Translational Control of Developmental Decisions

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At fertilization, the calm of oogenesis ends and the egg abruptly begins a flurry of activity. Many crucial steps—decisions concerning when and where to divide, specification of cell fates, and establishment of body axes—rely on materials the egg contains at that moment. In many animals, the first few hours of life proceed with little or no transcription. As a result, developmental regulation at these early stages is dependent on maternal cytoplasm rather than the zygotic nucleus. The regulatory molecules accumulated during oogenesis might, in principle, be of any type, including RNA and protein. It is clear that mRNAs present in the egg

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before fertilization—so-called maternal mRNAs—play a particularly prominent role in early decisions. Viewed from this perspective, it is not surprising that oocytes and early embryos display an impressive array of posttranscriptional regulatory mechanisms, controlling mRNA stability, localization, and translation.

The mechanisms by which translation of specific maternal mRNAs is controlled, and how those controls contribute to proper development, are the main focus of this chapter. Translational regulation is vital throughout development, in somatic as well as germ cells. The predominant mode of tissue-specific regulation in adult tissues is transcriptional; yet several of the examples we discuss hint that the importance of translational control may be currently underestimated, perhaps dramatically so.

One conclusion emerges exceptionally clearly from studies of translational control during early development: The region between the termination codon and the poly(A) tail—the 3′ untranslated region, or 3′UTR—is a key repository for the regulation of cytoplasmic mRNAs. Other regions of the mRNA will no doubt be found to play critical roles in developmental regulation, but thus far, the 3′UTR is preeminent.

Translational control is defined broadly in this chapter. Ideally, it is demonstrated by comparing the level of a specific, cytoplasmic mRNA to the rate of its translation. However, rates of translation can be difficult to measure directly in vivo. In several cases discussed in this chapter, only steady-state levels of the protein are known; however, translational control is inferred because the regulatory sequences responsible are located outside the protein-coding region. This argument is not airtight, however, and several examples suggest that caution is warranted.

In this chapter, we focus on translational controls that are vital for key developmental decisions. We do not discuss the role of modifications in the level or activity of translation factors, despite their importance in growth and differentiation (for review, see Gingras et al. 1999). Rather, we focus on mRNA-specific regulatory events and the roles of RNA–protein interactions. We first describe examples drawn from a range of biological contexts and organisms, with an emphasis on systems in which genetics has helped reveal biological function. The examples are not intended to be comprehensive, but to provide a reasonably detailed description of a small number of systems, selected to illustrate general points. Drawing on the examples, we consider possible molecular mechanisms and discuss emerging principles about the molecular circuitry of translational control and its regulatory niche.
TRANSLATIONAL CONTROL OF DEVELOPMENTAL EVENTS: SELECTED EXAMPLES

The diversity of developmental decisions in which translational regulation plays a key role is enormous, and the field is expanding explosively. We begin with three examples of regulatory cascades, drawing on the oocytes and embryos of *Caenorhabditis elegans* and *Drosophila melanogaster*.

Cell Fate and Patterning in the C. elegans Post-embryonic Germ Line: A Plexus of Controls

As development unfolds, cells assume specific fates and differentiate: For example, one cell becomes a neuron, whereas another becomes a lymphocyte. Although cell-fate regulators often act at the transcriptional level, they can also function at the level of translation. In this section, we describe how a plexus of translational controls regulates cell fates during the growth and differentiation of the *C. elegans* germ line.

Figure 1 summarizes the postembryonic development of the hermaphrodite germ line. *C. elegans* normally develops as either a hermaphrodite or a male, where a hermaphrodite is essentially a female that makes some sperm and then switches to oogenesis. During embryogenesis, two germ-line precursor cells arise from a single germ-line blastomere (Sulston et al. 1983); after the embryo hatches from its eggshell, these two germ-line precursor cells proliferate and differentiate as the animal progresses through four larval stages (L1, L2, L3, and L4) and enters adulthood. During this period of postembryonic development, a cluster of germ-line stem cells resides at the distal end of the growing germ-line tube (Fig. 1, yellow). Cells in meiotic pachytene are first observed during L3 in a “proximal” position (Fig. 1, green). During L4, the most proximal germ-line cells differentiate as sperm (Fig. 1, blue), and later in adulthood germ-line cells switch fates and become oocytes (Fig. 1, pink).

The regulation of germ-line proliferation, survival, and pattern of differentiation all appear to rely on translational controls. Best understood are two 3’UTR-mediated controls that influence the choice between spermatogenesis and oogenesis in the hermaphrodite germ line. The following sections review our current knowledge of these two controls as well as more preliminary studies of the controls governing germ-line proliferation and survival.
The Onset of Hermaphrodite Spermatogenesis: tra-2, GLD-1, and LAF-1

The \( \text{tra-2} \) sex-determining gene promotes female cell fates and is predicted to encode a large transmembrane protein, TRA-2A (Hodgkin and

A. Mitosis Meiotic pachytene Sperm Oogenesis

B. Germline development

L1 stage

L2 stage

L3 stage

\( \text{tra-2} \) 3'UTR

? / NOS-1
? / NOS-2
FBE/NOS-3

fem-3 3'UTR

Adult

Figure 1. (See facing page for legend.)
Brenner 1977; Kuwabara et al. 1992). Male development, including spermatogenesis in hermaphrodites, requires that tra-2 activity be repressed. Six dominant regulatory mutants, called tra-2(gf) (for gain-of-function), feminize the hermaphrodite germ line so that only oocytes are made (Doniach 1986; Schedl and Kimble 1988). The tra-2(gf) mutations therefore identify a site of regulation that is essential for hermaphrodite spermatogenesis. This site is of interest not only for its effect on cell fates, but also for its potential role in the evolution of controls that permit reproduction by hermaphroditism.

The tra-2(gf) mutations disrupt two tandemly repeated, cis-acting regulatory elements, called TGEs (formerly called DREs) (Fig. 2A). The TGEs are located in the tra-2 3'UTR and serve as translational repressor elements (Goodwin et al. 1993). Evidence supporting such a role includes polysome analyses of endogenous tra-2 mRNAs and a variety of experiments using chimeric reporter mRNAs (Goodwin et al. 1993; Jan et al. 1999). Although the TGEs only partially repress tra-2 mRNA translation, this is likely to be sufficient because the tra-2 locus is dosage-sensitive.

The GLD-1 protein appears to be a trans-acting repressor of tra-2 mRNA translation (Fig. 2B). GLD-1 belongs to the STAR family of RNA-binding proteins and is present in the hermaphrodite germ-line cytoplasm (Jones and Schedl 1995; Jones et al. 1996). The phenotype of gld-1 null mutants suggests that gld-1 regulates multiple aspects of hermaphrodite germ-line development, including promotion of hermaphrodite spermatogenesis and progression through meiosis during oogenesis (Francis et al. 1995a,b). In addition, gld-1 controls entry into the meiotic cell cycle (Kadyk and Kimble 1998). The conclusion that GLD-1 controls tra-2 translation rests on several lines of evidence (Jan et al. 1999). First, GLD-1 binds specifically to TGEs, in both yeast three-hybrid and in vitro

**Figure 1** Postembryonic development of the *C. elegans* germ line. (A) Pattern of cell fates in the adult hermaphrodite germ line. (Yellow) Mitotic germ-line stem cells; (green) region of germ line that has entered the meiotic cell cycle and is arrested in the pachytene stage of meiotic prophase I; (pink) oogenesis; (blue) spermatogenesis. (B) Larval development of the germ-line pattern. Color coding same as in A. (L1-4) First to fourth larval stages. Repression of the tra-2 sex-determining mRNA by laf-1 and GLD-1 is required for the onset of hermaphrodite spermatogenesis; repression of the *fem-3* sex-determining mRNA by FBF and NOS is required for the switch from spermatogenesis to oogenesis. The *mog* genes are also required for the sperm/oocyte switch, but it is not known whether their function is direct or indirect.
binding assays. Second, the level of TRA-2 protein is higher in \textit{gld-1} (null) mutants than in wild-type, without a commensurate increase in the level of \textit{tra-2} mRNA. Third, purified GLD-1 protein specifically represses the translation of TGE-bearing reporter RNAs in vitro. Finally, GLD-1 is a component of DRF, a TGE-specific RNA-binding activity present in crude worm extracts. These findings strongly support the hypothesis that GLD-1 is a translational repressor that acts through TGEs.

The \textit{laf-1} gene also influences TGE activity: loss-of-function mutations in \textit{laf-1} feminize the hermaphrodite germline and disrupt TGE-mediated regulation of reporter transgenes (Goodwin et al. 1997). However, \textit{laf-1} has not been cloned, and its molecular role remains unclear. Interestingly, \textit{laf-1}, like GLD-1, has a complex mutant phenotype, suggesting it too may control multiple mRNAs.

Translational control by TGEs has been broadly conserved in the animal kingdom. TGEs are found in the 3'UTRs of \textit{C. elegans tra-1}, \textit{C. brig-
gae tra-2, and the human oncogene GLI mRNAs (Jan et al. 1997). Moreover, TGEs repress translation in nematodes and mammalian cells (Jan et al. 1997), as well as in frog embryos (Thompson et al. 2000).

The mechanism by which translation is repressed by TGEs and GLD-1 is not understood. One clue is that repression is correlated with a change in poly(A) length, such that wild-type mRNAs possess shorter poly(A) tails than their mutant, derepressed counterparts. Similarly, TGEs promote deadenylation in frog embryos, where TGE-mediated repression requires a poly(A) tail (Thompson et al. 2000).

