via DICE elements (Ostareck et al 1997). hnRNP K also appears to have a dual function. In addition to its role in translation repression it also functions in the transcriptional activation of c-myc, which contains a CT-rich promoter (Takiamoto et al 1993, Michelotti et al 1996). Thus these proteins seem to be involved in the regulation of transcription, translation, and mRNA stability. This raises the possibility that regulation of one protein, or its partners, could affect a network of genes at several levels of gene expression.

IRP

IRPs are at the hub of a complex network of metabolic circuits (Figure 6). IRP was first identified as a regulator of IRE-containing genes involved in the control of iron homeostatis (reviewed in Hentze & Kuhn 1996). The interaction of IRPs with IREs in the 5′UTR of a number of mRNAs leads to their translational repression. In contrast, transferrin receptor mRNA, which contains multiple IREs within its 3′UTR, is stabilized against endonucleolytic cleavage by the presence of IRP (Binder et al 1994). In addition, IRP-1 is also a cytoplasmic aconitase (reviewed in Hentze & Kuhn 1996), which catalyzes the conversion between citrate and iso-citrate. The regulatory and enzymatic functions of IRP-1 are mutually exclusive and depend on the status of an iron-sulfur cluster within the protein (reviewed in Hentze & Kuhn 1996).

The IRE-binding activity of IRP-1 is regulated in response to at least two additional signals, nitric oxide and oxidative stress (in the form of hydrogen peroxide) (Figure 6) (Hentze & Kuhn 1996). This has fueled speculation that iron metabolism and oxidative stress, which result in the production of harmful free radicals, may be coordinately regulated. This speculation is supported by the presence of functional IREs in two Krebs cycle enzymes (Kohler et al 1995, Gray et al 1996, Melefors 1996, Schalinske et al 1998), the pathway that fuels production of mitochondrial reactive oxygen intermediates. Also, IRP-1 has been suggested to be a target of protein kinase C (Schalinske et al 1997).

A second IRP, IRP-2, is regulated in response to iron and nitric oxide but not by oxidative stress (Figure 6) (Hentze & Kuhn 1996). In contrast to IRP-1, IRP-2 is not modulated by post-transcriptional changes in its iron-sulfur cluster but instead is regulated by changes in its stability (Hentze & Kuhn 1996).

Thus IRP-mediated regulation allows a network of mRNAs to be coordinately regulated in response to a variety of signals. The differential expression of IRP-1 and IRP-2 may have physiological consequences: Cells in which IRP-1 is the most abundant may be more responsive to oxidative stress. IRP-1 and IRP-2 bind to distinct but overlapping sets of RNA sequences (Henderson 1996). Thus different cellular mRNAs may be regulated differentially by these proteins.

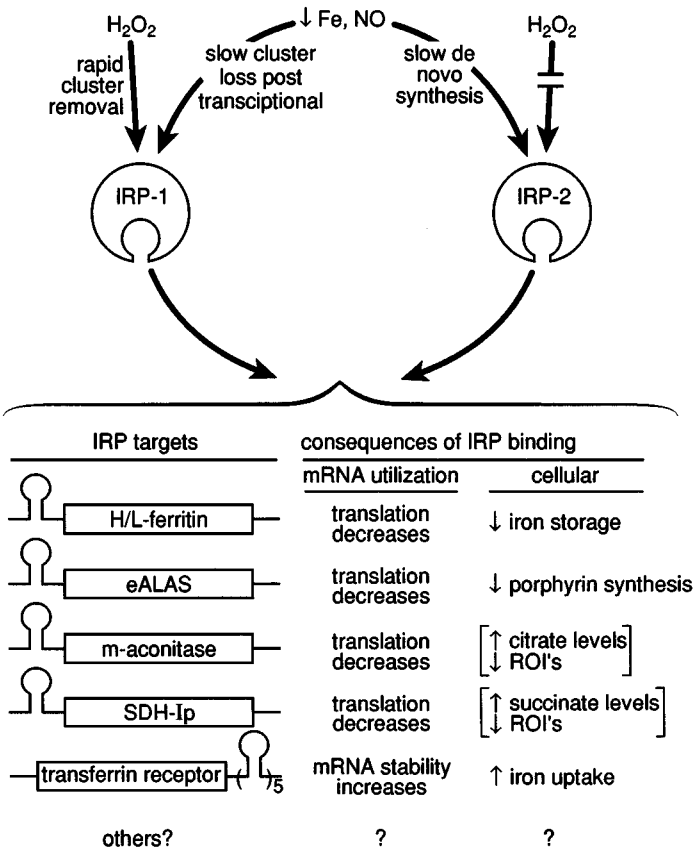

Figure 6 Regulation of gene expression by IRP-1 and IRP-2. The RNA-binding activities of IRP-1 and IRP-2 are activated in response to low cellular iron and nitric oxide. The activation of IRP-1 involves a slow post-transcriptional disassembly of its iron sulfur cluster, while activation of IRP-2 is also slow and involves synthesis of IRP-2 protein. IRP-1 binding activity is also activated by oxidative stress and involves a rapid disassembly of the iron-sulfur cluster. Binding of the IRPs results in translational repression of ferritin, erythroid 5-aminolevolinate synthase (eALAS), mitochondrial aconitase (m-aconitase), and succinate dehydrogenase iron protein (SDH-Ip) mRNA and in stabilization of transferrin receptor mRNA. The cellular consequences of IRP binding are indicated; however, the cellular consequences of IRP-regulation of aconitase and succinate dehydrogenase iron protein mRNAs are speculative. ROI, reactive oxygen intermediates. IREs do not function in the ORF (E Paraskeva, NK Gray, B Schlaeger, K Wehr, MW Hentze, submitted).

Sex Lethal

Sex lethal, a female-specific RNA-binding protein, regulates alternative splicing in the *Drosophila* sex determination hierarchy, determining splice-site choice for its own mRNA and transformer mRNA (Green 1991). Msl-2, part of a multi-subunit complex that functions in dosage compensation in male flies, is also regulated by Sex lethal. Sex lethal appears to act as a translational repressor of *msl-2* mRNA in females (Bashaw & Baker 1997, Kelly et al 1997) and is not present in male flies. The 5′UTR of *msl-2* mRNA contains two Sex lethal binding sites and the 3′UTR contains four (Bashaw & Baker 1997; Kelley et al 1997). Simultaneous interaction of Sex lethal with the 5′ and 3′ UTR binding sites appears to cause synergistic repression of *msl-2* (Bashaw & Baker 1997, Kelley et al 1997). The ability of Sex lethal to repress translation has recently been confirmed in vitro (Gebauer et al 1998). Thus this protein regulates both splicing in the nucleus and translation in the cytoplasm.

Sex lethal is not the only protein with a role in both regulated splicing and translation. Ribosomal protein L32 of *S. cerevisiae* regulates the splicing and translation of its own mRNA (Dabeva & Warner 1993). *Drosophila* Bicoid is a transcription factor but also represses the translation of *caudal* mRNA by binding to sites within its 3′UTR (Dubnau & Struhl 1996, Rivera-Pomar et al 1996).

GLOBAL CONTROL: REGULATION VIA INITIATION FACTORS

Changes in the rate or pattern of protein synthesis occur in response to such stimuli as heat shock, mitogenic stimulation, or growth. Such gross changes in translation are normally mediated by changes in the activity or abundance of the translation initiation factors. Modulation of the activity of the basal translation apparatus can preferentially affect the translation of specific mRNAs, as discussed below.

Numerous components of the translational machinery are phosphoproteins, including at least 13 initiation factors as well as 3 elongation factor subunits, 3 ribosomal proteins, and a number of amino-acyl tRNA synthases (Hershey 1991). Although the phosphorylation state of eIF-4B, eIF-4E, eIF-4G, eIF-2, and eIF-3 can all be modulated in vivo (reviewed by Merrick 1992, Morley 1994), a detailed understanding of the effect of these modifications is available only for eIF-2 and eIF-4E.

Analysis of the effects of initiation factor modifications in vivo is complicated by the multiplicity of responses that might in principle affect translation. For example, mitogen-stimulated T-cell activation leads to a two- to threefold increase in eIF-2 α and β subunits and eIF-4E levels, a similar increase in

mRNA and ribosome levels (Cohen et al 1990, Boal et al 1993), and an increase in eIF-4E phosphorylation and utilization (Boal et al 1993, Morley et al 1993). Similarly, in reticulocytes stimulated with phorbol esters, eIF-3, eIF-4B, eIF-4E, and eIF-4G are all phosphorylated (Morley & Traugh 1989). The situation has become even more complex recently with the discovery of factors that sequester initiation factors; at least one of these factors is regulated by phosphorylation. Dissecting the individual contributions of any one event on cellular growth and metabolism is a serious and central challenge.