The Hermaphrodite Switch from Spermatogenesis to Oogenesis: fem-3, FBF, NOS, and MOG

The fem-3 sex-determining gene directs male development (Hodgkin 1986; Barton et al. 1987). In a story that is remarkably parallel to that of tra-2 described in the previous section, genetic selections identified a regulatory element in the fem-3 3′UTR that mediates fem-3 repression and the switch from spermatogenesis to oogenesis. A series of dominant regulatory fem-3(gf) mutations masculinize the hermaphrodite germ line: Sperm are made to vast excess and the switch to oogenesis never occurs (Barton et al. 1987). These fem-3(gf) mutations therefore identify a site of regulation essential for the sperm/oocyte switch.

The fem-3(gf) mutations carry lesions in the fem-3 3′UTR: 17 are single nucleotide changes in a 5-bp region (Fig. 2C) (Ahringer 1991; Ahringer and Kimble 1991). The mutated region is presumed to be part of a regulatory element called the point mutation element, or PME. Several lines of evidence support the idea that the PME is a translational control element. First, the fem-3(gf) mutations do not detectably affect transcription, splicing, or stability of fem-3 RNA, and the fem-3(gf) mutant RNAs possess a longer poly(A) tail than their wild-type counterparts (Ahringer and Kimble 1991). Second, the FBF and NOS repressors that mediate fem-3 repression are homologs of Pumilio and Nanos, which are translational repressors in Drosophila (see below). Finally, overexpression of the fem-3 3′UTR in transgenic animals masculinizes the hermaphrodite germ line, perhaps by titration of the repressor (Ahringer and Kimble 1991). This effect requires the promoter and the PME, suggesting that it relies on the regulatory site in the RNA product.

FBF is a component of the trans-acting repressor that acts through the fem-3 PME (Fig. 2D). C. elegans contains two FBF proteins, FBF-1 and FBF-2, that are 91% identical in amino acid sequence; their functions to date are indistinguishable, and so they are often referred to collectively as
FBF-1 and FBF-2 are both RNA-binding proteins of the Puf family (for Pumilio and FBF) and are present in the germ-line cytoplasm (Zhang et al. 1997). Animals lacking both *fbf-1* and *fbf-2* make only sperm and fail to switch into oogenesis, consistent with a role for FBF-1 and FBF-2 in *fem-3* repression. Supporting this biological evidence for the role of FBF in *fem-3* repression, both FBF-1 and FBF-2 bind the *fem-3* PME and interact specifically with wild-type, but not mutant, forms of the PME (Zhang et al. 1997). Intriguingly, FBF-deficient germ lines are small, suggesting a broader role for FBF in germ-line proliferation (Zhang et al. 1997).

Three NOS proteins are likely to act together with FBF to repress *fem-3* translation (Fig. 2D) (Kraemer et al. 1999). On the basis of genetic studies, the three *nos* genes appear to be redundant in their regulation of the sperm/oocyte switch. One NOS protein, NOS-3, interacts directly with both FBF-1 and FBF-2, whereas NOS-1 and NOS-2 do not. In one simple model, FBF and NOS-3 function together in a macromolecular complex to repress *fem-3* translation and to regulate the switch from spermatogenesis to oogenesis (Fig. 2D). In this view, recruitment of NOS-3 by FBF either stabilizes a regulatory complex on the *fem-3* 3′UTR or confers repression. However, this model cannot explain involvement of NOS-1 and NOS-2 in the sperm/oocyte switch, since neither protein detectably binds FBF. NOS-1 and NOS-2 may form complexes with FBF and the *fem-3* 3′UTR indirectly, or they may act with other Puf proteins in the *C. elegans* genome to effect *fem-3* repression (Kraemer et al. 1999).

In addition to FBF and NOS, six *mog* genes also are critical for PME-mediated repression and the sperm/oocyte switch (Graham and Kimble 1993; Graham et al. 1993; Gallegos et al. 1998). Hermaphrodites defective in any one of these *mog* genes fail to switch from spermatogenesis to oogenesis. Furthermore, the *mog* genes are required maternally for embryogenesis, suggesting that they may control not only *fem-3*, but other maternal mRNAs as well. Three *mog* genes have now been cloned, and their molecular identity is unexpected and provocative. All three encode members of the DEAH-family of ATP-dependent helicases: *mog-1*, *mog-4*, and *mog-5* encode the *C. elegans* homologs of yeast *PRP16*, *PRP2*, and *PRP22*, respectively (Puoti and Kimble 1999, 2000). What does this tell us about the molecular function of the MOG proteins? The yeast Prp2p, Prp16p, and Prp22p proteins are integral components of the splicing machinery (Burge et al. 1999). Although a role for the *mog* genes in splicing has not been excluded, no general defect in splicing is observed in *mog-1* null mutants (Puoti and Kimble 1999). The *mog* genes may therefore be evolutionarily related to the *PRP* genes but have acquired a different function. One speculative idea is that the MOG pro-
teins may direct conformational changes in a ribonucleoprotein (RNP) complex involved in PME-mediated repression. MOG-1 is nuclear (Puoti and Kimble 1999), whereas both FBF and NOS-3 are cytoplasmic. Perhaps MOG proteins act in the nucleus to establish an RNP structure that can be accessed by FBF and NOS proteins in the cytoplasm. Such an RNP remodelling function may be analogous to the role of various complexes that remodel chromatin in an ATP-dependent manner (see, e.g., Pazin and Kadonaga 1997). Indeed, one such chromatin remodeler, SWI-2, is a DEAD-box helicase and has been assigned to the same superfamily of helicases as the DEAH-box proteins (Eisen et al. 1995).

**TGE- and PME-mediated Repression in Somatic Tissues**

TGE- and PME-mediated repression of *tra-2* and *fem-3*, respectively, has crucial roles in regulating germ-line development. However, these controls also occur in somatic tissues. Thus, the strongest *tra-2*(gf) mutation feminizes the intestine of older adult males (Doniach 1986), and the strongest *fem-3*(gf) mutation masculinizes the soma of *tra-1*(gf) XO females (Schedl and Kimble 1988). Although these effects are relatively minor, both demonstrate that TGE- and PME-mediated repression can occur in somatic tissues. In support of this idea, reporter transgenes controlled by TGE- or PME-containing 3'UTRs are translationally controlled in somatic tissues (Goodwin et al. 1997; Gallegos et al. 1998). Certain regulators affect both somatic and germ-line controls: *laf-1* is required for TGE-mediated repression and the *mog* genes for PME-mediated repression in both tissues. In contrast, GLD-1 and FBF are expressed predominantly in the germ line, suggesting that other members of the STAR or Puf gene families may mediate the somatic controls. Therefore, the regulatory machineries for translational controls are found in somatic tissues and are likely to be used there, a theme that is underscored by control of *lin-14* and *lin-41* in hypodermal cells of *C. elegans* (see below).

**tra-2 and fem-3 3'UTR Controls and Patterning the Germ Line**

The generation of the hermaphrodite pattern of gametes—first sperm, then oocytes—relies on controls exerted by the *tra-2* and *fem-3* 3'UTRs. How are these controls coordinated to generate the germ-line pattern? Do they act alone or in concert with other modes of regulation? The nature of the TRA-2 and FEM-3 proteins and their regulatory relationship provides some insight into these questions. In particular, TRA-2 protein is itself a *fem-3* repressor (Hodgkin 1986). The intracellular domain of the TRA-2 membrane protein binds FEM-3, suggesting that *fem-3* repression by
TRA-2 may rely on sequestration of FEM-3 (Mehra et al. 1999). By this model, the relative abundance of these two proteins is predicted to be critical for fate specification. Consistent with that idea, the levels of TRA-2 and FEM-3 appear to be poised in a delicate balance in the hermaphrodite germ line: tra-2(gf) mutants are predicted to make excess TRA-2 protein, swamp out available FEM-3, and thereby promote oogenesis. Similarly, fem-3(gf) mutants are predicted to make excess FEM-3, resulting in free FEM-3 and hence spermatogenesis. Perhaps most important for this discussion, tra-2(gf); fem-3(gf) double mutants can possess a self-fertile hermaphrodite germ line with sperm made first and then oocytes (Barton et al. 1987; Schedl and Kimble 1988). In this regard, the strength of the individual tra-2(gf) or fem-3(gf) allele is critical. Thus, an animal carrying a strong tra-2(gf) allele and a weak fem-3(gf) allele often makes only oocytes, but an animal carrying both strong fem-3(gf) and tra-2(gf) alleles is usually self-fertile. It seems likely that when gf allelic strengths are matched, the levels of TRA-2 and FEM-3 are comparable, albeit higher than normal, and balance between these two regulatory proteins is restored. The ability of the tra-2(gf); fem-3(gf) double mutant to develop a self-fertile hermaphrodite demonstrates that these 3′UTR controls can be bypassed to generate the sperm/oocyte pattern. We suggest, therefore, that this pattern does not rely only on 3′UTR controls, and we speculate that an alternative mechanism acts in parallel to ensure the proper pattern of sperm and then oocytes.

One major unanswered question is how the translational regulators of the tra-2 and fem-3 mRNAs are controlled to obtain more FEM-3 early and more TRA-2 later. A simple hypothesis is that all tra-2 germ-line mRNAs are repressed during larval development, but that tra-2 mRNAs synthesized in adults are not repressed. This change might rely on a change in activity of the translational repressor or a change in the relative abundance of tra-2 mRNA to repressor. Similar arguments can be made for fem-3.

Pattern Formation in Drosophila: A Translational Cascade

In Drosophila, asymmetries become evident during oogenesis and early embryogenesis that foreshadow the anterior–posterior and dorsal–ventral axes of the mature organism. Translational controls are critical for establishing body axes (for review, see Wharton 1992; Curtis et al. 1995). Each of the four maternal patterning systems (St Johnston and Nüsslein-Volhard 1992) requires the translational control of one or more mRNAs, representative examples of which are provided in Table 1.
Table 1 Translational control in the four maternal patterning systems of *Drosophila*: Representative examples

<table>
<thead>
<tr>
<th>Maternal system</th>
<th>Translationally controlled mRNA</th>
<th>Role of protein product</th>
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<tbody>
<tr>
<td>Anterior</td>
<td><em>bicoid</em> (Driever and Nüsslein-Volhard 1988a,b)</td>
<td>Anterior determinant, activates genes required for head and thorax formation (Frohnhöfer and Nüsslein-Volhard 1986; Driever and Nüsslein-Volhard 1988a,b); also required to repress translation of <em>caudal</em> mRNA (Struhl 1989), which encodes a homeobox protein (Mlodzik et al. 1985)</td>
</tr>
<tr>
<td>Terminal</td>
<td><em>torso</em> (Casanova and Struhl 1989; Sprenger et al. 1989)</td>
<td>Cell-surface receptor that responds to localized extracellular ligand to generate terminal structures (Stevens et al. 1990; Martin et al. 1994)</td>
</tr>
<tr>
<td>Dorso-ventral</td>
<td><em>toll</em> (Gay and Keith 1992)</td>
<td>Cell-surface receptor that responds to localized extracellular ligand to generate ventral structures (Hashimoto et al. 1988; Stein et al. 1991; Morisato and Anderson 1994)</td>
</tr>
</tbody>
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**Coordinate Activation**

The maternal transcripts of several axis-determining genes are translationally dormant in oocytes but are activated soon after fertilization. This coordinate activation often requires cytoplasmic polyadenylation. mRNAs that encode key regulatory proteins for the anterior, terminal, and dorsal–ventral patterning systems, respectively—*bicoid*, *torso*, and *toll* (Table 1)—undergo polyadenylation concomitant with their activation.
For \textit{bicoid} mRNA, polyadenylation after egg deposition is critical for translation. Early evidence supporting this idea came from specific (Bic-D) mutant embryos that lack anterior structures. These embryos inappropriately express the posterior morphogen \textit{nanos} in the anterior, which blocks production of the anterior determinant \textit{bicoid}. The lack of Bicoid protein production was correlated with a \textit{bicoid} mRNA that has a shortened poly(A) tail (Wharton and Struhl 1991). Subsequent work directly showed that the polyadenylation of \textit{bicoid} mRNA is necessary for its activation (Sallés et al. 1994). Similar experiments have documented that translation of Toll protein, a crucial regulator of dorsal–ventral patterning, is also dependent on poly(A) addition (Schisa and Strickland 1998).

Unlike \textit{bicoid} mRNA, translational activation of mRNAs encoding \textit{nanos} and \textit{oskar}, two crucial posterior determinants, does not involve a detectable change in poly(A) tail length upon fertilization (Sallés et al. 1994; Lie and Macdonald 1999b). However, the ultimate effect of Nanos protein is to control the poly(A) status and translation of maternal \textit{hunchback} mRNA in the posterior (Wreden et al. 1997; considered in detail in the next section). Polyadenylation thus plays a critical role in the anterior, posterior, and dorsal–ventral patterning systems in \textit{Drosophila}.

\textit{Translational Cascades: Posterior Patterning}

Anterior–posterior patterning hinges in part on a regulatory cascade of translational control. A series of opposing protein gradients help determine the axis, and they are established by regulated mRNA localization and translation, events that are linked in the embryo.

Posterior development of the \textit{Drosophila} embryo is critical both for abdomen formation and for providing the correct environment for germ-cell development (Lehmann and Nüsslein-Volhard 1991). Both of these processes must be restricted to the posterior for normal development to occur; misexpression of the posterior determinant \textit{nanos} in the anterior is lethal to embryos (Wharton and Struhl 1989; Gavis and Lehmann 1992). The difficulties in restricting expression of proteins to the posterior in the absence of transcription illustrates two central features of translational control: regulation in space and in time.

Spatial regulation is necessary since certain mRNAs critical for posterior patterning are found not only in the posterior, but throughout the embryo. Repression of unlocalized mRNAs, coupled with the selective translation of posterior mRNA, ensures that protein production is region-specific. Temporal regulation is also required for these mRNAs. Once they
accumulate in the posterior of the oocyte/embryo, their expression must be coordinated with the onset of embryogenesis in the rest of the embryo.

To accomplish posterior regulation, Drosophila has evolved a mechanism that coordinates spatial and temporal controls. It is easiest to conceptualize this pathway by starting at the end point. The ultimate goal of the entire system is to repress the translation of maternal hunchback mRNA in the posterior. If the posterior determinant nanos is lacking, embryos die from a lack of posterior structures, but embryos lacking both nanos and maternal hunchback are viable (Hülskamp et al. 1989; Irish et al. 1989; Struhl 1989).

Posterior repression of maternal hunchback mRNA requires both Nanos and Pumilio (Fig. 3) (Lehmann and Nüsslein-Volhard 1991; Barker et al. 1992). Pumilio is uniformly distributed (Macdonald 1992) and thus cannot account for the restriction of the process to the posterior (Fig. 3A). Pumilio, a protein structurally related to FBF, binds specifically to nanos response elements (NREs) in hunchback mRNA's 3'UTR (Murata and Wharton 1995) and likely saturates hunchback mRNAs throughout the embryo (Zamore et al. 1999). However, Nanos protein expression is limited to the posterior (Wang and Lehmann 1991; Wang et al. 1994), and this localization underlies the asymmetric repression.

Pumilio recruits Nanos protein to a ternary complex containing the NREs (Fig. 3B) (Sonoda and Wharton 1999). The formation of the ternary complex is critical: Mutant forms of each component that do not regulate in vivo do not form the complex (Sonoda and Wharton 1999). The complex promotes repression and deadenylation of maternal hunchback mRNA in the posterior. Shortening of the poly(A) tail is one important factor in repressing its translation (Wreden et al. 1997).

Regions of the ternary complex that may contact the translation or deadenylation machinery have been identified: For example, specific Pumilio mutations permit complex formation but fail to repress (Sonoda and Wharton 1999). Recruitment of Nanos requires specific nucleotides within the NRE as well as specific amino acids in Pumilio, implying that either Pumilio or the RNA undergoes a conformational change upon forming the Pumilio/NRE complex, which then is recognized by Nanos (Fig. 3B) (Sonoda and Wharton 1999).

From the biological standpoint, these results raise the question of how Nanos protein, the localized posterior determinant, is restricted to the posterior. Although nanos mRNA is highly concentrated at the posterior pole, there are substantial levels of the transcript throughout the embryo (Bergsten and Gavis 1999). Translation of all the mRNA would be disastrous, since ectopic expression of Nanos protein is lethal (Ephrussi and
Figure 3  Spatial regulation of *Drosophila hunchback* mRNA by Nanos and Pumilio. (A) Distributions of *hunchback* mRNA and of Hunchback, Pumilio, and Nanos proteins in an early syncitial *Drosophila* embryo; anterior to the left, posterior to the right. *hunchback* mRNA is distributed throughout the embryo, but the protein appears only in the anterior portion (purple). Pumilio protein (blue) is uniformly distributed, while Nanos protein (green) is present in a gradient emanating from the posterior pole. (B) Pumilio (blue), Nanos (green), and the NRE (red) interact to form a tertiary complex that represses the mRNA. NRE-bound Pumilio is insufficient for repression, leaving *hunchback* mRNA on, and promoting anterior development. Recruitment of Nanos results in the formation of a tertiary complex that represses *hunchback* mRNA and permits posterior development. Formation of the tertiary complex involves an alteration in either the NRE, Pumilio, or both, and is represented by the altered shape of Pumilio in the tertiary complex.
Lehmann 1992; Gavis and Lehmann 1992; Smith et al. 1992). However, translation of the unlocalized mRNA is repressed (Gavis and Lehmann 1994). This repression depends on the uniformly distributed protein, Smaug (Dahanukar et al. 1999), which binds to translational control elements in the 3′ UTR of nanos mRNA (Smibert et al. 1996; Dahanukar et al. 1999). Once in the posterior, translational repression of nanos mRNA by Smaug is overcome by activation of the mRNA by localized Oskar protein (Dahanukar et al. 1999). Thus, nanos mRNA is activated in the embryo only in the correct locations at the correct time.

Working backward in the cascade (Fig. 4) prompts the following question: How is the localization of Oskar protein accomplished? The situation here is analogous to regulation of nanos: Unlocalized oskar mRNA is repressed by repressor proteins that bind to the Bruno response element (BRE) in the 3′ UTR (Kim-Ha et al. 1995; Lie and Macdonald 1999a; Castagnetti et al. 2000). However, posterior localization does not automatically trigger oskar mRNA’s translation. Rather, a 5′ region of oskar mRNA is absolutely required to relieve BRE-mediated repression (Gunkel et al. 1998). This activator region is located between the 5′-most AUG and the second AUG of oskar mRNA and does not appear to bind Bruno itself. The activator region is not required for the translation of mutant oskar mRNAs in which the BREs have been deleted or inactivated. It only functions at the posterior pole, suggesting that at least one limiting component of an active derepressor machinery must be located in this region of the oocyte cytoplasm.