The signaling pathways that control initiation factor phosphorylation are discussed in recent reviews (Morley 1994, Clemens 1996, Pain 1996, Sonenberg 1996, Flynn et al 1997). We focus here on the mechanisms by which initiation appears to be modulated.

eIF-2

eIF-2 binds GTP and the initiator tRNA and delivers this complex to the small ribosomal subunit (Figure 2). The GTP is hydrolyzed at the initiator AUG, prior to joining of the large ribosomal subunit; eIF-2-GDP is released, leaving a charged initiator tRNA in place (Merrick 1992, Clemens 1996). In addition, eIF-2 appears to function in identification of the initiator codon (Donahue et al 1988, Cigan et al 1989). Phosphorylation of eIF-2, which consists of three subunits (α, β, and γ), results in an inhibition of translation and is a central control point in the initiation pathway (Clemens 1996). The α subunit of eIF-2 can be phosphorylated at serine 51 by a hemin-controlled repressor kinase (HCR) in response to heme deficiency in reticulocytes (Clemens 1996) or by yeast *GCN2* kinase in response to uncharged tRNA (Hinnebusch 1996). Finally, it can be phosphorylated by double-stranded RNA-regulated protein kinase (PKR) in response to double-stranded RNA (Clemens 1996, Clemens & Elia 1997). Phosphorylation of eIF-2 does not inhibit its activity per se but instead inhibits its recycling by the GDP/GTP exchange factor, eIF-2B (Clemens 1996). Recycling of eIF2-GDP to eIF2-GTP is required for the next round of initiation (Merrick 1992). The interaction of phosphorylated eIF-2 with the recycling factor eIF-2B forms a stable complex in which the bound GDP cannot be exchanged for GTP (Rowlands et al 1988, Dholakia & Wahba 1989, Kimball et al 1998). As eIF-2 is normally in excess of eIF-2B, this essentially sequesters the cellular eIF-2B activity and leads to a general inhibition of translation.

Regulation of eIF-2 activity by HCR and Gcn2p kinase are generally considered to represent specialized forms of regulation. However, several observations suggest that regulation by PKR may play a more generalized role in growth control. First, interleukin-3 stimulation of cell growth involves the dephosphorylation of PKR and eIF-2 (Ito et al 1994). Second, PKR activity is inhibited in ras-transformed cells (Mundschau & Faller 1992). Third,

dominant-negative mutants of PKR, and mutants of eIF-2α that cannot be phosphorylated, cause transformation of transfected cells (Koromilas et al 1992b, Donze et al 1995). Fourth, these transformed cell lines can form tumors in nude mice (Meurs et al 1993). However, because PKR phosphorylates several transcription factors (Clemens & Elia 1997), only some of its effects may be through eIF-2α.

PKR is not essential in vivo. Homozygous knockout PKR mice display only a limited deficiency in their interferon response, not aberrant growth or high tumor incidence (Yang et al 1995). Similarly, a survey of human hematological malignancies suggest that PKR activity is not abnormally low (Basu et al 1997). These results may indicate that eIF-2 phosphorylation and PKR have little function in growth control in vivo. On the other hand, an alternative kinase pathway may exist, as may other modes for controlling the activity of eIF-2 in response to growth signals. Indeed, the levels and activity of the recycling factor eIF2B are regulated (Clemens 1996, Welsh et al 1998).

eIF-4E

The cytoplasmic cap-binding protein, eIF-4E, has been suggested to be limiting for translation in vivo and appears to be a critical regulator of cell growth, development, and differentiation (reviewed by Flynn & Proud 1996, Sonenberg 1996, Morley 1997). For example, eIF-4E overexpression induces aberrant cell growth, transformation of cells to form foci in soft agar, and tumors in nude mice (reviewed in Sonenberg 1996). Moreover, elevated eIF-4E levels are found in several transformed tumor cell lines (Miyagi et al 1995) and in virtually all breast carcinomas (Kerekatte et al 1995, Li et al 1997, Nathan et al 1997a) and may be a marker for predicting the recurrence of head and neck cancer (Nathan et al 1997b). Increased levels of eIF-4E phosphorylation correlate with an increased translation during oocyte maturation in starfish (Xu et al 1993), *Xenopus* (Morley & Pain 1995a), and mouse oocytes (Gavin & Schorderet-Slatkine 1997). Additionally, microinjection of eIF-4E into *Xenopus* embryos causes mesoderm induction (Klein & Melton 1994).