Bruno was the first protein found that acts as a repressor of oskar mRNA (Kim-Ha et al. 1995), but it appears to have collaborative partners. Apontic can bind both Bruno protein and to the BREs in the oskar mRNA 3′ UTR, and there are genetic interactions between the apontic and bruno genes (the aret locus) (Lie and Macdonald 1999a). A 50-kD protein binds both the 5′ end and the 3′ BREs of oskar mRNA, and BRE mutants that bind Bruno but not this 50-kD protein have reduced translational repression (Gunkel et al. 1998). Finally, mutants in the Bic-C gene, which encodes an RNA-binding protein, prematurely translate oskar mRNA (Saffman et al. 1998). Thus, it appears that a multicomponent protein assemblage may regulate translation of unlocalized oskar mRNA.

In the posterior, activation of oskar mRNA translation also is complex. There are several collaborators: Oskar protein itself, Vasa (Markussen et al. 1997), Orb (a CPEB homolog; Chang et al. 1999), Staufen (St Johnston et al. 1991), and Aubergine (Wilson et al. 1996).
Figure 4 Cascades of translational regulation and localization that control formation of the anterior–posterior axis in *Drosophila*. Arrowheads depict positive events, and blunt ends indicate repressive events. Citations are provided in the text. The events depicted occur either in the growing oocyte or in the 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 and repressed during oogenesis, requires Staufen protein. Bicoid protein then represses the translation in the anterior of uniformly distributed *caudal* mRNA. In the posterior, the initial event is localized expression of Oskar protein. Translation of *oskar* mRNA during its transit from the anterior end of the oocyte is repressed by Bruno in collaboration with Apontic, Bic-D, and p50 (see text). Its localization and activation at the posterior requires Staufen, Vasa, and Oskar protein itself. *nanos* mRNA is also localized to the posterior pole, a process that requires the presence of Oskar protein. Its mis-localized expression is prevented by Smaug, and its activation in the posterior requires Vasa. *hunchback* mRNA is present throughout the embryo, as is Pumilio. Posteriorly localized Nanos acts in concert with Pumilio to repress the *hunchback* mRNA in the posterior. We include in the figure 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.

Vasa is an ATP-dependent RNA helicase (Liang et al. 1994), suggesting that its effects may be directly on *oskar* mRNA, altering an RNA structure or promoting RNA–protein transactions. Other proteins required to
activate oskar mRNA expression act indirectly through their role in mRNA localization. These include gene products that affect cytoskeletal organization and function (see, e.g., Cappuccino and Spire; Ephrussi et al. 1991; Theurkauf 1994; Kim-Ha et al. 1995), as well as proteins that interact with specific mRNAs (e.g., Staufen; St Johnston et al. 1991). Thus, with both nanos and oskar, the regulatory pathway involves repression in all regions except the posterior, and a separate mechanism to ensure activation in the posterior (Fig. 4).

As a general consideration, repression of unlocalized mRNA translation is the most parsimonious way to achieve posterior specific protein expression. If all mRNA molecules were translated equivalently, a trail of protein would be produced that would have to be either transported posteriorly or destroyed: The spatial control mechanisms provide an intuitively satisfying solution. However, if the oocyte relied on this mechanism alone, with repressor molecules excluded from the posterior, once the mRNA reached this region its translation would commence. A separate activation mechanism in the posterior gives the embryo the temporal control needed to coordinate the patterning systems.

Parallel Cascades in the Anterior and Posterior

As if this complexity were not enough, Bicoid protein, in addition to its role as a transcriptional factor, is required for translational repression of caudal mRNA, another mRNA important in axis formation (Fig. 4). In the absence of bicoid activity, the normal gradient of Caudal protein—low in the anterior to high in the posterior—is disrupted, with high Caudal now found at the anterior as well (Macdonald and Struhl 1986; Mlodzik and Gehring 1987; Driever and Nüsslein-Volhard 1988b). Bicoid protein binds to caudal mRNA, and this interaction appears to be essential for translational repression (Dubnau and Struhl 1996; Rivera-Pomar et al. 1996; Chan and Struhl 1997; Niessing et al. 1999). The key regulatory elements lie in the 3′UTR of caudal mRNA. Remarkably, the homeodomain region of Bicoid protein is required to bind both to caudal mRNA and to DNA targets in its role as transcriptional activator.

The regulation of the anterior–posterior axis thus involves two parallel cascades of translational control at opposite ends of the embryo (Fig. 5). Many of the key players—bicoid, nanos, caudal, and hunchback—initially are translationally dormant and are activated only after fertilization. At the anterior, newly synthesized Bicoid protein represses caudal mRNA; at the posterior, Nanos protein represses hunchback mRNA. Ultimately, the posteriorly localized hunchback mRNA is destroyed (Tautz and Pfeifle 1989). Thus, this web of interactions establishes opposing gradients of Hunchback and Caudal proteins.
Translational Controls in the *C. elegans* Early Embryo

In *C. elegans*, several key regulators of body axes and blastomere fates are controlled translationally. Some of the controls parallel analogous controls in *Drosophila*; others do not. Perhaps the most important similarity between *Drosophila* and *C. elegans* is the presence of cytoplasmic RNA-enriched granules that are localized to the future posterior of the fertilized zygote, and then segregated into germ-line precursor cells as they are born. These granules, called P granules in *C. elegans*, polar granules in *Drosophila*, and germ plasm in *Xenopus*, appear to be central hubs for translational control and contain at least some related RNAs and proteins.

Figure 6A introduces the *C. elegans* early embryo. The first division establishes the anterior–posterior axis, to a first approximation, and the second division similarly establishes the dorsal–ventral axis. We refer readers to a recent review of *C. elegans* embryogenesis for details (Schnabel and Priess 1997).

*Translational Control of glp-1*

The *glp-1* gene encodes a Notch-related receptor critical for a cascade of cell–cell interactions specifying dorsal–ventral and left–right axes of the *C. elegans* embryo (for review, see Schnabel and Priess 1997). As shown in Figure 6B, GLP-1 protein first appears at the 2- to 4-cell stage in anterior,
but not posterior, blastomeres; in contrast, *glp-l* maternal mRNA is uniformly distributed at this time (Evans et al. 1994). Therefore, *glp-l* mRNA must be subject to at least two distinct translational controls. One is temporal: *glp-l* mRNA is translationally silent in oocytes and the fertilized one-cell embryo, but its translation is activated after the first embryonic division. The second is spatial: *glp-l* is translated only in anterior blastomeres and is kept silent in posterior blastomeres. The elements that mediate both controls reside in the *glp-l* 3′UTR. A U-rich region at the 3′ end of the 3′UTR is required to repress translation in oocytes, and a centrally located stretch of 39 nucleotides is responsible for spatial regulation (Evans et al. 1994). At present, the trans-acting factors controlling *glp-l* translation are not known. Such trans-acting factors might include posterior repressors, anterior activators, or both. To date, the only genes known to be essential for the asymmetric expression of *glp-l* are the *par* genes, which are critical for asymmetry of the embryo per se (Crittenden et al. 1997).

**Translational Control of pal-1 mRNA by MEX-3**

The PAL-1 homeodomain transcription factor is required for certain posterior fates; it is expressed in posterior blastomeres, largely due to translational regulation conferred by its 3′UTR (Hunter and Kenyon 1996). MEX-3 is a KH-domain RNA-binding protein that may repress *pal-l* mRNA. The location of MEX-3 protein within the embryo complements that of PAL-1 protein (Fig. 6C). MEX-3 is first detected in the cytoplasm of developing oocytes, where it is expressed at high levels, and becomes enriched in AB and its daughters after fertilization (Draper et al. 1996). In contrast, PAL-1 protein is detected for the first time at the 4-cell stage and then only in EMS and P2 (Hunter and Kenyon 1996). Because *pal-l* maternal RNA is evenly distributed in developing oocytes and early embryos, it must be controlled both temporally and spatially, a theme also observed for *glp-l* (see above). In mex-3 mutants, *pal-l* mRNA is released from those controls: It is expressed early and uniformly, being present throughout oocytes and early embryos (Hunter and Kenyon 1996). A reporter RNA bearing a *pal-l* 3′UTR is expressed in a *pal-l*-like pattern and is similarly derepressed in mex-3 mutants (Hunter and Kenyon 1996). The simplest interpretation is that MEX-3 acts directly through regulatory elements in the *pal-l* 3′UTR to repress translation of the *pal-l* mRNA.