In vivo, phosphorylation of eIF-4E at serine 209 occurs in response to growth factors, hormones, and mitogens (reviewed by Sonenberg 1996). Conversely, dephosphorylation is triggered by serum deprivation, viral infection, and heat shock (reviewed by Sonenberg 1996). Phosphorylation at the same position can be achieved by a number of kinases; in vivo, protein kinase C and a kinase in the MAP-kinase cascade (possibly Mnk1) (Waskiewicz et al 1997) are likely candidates (Flynn & Proud 1996, Sonenberg 1996, Morley 1997). It should be noted that phosphatase activity may also contribute to eIF-4E regulation (Sonenberg 1996, Morley 1997).

Phosphorylation of eIF-4E appears to enhance its activity, because the phosphorylated form of eIF-4E is predominately found in 48S pre-initiation

complexes (Joshi-Barve et al 1990). However, a large proportion of phosphorylated eIF-4E is not associated with mRNAs (Rau et al 1996). Phosphorylation has been reported to increase both its affinity to the cap (Minich et al 1994) and its association with eIF-4A and eIF-4G to form eIF-4F complexes (e.g. Lamphear & Panniers 1990, Bu et al 1993, Morley et al 1993, Morley & Pain 1995b). Since eIF-4F has been reported to have a greater affinity for the cap than eIF-4E alone (Bu et al 1993, Haghihat & Sonenberg 1997), effects of eIF-4E phosphorylation may result from a combination of an increase in the amount of eIF-4F and from enhanced cap-binding capacity.

Limiting eIF-4E levels may regulate translation of specific mRNAs by forcing all mRNAs to compete for the translation apparatus (Hiremath et al 1985, Duncan et al 1987). In this view, when eIF-4E (and therefore eIF-4F) is limiting, mRNAs that have structure-rich 5′ UTRs will be most poorly translated. The increased availability of eIF-4F will result in an increased delivery of RNA helicase activity to the 5′UTR, disrupting the secondary structures. Indeed, in mammalian cells, overexpression of eIF-4E results in a more efficient translation of reporter mRNAs containing structured 5′UTRs (Koromilas et al 1992a).

The importance of eIF-4E in cell growth regulation is illustrated by its connections to ras. eIF-4E can substitute for ras in a two-oncogene transformation assay (Lazaris-Karatzas & Sonenberg 1992). A number of approaches suggest that ras is an upstream activator of eIF-4E (e.g. Frederickson et al 1992, Rinker-Schaeffer et al 1992). For example, overexpression of ras can elevate eIF-4E phosphorylation in the absence of extracellular stimuli (Rinker-Schaeffer et al 1992). Yet ras also appears to be downstream of eIF-4E; eIF-4E overexpression increases ras activity, and eIF-4E's transforming ability is prevented by blocking the activation of ras (Lazaris-Karatzas et al 1992). These data suggest a simple positive feedback loop: Ras activates eIF-4E phosphorylation, which leads to increased translation of growth factors that are then secreted, bind cell surface receptors, and thus induce further ras activation (reviewed by Rhoads 1991, Sonenberg 1996). A similar pathway may explain the induction of mesoderm caused by eIF-4E overexpression in *Xenopus* embryos, since that process can be inhibited by co-expression of a dominant-negative ras mutants (Klein & Melton 1994). In yeast, however, overexpression of eIF-4E is without pronounced effect and does not enhance translation of mRNAs with structured 5′UTRs (Lang et al 1994).