**Translational Control of apx-1**

APX-1 is a transmembrane protein that serves as a ligand for the GLP-1 receptor. In the early embryo, APX-1 signals from the P2 blastomere to
its neighbor ABp and thereby induces the normal ABp fate (Mello et al. 1994). The \textit{apx-1} maternal mRNA is uniformly distributed in early embryos, but APX-1 protein is found only in specific blastomeres (Fig. 6D) (Mickey et al. 1996). \textit{mex-1} and \textit{pos-1} genes may act in a cascade to control the translation of \textit{apx-1} mRNA (Tabara et al. 1999). MEX-1 and POS-1 are both cytoplasmic proteins that contain two copies of a CCCH “finger” motif (Guedes and Priess 1997; Tabara et al. 1999). A biochemical function for the CCCH motif is unknown, but several proteins with CCCH motifs have been implicated in different aspects of RNA metabolism (Zhang et al. 1992; Barabino et al. 1997; Carballo et al. 1998), sug-

![Diagram](attachment:image.png)

Figure 6 (See facing page for legend.)
gesting that it binds RNA. *mex-1* mutant embryos fail to produce both POS-1 and APX-1, and *pos-1* mutant embryos lack APX-1. These findings are consistent with *mex-1* working upstream to control *pos-1* translation, with POS-1 in turn activating translation of *apx-1* mRNA. The temporal expression of the three proteins is also consistent with a regulatory cascade (Fig. 6D). MEX-1 is first detected in oocytes (Guedes and Priess 1997), whereas POS-1 is initially detected at low levels in 1-cell embryos (Tabara et al. 1999). APX-1 is the last protein produced, as it is first detected in P2 of the 2-cell embryo (Mickey et al. 1996). Although these data suggest a linear pathway, it is also possible that MEX-1 and POS-1 act in separate pathways to affect APX-1 expression. Mutations that reduce MEX-1 and POS-1 activities result in different phenotypes, indicating that the two proteins do not only affect APX-1 expression but that they likely have different targets or function at different developmental times. Indeed, MEX-1 is also required for PIE-1 localization, which is essential for germ-line specification (Guedes and Priess 1997; see below).

*Translational Control in Germ-line Blastomeres*

Specification of the germ-line precursor cells in the early *C. elegans* embryo relies on a combination of transcriptional and posttranscriptional controls. These germ-line precursor cells arise by the segregation of germ-line blastomeres, P1, P2, P3, and P4, in consecutive divisions. The

*Figure 6* Translational controls and patterning in the early *C. elegans* embryo. All embryos are oriented with anterior to left and posterior to right. Blastomere names are provided in *A* only; in *B–E*, nuclei are depicted as a circle within the cell. P granules are represented as a cluster of black dots at the posterior end of fertilized zygotes, P1 blastomeres at the 2-cell stage, and P2 blastomeres at the 4-cell stage. (*A*) The fertilized zygote harbors P granules at the posterior end. The 2-cell embryo possesses one larger blastomere, AB, and one smaller one, P1. The 4-cell embryo harbors the daughters of AB, which are called ABa and ABp, and the daughters of P1, which are called EMS and P2. The AB blastomere generates somatic cells that are, for the most part, anterior; the EMS blastomere generates somatic cells, including the intestine, muscle, and hypodermis; P1 and P2 both carry P granules. (*B–D*) Diagrams showing distribution of GLP-1 (*B*), MEX-3 and PAL-1 (*C*), and MEX-1, POS-1, and APX-1 (*D*) proteins at individual stages during early embryogenesis. Maternal mRNAs encoding GLP-1, PAL-1, and APX-1 are uniform in oocytes and early embryos; maternal mRNAs encoding MEX-3, MEX-1, and POS-1 are uniform in oocytes and 1-cell embryos, but become asymmetrically distributed in late-stage 1-cell embryos (MEX-3, POS-1) or later (MEX-1).
RNA-rich P granules are localized to these germ-line blastomeres and are critical for the germ-line fate (Kawasaki et al. 1998; Seydoux and Strome 1999). The germ-line fate relies in part on repression of polymerase II-mediated transcription in germ-line blastomeres by the PIE-1 protein (Seydoux and Fire 1994; Seydoux et al. 1996; Seydoux and Dunn 1997; Batchelder et al. 1999). However, the germ-line fate also appears to rely on posttranscriptional, and likely translational, controls. Specifically, the putative translational regulator pos-1 is required for specifying the germ-line fate (Tabara et al. 1999). Furthermore, several proteins predicted to control RNA activity or to bind RNA are colocalized with P granules. These include GLH-1 and GLH-2, two homologs of Drosophila Vasa that contain DEAD-box helicase motifs (Gruidl et al. 1996); PGL-1, a protein bearing multiple RGG boxes (Kawasaki et al. 1998); the GLD-1 translational regulator (Jones and Schedl 1995; Jan et al. 1999); MEX-1 (Guedes and Priess 1997); MEX-3 (Draper et al. 1996); POS-1 (Tabara et al. 1999); and PIE-1 (Mello et al. 1996). Finally, maternal RNA encoding the translational regulator NOS-2 colocalizes with P granules (Subramaniam and Seydoux 1999). Although the functions of these various proteins and RNAs are not yet fully understood, one idea is that the P granules serve as an RNA control hub in the germ-line blastomere.

The Early Embryonic Cell Cycle and Meiotic Maturation

A dramatic transition from cell cycle arrest to mitotic cleavage occurs upon fertilization. In some species, it is immediately preceded by completion of the meiotic cell cycle, referred to as oocyte maturation. To regulate these transitions, eggs of many species contain mRNAs that encode cell cycle regulators, such as cyclins and cyclin-dependent kinases (CDKs). Control of their translation helps orchestrate the transition from quiescence to meiosis and mitosis, as does their posttranslational modification. For the purposes of this discussion, it is necessary only to know that cyclins and CDKs form complexes that promote the cell cycle. Activation of the complex requires dephosphorylation of the kinase at certain positions by the CDC25 phosphatase, and lack of phosphorylation by the WEE1 kinase.

Translation of cyclin mRNAs appears to be important for proper postfertilization mitoses in many species, and perhaps for meiotic maturation as well. The analysis of cyclin regulation and function is complicated by
the mixed contributions of proteolysis and regulated synthesis to changes in cyclin protein levels, and by the presence of multiple cyclins with overlapping roles. Nevertheless, intensive studies of the translational control of these critical regulators have been informative.

Frog oocytes contain mRNAs encoding several different cyclins. *Xenopus* cyclin A1, B1, and B2 mRNAs are activated at different times during maturation, and to different extents (Kobayashi et al. 1991). Each mRNA receives poly(A) concomitant with its translational stimulation (Sheets et al. 1994). To identify signals involved in these controls, chimeric mRNAs were injected that contained each 3′UTR joined to a translational reporter. The different cyclin 3′UTRs determined when, and how much, translation was stimulated during oocyte maturation. Invariably, translational stimulation required poly(A) addition (Sheets et al. 1994). Thus, 3′UTRs, by controlling polyadenylation, can impose different patterns of translation, stimulating translation at different times and to different extents. Similar results have been obtained with a variety of other mRNAs unrelated to the cell cycle (Chapter 27).

Full translational control of cyclin B1 mRNA appears to be achieved through two separate but related mechanisms: translational repression and polyadenylation. Repression of cyclin B1 mRNA in resting oocytes apparently requires specific sequences in the 3′UTR that overlap with (and may be identical to) those that are required for its subsequent polyadenylation and activation (see below). The role of polyadenylation in derepression of the endogenous mRNA is uncertain, since cyclin B1 protein levels can increase when polyadenylation is blocked by inhibition of the cyclin/CDK complex (Frank-Vaillant et al. 1999), yet injected mRNAs require a poly(A) tail to be derepressed (de Moor and Richter 1999; Barkoff et al. 2000).

Regulation of maternal cyclin mRNAs at the translational level may be common. In *Drosophila* embryos, for example, maternal cyclin B mRNA is localized to pole cells (the presumptive germ line) and is repressed until mitoses resume in the developing gonad, well after fertilization (Dalby and Glover 1993). The regulatory elements responsible for translational control and localization reside in its 3′UTR (Dalby and Glover 1993). *Drosophila* cyclin B1 mRNA is not repressed in *nanos* or *pumilio* mutants: The precocious expression that results may underlie the failure of *nanos* mutant animals to slow the cell cycle and enter mitotic quiescence at the start of germ-cell development (Asaoki-Taguchi et al. 1999; Deshpande et al. 1999).

In surf clams and sea urchins, certain cyclin mRNAs are repressed during oogenesis, then activated dramatically at fertilization, when they
receive poly(A) (Rosenthal et al. 1980; Standart 1992). The common regulation of cyclin mRNAs presumably reflects their role after the cell cycle resumes at fertilization, and the deleterious consequences of their premature expression. Other maternal mRNAs that participate in cell-cycle-related events, such as DNA replication and the synthesis of DNA precursors, are also subject to translational control (e.g., histones, ribonucleotide reductase, HGPRT; for review, see Standart 1992).

Proteins that regulate CDK activity by covalent modification are also controlled at the translational level. For example, translation of *Drosophila* CDC25 (*twine*), a phosphatase required to activate CDK2, requires *boule*, an RNA-binding protein of the DAZ family. In the absence of either protein, *Drosophila* oocytes arrest in meiosis. In *Xenopus*, CDC25 levels are constant through maturation and early development, but the level of the inhibitory kinase, WEE1, increases during meiosis (Murakami and Vande Woude 1998). This likely reflects its translational activation.

c-mos mRNA

The c-mos proto-oncogene encodes a protein kinase that is critical in the control of vertebrate meiosis and the early embryonic cell cycle (for review, see Yew et al. 1993; Vande Woude 1994; Gebauer and Richter 1997; Sagata 1997). Consistent with these roles, c-mos mRNA is normally found only in the germ line. In frog oocytes, removal of c-mos mRNA prevents maturation, whereas its overexpression induces it (Sagata et al. 1988, 1990). Female mice lacking a functional c-mos gene display reduced fertility, as well as ovarian cysts and teratomas, consistent with a crucial role in oocyte growth (Colledge et al. 1994; Hashimoto et al. 1994).

In frogs, translation of c-mos mRNA apparently increases during oocyte maturation (Sagata et al. 1988). Fox et al. (1989) noted, by sequence inspection, that *Xenopus* c-mos mRNA contained signals that could cause cytoplasmic polyadenylation, and proposed that cytoplasmic polyadenylation of c-mos mRNA therefore might be a critical control point in meiotic maturation. This hypothesis has since gained substantial support. c-mos mRNA receives poly(A) during maturation. Furthermore, the c-mos 3'UTR contains signals sufficient for cytoplasmic polyadenylation (Paris and Richter 1990; Sheets et al. 1994), and when linked to a reporter, stimulates translation during maturation (Sheets et al. 1994). Removal of cytoplasmic polyadenylation signals from endogenous c-mos mRNA, achieved by targeted RNase H cleavage, prevents maturation
The amputated mRNA, lacking its polyadenylation signals, is stable. Maturation, and the increase in c-mos protein levels, can be restored by injection of synthetic c-mos mRNA carrying polyadenylation signals, or of a "prosthetic RNA" that brings polyadenylation signals to the amputated endogenous mRNA by base-pairing (Sheets et al. 1995). These experiments strongly argue that polyadenylation, or the presence of a poly(A) tail, is critical in the activation of c-mos mRNA.

These studies do not argue that polyadenylation is the only process triggered by progesterone that is critical for c-mos activation. The mere presence of a long poly(A) tail, provided by a prosthetic RNA, is sufficient to activate amputated c-mos mRNA after addition of progesterone. This rescue by poly(A) is length-dependent: 130 adenosines rescue, whereas 30 do not, corresponding reasonably well with the lengths of poly(A) on c-mos mRNA before and after maturation (Barkoff et al. 1998). However, in the absence of progesterone, the presence of a long poly(A) tail does not elevate c-mos protein levels, demonstrating that a long tail alone is insufficient to activate. c-mos protein levels are controlled not only by changes in translation of c-mos mRNA, but also by regulated proteolysis, as is the case with certain cyclins (Nishizawa et al. 1993).

Cytoplasmic polyadenylation of c-mos mRNA is also required for the maturation of mouse oocytes (Gebauer et al. 1994). In mouse oocytes, removal of the polyadenylation signals from c-mos mRNA does not block completion of first meiosis as in frogs. Rather, these oocytes complete the first meiotic division but fail to progress normally to meiosis II. This phenotype resembles that observed in oocytes derived from females homozygous for a disrupted c-mos gene, which undergo parthenogenetic activation after completing first meiosis (Colledge et al. 1994; Hashimoto et al. 1994).

Recent results suggest that cytoplasmic polyadenylation elements (CPEs) are bifunctional, first repressing translation prior to maturation, and later activating. The requirement for polyadenylation may sometimes be simply to prevent removal of the tail due to cytoplasmic deadenylation: For example, it appears that tPA mRNA needs a short poly(A) tail, rather than poly(A) extension per se, to be activated during maturation (Stutz et al. 1998).

In addition to c-mos, translational control of at least one other mRNA is likely to be critical in activating maturation in response to progesterone (Nebreda et al. 1995; Barkoff et al. 1998; Frank-Vaillant et al. 1999). Indeed, the translation of cyclin B1 (Frank-Vaillant et al. 1999) and Ringo/Speedy (Ferby et al. 1999; Lenormand et al. 1999) proteins may be critical in inducing maturation.
Perspective

The idiosyncrasies of cell cycle control in the early embryo vary widely among species. c-mos, for example, appears to be a vertebrate adaptation (Yew et al. 1993; Gebauer and Richter 1997; Sagata 1997). It is unclear whether there is a widespread and conserved strategy of translational control of a cell cycle component—for example, a common regulator and mRNA target among many species. The apparent conservation of DAZ function in regulating meiosis in both vertebrates and invertebrates suggests this may be such a case, as may control of certain of the cyclins (see above). Regardless, it is clear that many species exploit translational control of specific cell-cycle related mRNAs to help thrust the idling egg through the completion of meiosis and the onset of mitotic cleavage.

Temporal Control of Developmental Events: RNA Regulators

Translational controls are not restricted to maternal mRNAs and early embryos. Indeed, a particularly provocative form of translational control directs progression through the life cycle in the somatic tissues of the nematode C. elegans. Normally, C. elegans passes through four distinct larval stages, called L1, L2, L3, and L4, to reach maturity as adults (Fig. 1). This progression depends on several “heterochronic” genes, including lin-14 and lin-41 (for review, see Ambros and Moss 1994). The key regulators of these two mRNAs appear to be short, repressive RNAs.

lin-14 is required for L1-specific events (Ambros and Horvitz 1984). LIN-14 protein is abundant at the L1 stage, but rare at later stages (Ruvkun and Giusto 1989); in contrast, lin-14 mRNA is equally abundant throughout larval development (Wightman et al. 1993). Two lin-14(gf) mutants, which disrupt the 3′UTR and cause lin-14 protein levels to remain high throughout larval development (Ambros and Horvitz 1984; Ruvkun et al. 1989; Ruvkun and Giusto 1989; Wightman et al. 1991), reiterate patterns of cell lineage and cell fate normally associated with the L1 larval stage. Temporal repression of lin-14 at the L1 and later stages requires sequences in its 3′UTR, as well as the lin-4 gene product (Ambros 1989; Arasu et al. 1991; Wightman et al. 1991, 1993; Lee et al. 1993). Animals lacking lin-4 activity reiterate L1-specific events (Chalfie et al. 1981), as do lin-14(gf) mutants. Remarkably, lin-4 encodes two short RNAs (22 and 61 nucleotides) with no apparent protein-coding capacity. Instead, both RNAs are complementary to each of seven conserved elements present in lin-14 mRNA, prompting the proposal that lin-4/lin-14 RNA duplexes cause translational repression (Fig. 7A) (Lee
et al. 1993; Wightman et al. 1993). Indeed, the regions of complementarity are required for repression in vivo, and for base-pairing between the RNAs in vitro (Fig. 7B) (Ha et al. 1996). lin-28, another gene that regulates timing of early 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).

A later temporal transition in cell fates, from L4 to adult, requires another set of heterochronic genes, including lin-41 and let-7. lin-41 encodes a RING finger protein of the RBCC subfamily (Slack et al. 2000). Lack of lin-41 leads to precocious adult fates at the L4 stage without affecting the L1 to L2 transition (Abrahante et al. 1998). let-7 mutants exhibit a reciprocal phenotype, reiterating L4-stage events in adults. Furthermore, increased let-7 dosage causes precocious expression of adult events in L4-stage animals (Reinhart et al. 2000). The 3’UTR of lin-41 causes repression of a reporter gene at the L4/adult transition. These data suggest that let-7 represses lin-41 via its 3’UTR (Fig. 7C).

The molecular identity of let-7 reveals startling parallels with lin-4. let-7 encodes a 21-nucleotide RNA without an open reading frame that is complementary to two segments of the lin-41 3’UTR. The structures of the two potential let-7/lin-41 duplexes are similar (Fig. 7D). Although base-pairing has not been demonstrated directly, the complementary sites in the lin-41 3’UTR greatly enhance repression of a transgene at the adult stage, and this repression requires let-7 (Reinhart et al. 2000).

In the simplest view, the early and late developmental transitions are triggered just by the expression of the regulatory RNAs (Fig. 7B). lin-4 RNA increases in abundance early, as lin-14 and lin-28 are repressed (Feinbaum and Ambros 1999). Similarly, abundant let-7 RNA is first detected at the L4 stage, as lin-41 is extinguished (Reinhart et al. 2000).

Although regulatory RNAs are critical here, they may not be the whole story. The secondary structures of each potential lin-4/lin-14 hybrid, and the sequence of the “looped-out” regions, are quite similar (Fig. 7B). In particular, they include a bulged C residue whose presence and identity are critical for repression, and which may be part of a protein-binding site (Ha et al. 1996). Similarly, the two putative let-7/lin-41 duplexes are closely related, including bulges with similar sequences (Fig. 7D). Thus the lin-4/lin-14 and let-7/lin-41 interactions may create two distinct RNA structures that are specifically discriminated by proteins. Put another way, the short RNAs create new RNA structures in their targets. Perhaps ATP-dependent RNA helicases implicated in translational regulation (e.g., the Mog and Vasa proteins) act similarly, creating new binding sites for repressor proteins.
The biochemical mechanism by which base-pairing leads to repression is unknown, but appears not to be “simple” interference with initiation: Neither the rate of synthesis of lin-14 mRNA, its state of polyadenylation, its apparent abundance in the cytoplasm, nor its distribution in a polysome profile changes in response to the accumulation of lin-4 RNA. These findings suggest that association of lin-4 RNA with the 3’ UTR of lin-14 mRNA inhibits a step(s) after initiation, such as translational elongation and/or the release of stable LIN-14 protein (Olsen and Ambros 1999).

The identification of the lin-4 and let-7 repressors is unambiguous and emphasizes the importance of considering RNA in searching for

Figure 7 (See facing page for legend.)
activities or genes that repress. *lin-4* and *let-7*, and their apparent noninitiation mode of repression, are not likely to be mere deviants, but rather harbingers of other regulatory RNAs and widely used mechanisms (Wickens and Takayama 1994).

**Dosage Compensation in *Drosophila***

Dosage compensation balances the transcriptional output of the two female and the single male X chromosomes. In mammals, inactivation of one of the two female X chromosomes implements dosage compensation by adjusting the transcriptional output to that of the male. In *Drosophila*, the transcriptional output from the single male X chromosome is approximately doubled, thus allowing an equal level of expression of X-linked genes in males and females (Baker et al. 1994; Kelley and Kuroda 1995).