A prediction of the feedback models above is that overexpression of eIF-4E will lead to the activation of one or more mRNAs encoding growth factors and mitogenic proteins with structure-rich 5′UTRs. Indeed, the translation of a number of such cellular mRNAs in response to elevated eIF-4E levels has been observed. These include the growth-promoting proteins, cyclin D1 (Rosenwald et al 1993) and ornithine decarboxylase (Shantz & Pegg 1994), and the growth-related protein, P23 (Bommer et al 1994).

eIF-4EBP/PHAS-I

eIF-4E activity can be modulated by interaction with a regulatory protein, designated eIF-4EBP1 or PHAS-I (Lin et al 1994, Pause et al 1994a). This 118-amino acid phosphoprotein competes with eIF-4G for binding to eIF-4E and prevents formation of eIF-4F (Haghighat et al 1995). eIF-4EBP1 and eIF-4G share a motif involved in binding eIF-4E, which explains their competitive binding (Haghighat et al 1995, Mader et al 1995). Under conditions such as serum deprivation and heat shock, eIF-4EBP1 becomes dephosphorylated and sequesters eIF-4E, globally limiting cap-dependent translation (reviewed in Flynn & Proud 1996, Sonenberg 1996, Lawrence & Abraham 1997). The effects of eIF-4EBP1 may be most severe on specific subsets of mRNAs with secondary structure in their 5′UTR. Interestingly, the modulation of eIF-4E and eIF-4EBP1 activity does not always act in concert but may vary in their response to stimuli, for example during certain stages of adenovirus infection (Feigenblum & Schneider 1996) and heat shock in some cell types (Scheper et al 1997). Phosphorylation of eIF-4EBP1 by the mTOR/FRAP protein kinase pathway, indicated by sensitivity to the immunosuppressant rapamycin, leads to its dissociation from eIF-4E (reviewed in Lawrence & Abraham 1997). Recent evidence suggests that mTOR may directly phosphorylate eIF-4EBP1 (Brunn et al 1997). A second protein, eIF-4EBP2, appears to share many of the properties of eIF-4EBP1 (Pause et al 1994a) but exhibits a different pattern of expression (Tsukiyama-Kohara et al 1996).

A yeast homologue of eIF-4EBP, called *CAF20*, shares the common motif thought to be involved in eIF-4E binding (Altmann et al 1997). As expected, yeast strains deficient in Caf20p exhibit accelerated growth, whereas overexpression of Caf20p decreases growth rate (Altmann et al 1997).

Control of eIF-4E activity is exerted at two levels, via its phosphorylation status and by its sequestration via eIF-4EBP1. The cellular importance of the eIF-4EBP1 interaction is suggested by the finding that eIF-4EBP1 over-expression in eIF-4E transformed fibroblasts significantly inhibited proliferation and the ability of these cells to grow in soft agar (Rousseau et al 1996). However, even though rapamycin treatment results in rapid and complete dephosphorylation of eIF-4EBP1, its effect on protein synthesis and growth rates manifest more slowly and are reduced by at most 50% (Berretta et al 1996, Feigenblum & Schneider 1996). The importance of eIF-4EBP1 may vary in different cell types and conditions and may primarily act through specific subsets of mRNAs.

eIF-4G Decoys

eIF-4G is phosphorylated in response to many stimuli but the sites and consequences of phosphorylation are not yet well defined (reviewed by Morley et al 1997). eIF-4G, like eIF-4E, has been suggested to be limiting in cells,

and amplification of its gene has been found in independent squamous cell lung carcinomas (Brass et al 1997). Moreover, levels of eIF-4G appear to be tightly regulated in cells with control occurring both at the level of synthesis and turnover (discussed in Morley et al 1997).

Levels of free eIF-4G may be regulated by proteins analogous to those that prevent the interaction of eIF-4E with its partners. A novel 97-kDa protein, referred to as NAT1, DAP-5, Eif4g2, or p97, has 30% homology to the C-terminal two thirds of eIF-4G (Morley et al 1997). This portion of eIF-4G does not interact with eIF-4E but can promote internal initiation and contains binding sites for eIF-3 and eIF-4A and has RNA-binding activity. This protein appears to act as a eIF-4G decoy through its affinity for eIF-3 and/or eIF-4A, both of which it can interact with in vitro (Imataka et al 1997). Thus the eIF-4G decoy suppresses both cap-dependent and IRES-mediated translation. In principle, eIF-4G decoys could also activate specific mRNAs (Hentze 1997). Overexpression of the eIF-4G decoy inhibits cell growth. Surprisingly, although the N-terminal part of the eIF-4G decoy is most homologous to eIF-4G, it is the C-terminal region that seems to result in growth inhibition (Levy-Strumpf et al 1997). As a result, it is unclear whether these proteins repress growth by titrating initiation factors or through another mechanism.