The major dosage compensation pathway in *Drosophila* is controlled by a heteromeric complex consisting of the four proteins Maleless (Mle) and Male-Specific Lethal (MSL)-1, -2, and -3. The MSL complex associates with numerous sites along the male X chromosome and probably stimulates transcription by promoting histone acetylation. Although three of the four subunits are expressed in both sexes, MSL complex formation

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**Figure 7** Repressive RNAs in *C. elegans*: *lin-4* and *let-7*. *(A)* Model for the role of *lin-4* in translational repression of *lin-14* mRNA. (*Shaded circles*) Ribosomes; *(thin lines)* *lin-14* mRNA; *(small open rectangles)* putative regulatory sites to which *lin-4* RNA may bind; *(thick black arrow)* *lin-4* RNA (arrowhead is at the 3' end of the short [21 nucleotides] *lin-4* RNA). The *lin-14 3'UTR* possesses 7 conserved elements (1–7) that are likely to be translational regulatory elements. During the L1 larval stage, *lin-14* is translated; then the translational repressor, *lin-4*, associates with regulatory elements and *lin-14* becomes translationally repressed. mRNA not drawn to scale. *(B)* Potential hybrids between *lin-14* mRNA and *lin-4* RNA. *(Open rectangles)* Elements in *lin-14* mRNA; *(black rectangles)* *lin-4* RNA. The location of a point mutation in *lin-4* that reduces its activity is indicated by a triangle in hybrids 1, 2, 4, and 6. In addition to the 21-nucleotide *lin-4* RNA, a longer (~60 nucleotides) RNA also is present, with additional sequence beyond the 3' end of the 21-nucleotide RNA. The 3' of the short (21 nucleotides) RNA is indicated by a vertical line; any additional complementarity in the longer *lin-4* RNA to the *lin-14* sites is indicated. Note that only a subset of these structures may be needed for repression. *(C)* Model for the role of *let-7* RNA in translational repression of *lin-41* mRNA. See A for key. *(D)* Potential hybrids between *lin-41* and *let-7* RNA. The location of a triangle indicates the position of a point mutation that reduces its activity.
is restricted to males by the male-specific expression of the MSL-2 protein. Experimental expression of MSL-2 in females triggers MSL complex assembly, showing that MSL-2 is the limiting subunit (Bashaw and Baker 1995; Kelley et al. 1995).

Expression of MSL-2 is under negative control. In the female, it is inhibited by the female-specific RNA-binding protein Sex-Lethal (SXL). SXL expression is limited to female flies by a combination of transcriptional control and autoregulated splicing (for review, see Gebauer et al. 1997). The SXL protein is composed of two ribonucleoprotein consensus motifs (RRMs) and a glycine/asparagine-rich amino terminus. It binds long oligouridine stretches for high-affinity binding. Affinity may also be modulated by flanking RNA sequences and possible associations with other factors. SXL has been shown to function as a female-specific regulator of splicing that controls the expression of the transformer (tra) and its own mRNA.

How does SXL inhibit MSL-2 expression? msl-2 pre-mRNA harbors two consensus high-affinity SXL-binding sites in its 5′UTR and four in its 3′UTR (egg-shaped symbols in Fig. 8). Interestingly, the two sites in the 5′UTR are both located within an intron that is spliced in a sex-specific fashion. The intron is removed in males but retained in females, due to SXL's effects on splicing (for review, see Gebauer et al. 1997). msl-2 mRNA is efficiently exported into the cytoplasm in both sexes, but MSL-2 protein is only expressed in males. The retained intron cannot suppress MSL-2 expression per se, because constructs in which the intron is retained due to splice-site mutations are expressed in transfected cells and in transgenic flies if SXL is absent. Several lines of evidence show that SXL acts as a translational repressor in the cytoplasm and that both the 5′ and the 3′UTR-binding sites are important for this (Bashaw and Baker 1997; Kelley et al. 1997; Gebauer et al. 1998). First, reporter constructs bearing the SXL-binding sites only in either the 5′UTR or 3′UTR are not efficiently repressed by SXL in transfected cell lines and transgenic flies. Second, SXL-mediated inhibition of msl-2 expression does not affect the cytoplasmic levels of msl-2 mRNA. Third, the regulation of msl-2 mRNA translation by SXL has recently been recapitulated in a cell-free system from Drosophila embryos with recombinant SXL protein and in-vitro-transcribed reporter mRNAs bearing both untranslated regions of msl-2 mRNA (Gebauer et al. 1999). Mutation of sites in either UTR drastically reduces repression, indicating that the two regions act synergistically (Bashaw and Baker 1997; Kelley et al. 1997). Of the two sites in the 5′UTR, the downstream site is more important, at least in vitro (Gebauer et al. 1999). This indicates that, unlike the IRE/IRP system, cap-proxim-
Figure 8 Regulation of msl-2 expression by sex-lethal (SXL). The nuclear msl-2 pre-mRNA is depicted with two introns and bearing six SXL binding sites (red). SXL is only expressed in female (XX) embryos, where binding to two sites in the intron within the 5'UTR inhibits splicing, enforcing intron retention. Following export into the cytoplasm, SXL binding to the sites within both UTRs represses translation. In male embryos (XY), the MSL-2 protein is expressed following unimpeded splicing and translation.

ity is not important. Numerous other mRNAs from *Drosophila* bearing 3'UTR-binding sites for SXL have been identified (Kelley et al. 1995). Their biological functions remain to be clarified.

In summary, SXL inhibits msl-2 expression in *Drosophila melanogaster* by an integrated two-step mechanism that involves splicing and translation. Interestingly, the latter but not the former is conserved in evolution: In *Drosophila virilis*, the splice sites are not maintained so that this related organism apparently relies entirely on translational regulation to achieve dosage compensation by the MSL complex.

**Mesoderm Specification in Xenopus**

In frogs, mesoderm arises through a process termed "induction," in which a signal is secreted from endodermal cells at the bottom of the embryo to
overlying cells, causing those cells to follow mesodermal fates (for review, see Melton 1994). Members of the fibroblast growth factor (FGF) and transforming growth factor β (TGF-β) families of secreted polypeptides are likely signals in this process, as are the cell-surface receptors to which they bind (for review, see Melton 1994).

Two forms of translational control have been implicated in mesoderm induction. The first involves a maternal mRNA encoding an FGF receptor, FGFR-1 (Robbie et al. 1995). Expression in embryos of a dominant inhibitory form of the FGF receptor interferes with mesoderm induction in vivo, presumably by titrating wild-type receptors into inactive complexes (Amaya et al. 1991, 1993). These and other results strongly suggest that the FGF receptor and its ligand play a key role in mesoderm induction (for review, see Melton 1994). FGFR-1 mRNA is silent in oocytes, but activated during oocyte maturation, prior to fertilization (Musci et al. 1990). The repression is due to a negative regulatory element in the 3'UTR of FGFR-1 mRNA, in the 180 nucleotides immediately downstream from the termination codon. The temporal or spatial control of its de-repression may be important in embryonic induction, although the existence of multiple receptors for FGF-related ligands may complicate the issue.

A second speculative role for translational control in mesoderm induction may be that increased activity of eIF4E in the embryo specifically stimulates the translation of activin, a member of the TGF-β superfamily and a potent inducer of mesoderm (for review, see Melton 1994). This idea is based, in part, on the finding that overexpression of the general translation factor eIF4E in frog embryos induces mesodermal fates in cells that would otherwise form ectoderm (Klein and Melton 1994). Moreover, eIF4E overexpression specifically stimulates translation of injected activin mRNA without affecting either total protein synthesis or other injected mRNAs (Klein and Melton 1994).

Activin and eIF4E may comprise a positive feedback loop. Mesoderm induction by eIF4E is blocked by coexpression of a dominant inhibitory form of the activin receptor (Klein and Melton 1994). Since mRNA injection experiments imply that activin translation may be stimulated by eIF4E, these data suggest a simple autocrine loop: Activin elevates eIF4E levels, which further enhances activin synthesis. A circuit of this type could both amplify the initial inducing signal and explain how one cell that has been induced to form mesoderm can induce mesoderm in an adjacent cell. This model predicts that the level of eIF4E activity is elevated during early development, at least in certain blastomeres, and that specific mRNAs involved in mesoderm induction should be stimulated as a result. Those mRNAs might encode activin or other mesoderm inducers.
Although the activin/eIF4E circuit is speculative, it closely parallels an apparent mechanism of neoplastic transformation of mammalian cells by overexpression of eIF4E (Lazaris-Karatzas et al. 1990; Chapter 6). In that case, as in mesoderm induction, elevation of the levels of a general translation factor has dramatic effects on cell fate.

**Terminal Differentiation**

Certain genes are expressed late in differentiation, as cells take on their ultimate fates. In the examples of terminal differentiation described below—late spermatogenesis and red blood cell differentiation—the nucleus is effectively silenced: The spermatid pronucleus is highly condensed and inactive, and in mammals, red blood cells lose their nucleus entirely. In these cases, as in the early embryo, the cell must exploit translational control to change the proteins it contains.

**Mammalian Spermatogenesis: Protamine mRNAs and DAZ Proteins**

Spermatogenesis is a highly conserved process that involves both cell division and cell differentiation. The germ-cell population first expands through mitosis, generating “spermatocytes” that enter meiosis. The haploid products of meiosis (“round spermatids”) then differentiate into “elongating spermatids” and “spermatozoa.” The entire process takes approximately 3 weeks and occurs throughout adult life.

Regulation of mRNAs appears to play a major role during spermatogenesis. Multiple mRNAs are regulated (for review, see Hecht 1998). Here we focus on two intensely studied examples: protamine mRNAs and their regulators, and the DAZ family of proteins and their likely targets.