Ribosomal Protein S6

The activation of a number of mRNAs whose translation and function are closely correlated to cell growth is partially inhibited by rapamycin (Jefferies et al 1994b, Pedersen et al 1997), which prevents the activation of $p70^{S6k}$ and phosphorylation of eIF-4EBP1. These mRNAs are controlled through sequences in their 5′UTR, which typically contain short ($\approx$8 nt) polypyrimidine tracts (terminal oligopyrimidine tracts, TOP) (Meyuhas et al 1996) near their caps. TOP mRNAs include vertebrate ribosomal proteins and two translation elongation factors, eEF-1α and eEF-2 (Jefferies et al 1994a, Terada et al 1994, Meyuhas et al 1996). Although the absolute requirement for the TOP element to be cap proximal (Hammond et al 1991) suggested that eIF-4E may regulate these mRNAs, this does not appear to be the case; for example, overexpression of eIF-4E does not increase their translation (Shama et al 1995).

Studies of a number of ribosomal protein mRNAs suggest that proteins that bind to their 5′UTRs may control translation; however, binding does not correlate with translational regulation (Gray & Hentze 1994b, Amaldi et al 1995, Meyuhas et al 1996). Control may be achieved by regulated recruitment of repressor or activator proteins by these constitutively bound factors. Alternatively, sensitivity to rapamycin raises the possibility that modification of ribosomal protein S6 by $p70^{S6k}$ directly enhances TOP mRNA translation (Jefferies et al 1994b, Terada et al 1994). Mutants of $p70^{S6k}$ support

the idea that rapamycin is acting through this and not another kinase in the mTOR pathway (Jefferies et al 1997). Modification of S6, which lies within the mRNA-binding site of the ribosome (Jefferies & Thomas 1996), increases the affinity of the small ribosomal subunits for poly(U) (Gressner & van de Leur 1980). This may indicate that S6 modification results in more efficient interactions with TOP mRNAs via the polypyrimidine tract. Regulation may be achieved by a combination of these mechanisms. For instance, modification of S6 may enable 40S ribosomal subunits to overcome putative repressors. Alternatively, putative repressors may be targets for $p70^{S6k}$ phosphorylation, possibly inducing changes in protein-protein interactions that result in translation.

Non-Identical Twins

Each of the three subunits of eIF-4F has different iso-forms arising from different genes or alternative splicing, further enhancing the opportunities for translational regulation.

eIF-4A was first found to be encoded by two genes in mice and is also encoded by two genes in yeast (Nielsen & Trachsel 1988, Linder & Slonimski 1989); the two products may vary in abundance between cell types and growth states (Nielsen & Trachsel 1988, Williams-Hill et al 1997) and may form eIF-4F complexes with different cap affinities (Conroy et al 1990).

eIF-4G is encoded by two genes with nonidentical functions in *S. cerevisiae* (Goyer et al 1993, Tarun et al 1997). In yeast, the two genes have non-identical functions (Goyer et al 1993, Tarun et al 1997). Recently, a second eIF-4G gene, called eIF-$4G_{II}$, has been found in mammals (humans) (Gradi et al 1998). eIF-$4G_{II}$ shares many characteristics of the previously identified human eIF-4G factor but may be more resistant to cleavage during picornaviral infection (Gradi et al 1998), and the two forms differ in abundance among cell types (Gradi et al 1998).

Only one eIF-4E gene has been identified in yeast and flies. Recently, a second gene, *EIF4E2,* was described in humans (Gao et al 1998); however it is not clear whether the mRNA from this gene gives rise to protein. In *Drosophila*, alternative splicing generates two different iso-forms, called eIF-$4E_I$ and eIF-$4E_{II}$ (Lavoie et al 1996). Only eIF-$4E_I$ is detected in embryos, whereas both are detected in adults (Lavoie et al 1996).

IN CLOSING

We have tried to encompass many of the broad themes in the regulation of translation initiation, with special emphasis on its pertinence to the control of development and cell growth. We have highlighted important unresolved issues

and likely upcoming developments, as have other recent reviews in specific areas of consideration.

The enormous biological breadth of translational regulation and its emergence as a central means by which genes are controlled has led to an enhanced appreciation of its complexities. The effort to consolidate the rich biology with detailed understanding of the underlying biochemical mechanisms promises an exciting and surprising future.

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