**Translational Regulation of Protamine Expression.** During the terminal stages of spermatogenesis, chromosomes are repackaged with protamines rather than histones to facilitate chromosome condensation. Protamine mRNAs (*Prm-1* and *Prm-2*) that had previously been silent become active. Protamine mRNAs are synthesized in round spermatids, are stored as cytoplasmic ribonucleoprotein (RNP) particles for up to a week, and finally translated in elongated spermatids. Repression of *Prm-1* is imposed by a 3′UTR-mediated mechanism and is essential for normal spermatid differentiation: premature translation of *Prm-1* leads to precocious nuclear condensation and sterility (Braun et al. 1989; Lee et al. 1995).

Several 3′UTR sequences have been implicated in *Prm* mRNA translational control, suggesting redundancy. Sequences at the 5′ and 3′ ends of the *Prm-1* 3′UTR are sufficient to confer *Prm-1*-like translational reg-
ulation on a reporter transgene in mice (Fajardo et al. 1997). A protein, called Prbp, binds the 3′ sequence and is present in the cytoplasm of round spermatids, but not in elongated spermatids (Lee et al. 1996). However, Prbp-deficient mice do not prematurely express Prm-1, Prm-2, or a transgene carrying the 3′ end of the Prm-1 3′ UTR (Zhong et al. 1999); rather, the activation of these mRNAs in elongated spermatids is defective (Zhong et al. 1999). This suggests a role for Prbp in activation, not repression (Zhong et al. 1999).

In addition, both the Prm-1 and Prm-2 3′ UTRs contain two conserved regions, called Y and H boxes. The Y box cross-links to an 18-kD protein present in male germ cells and in testicular RNP particles (Kwon and Hecht 1991). An extract enriched for the 18-kD protein represses translation of reporter RNAs containing the Y and H boxes in vitro (Kwon and Hecht 1993). Interestingly, the 18-kD protein present in round and elongating spermatids binds RNA, whereas the protein found in elongated spermatids does not. Phosphorylation may control the RNA-binding activity, and hence, translation (Kwon and Hecht 1993). The ability of the Y and H boxes to mediate repression in vivo has not been examined.

Efforts to identify proteins responsible for targeting Prm-1 and related mRNAs to mRNP particles have identified several spermatid mRNP-associated proteins, including poly(A)-binding protein (Gu et al. 1995), spermatid perinuclear RNA-binding protein (Spnr; Schumacher et al. 1995b), testis nuclear RNA-binding protein (Tenr; Schumacher et al. 1995a), and the Y-box proteins (Tafuri et al. 1993). Although their functions are unclear, Y-box proteins nonspecifically bind RNA and may play an important role in forming repressive mRNP particles.

**DAZ Proteins and the Regulation of Meiosis.** Three regions on the human Y chromosome, called AZFa, AZFb, and AZFc (Azoospermia Factor) are required for proper spermatogenesis (for review, see Elliot and Cooke 1997). Candidate spermatogenesis genes that encode RNA-binding proteins have been identified in AZFb and AZFc. Deletion of AZFc removes a small family of genes named **DAZ** (Deleted in Azoospermia; Reijo et al. 1995). Deletion of AZFb region removes another gene family called **RBM** (Ribosomal Binding Motif; Ma et al. 1993; Elliot et al. 1997). Although good correlative evidence suggests that the **DAZ** and **RBM** families are involved in spermatogenesis in humans, mutations that cause spermatogenic defects by affecting only one **DAZ** or **RBM** gene have not been reported.

In mice and flies, genetic evidence demonstrates that **DAZ** family members are required for spermatogenesis. Both mice and flies each have an autosomal **DAZ**-related gene, called Dazla and **boule**, respectively.
Disruption of the mouse Dazla gene results in infertility in both sexes, due to a reduction in the number of germ cells. Thus, Dazla is necessary for development and survival of germ cells in both the ovary and testis (Ruggiu et al. 1997). Loss of *boule* in flies also results in male-specific infertility, in which spermatogenesis arrests at the G$_2$/M transition of meiosis I (Castrillon et al. 1993; Eberhart et al. 1996).

*twine* mRNA is a likely target of Boule protein during male meiosis. *twine* encodes a meiotic, *cdc25*-like phosphatase. *twine* mRNA, but not protein, is present in premeiotic cells, suggesting that *twine* mRNA is repressed at this stage (Alphey et al. 1992; Courtot et al. 1992; White-Cooper et al. 1998). Several lines of evidence suggest that Boule is needed to activate *twine* translation. Spermatocytes in *twine* mutants fail at the G$_2$/M transition, as do *boule* mutants, and *boule* acts genetically before *twine* in spermatogenesis. Moreover, *boule* is required for translation of a *twine*–*lacZ* reporter construct (Maines and Wasserman 1999), although a direct interaction between Boule and *twine* mRNA has not been reported. Since *boule* and *twine* have different phenotypes, *boule* probably has other targets (Eberhart et al. 1996).

The function of DAZ family proteins may be conserved. Defects in family members in man, mice, frogs, and flies give similar phenotypes. Moreover, DAZ proteins can function across species: *Xenopus* Xdazl rescues the meiotic defect of *boule* mutant flies (Houston et al. 1998), and human DAZ partially rescues the spermatogenic defect of Dazl mutant mice (Slee et al. 1999). Given the similarities in sequence, function, and expression patterns, it seems likely that these proteins commonly control spermatogenesis by regulating translation of specific mRNAs: to date, *Drosophila twine* is the only target mRNA identified.

**Red Blood Cell Differentiation: 15-Lipoxygenase mRNA**

As mammalian reticulocytes differentiate into erythrocytes, their mitochondria are destroyed. The enzyme 15-lipoxygenase (LOX) catalyzes deoxygenation of polyenoic fatty acids, even in intact membranes, and is thought to be critical for the destruction of internal membranes and mitochondria (Rapoport and Schewe 1986). Although LOX mRNA apparently is present even at early stages of erythropoiesis, it is not translated until reticulocytes mature into erythrocytes (Thiele et al. 1982). This translational silencing is critical in early erythroid precursor cells and young reticulocytes, which require intact mitochondria for their metabolism.

The 3′UTR of rabbit LOX mRNA contains ten nearly perfect repeats of a 19-nucleotide sequence, whereas the mouse mRNA contains four
similar repeats in a comparable location (Hunt 1989; Ostareck-Lederer et al. 1994). These repeats, called differentiation control elements (DICE), mediate translation repression, as demonstrated in vitro (Ostareck-Lederer et al. 1994). Two proteins, hnRNP K and hnRNP E1, can interact with each other, bind to the DICE, and silence LOX mRNA translation both in vitro and in transfected HeLa cells. Importantly, silenced LOX mRNA in early erythroid cells is associated with hnRNP K (Ostareck et al. 1997). Interestingly, repression in vitro appears to be independent of any change in poly(A) length and of the 5′ terminal cap, points to which we later return. Although rabbit LOX mRNA contains ten tandem repeats, two are sufficient for repression (Ostareck et al. 1997).

Masking and CPEB

Masking

The “masking” hypothesis, initially proposed by Spirin more than 30 years ago (Spirin 1966), suggests that specific mRNAs are repressed through the action of proteins that hide them from the translational apparatus. In response to a stimulus, such as fertilization, the masking proteins are removed, the mRNA is revealed, and its translation begins. In its initial formulation, masking was proposed to explain the dramatic increase in protein synthesis observed in sea urchin eggs at fertilization. Classically, masking is defined operationally, using extracts derived from eggs and early embryos. In vivo, a specific mRNA is repressed in the egg but becomes active at fertilization. The patterns of protein synthesis are maintained in extracts of eggs and early embryos; in particular, mRNAs that are repressed in vivo continue to be repressed when translated in vitro, provided they are presented as mRNPs (i.e., with proteins still attached). Removal of the proteins from the mRNPs activates (i.e., “unmasks”) the mRNA in vitro. Protein removal can be accomplished crudely, for example, by extraction with organic solvents, or by more subtle means, as described below (for review, see Standart 1992; Standart and Jackson 1994).

Thus, masking is followed by activation, or unmasking. Only some of the mRNAs we have discussed in previous sections—bicoid, for example—appear to behave in this way. In contrast, frog cyclin B1 mRNA is already expressed at a low level before oocyte maturation begins, and so, at the least, may not be fully masked; lin-14 and lin-41 mRNAs are initially translationally active, and then are shut off, the opposite of the situation in classic masking. It is uncertain whether these different mRNAs
are repressed by the masking mechanism used to silence mRNAs from their birth. However, masking is repression, and the differences are likely semantic and historical, not biological.

Clam ribonucleotide reductase mRNA provides a well-studied paradigm for masking (Standart et al. 1990). Unmasking of this mRNA in an extract of surf clam oocytes can be achieved by incubation in 0.5 M KCl and gel filtration, which presumably removes the masking factor. Masking can be restored in the extract by removal of the salt prior to gel filtration, which presumably permits the factor to rebind. Remasking in this fashion requires sequences in the 3'UTR (Standart et al. 1990). Masked ribonucleotide reductase mRNA can be derepressed in oocyte extracts by severing the 3'UTR from the body of the mRNA, using targeted RNase H-cleavage (Standart et al. 1990). The activation appears to be independent of polyadenylation, even though the mRNA receives poly(A) as it is activated in vivo. These data imply that removal of 3'UTR-bound factors is sufficient for derepression, and that derepression in vitro can be uncoupled from poly(A) addition.

**CPEs and CPEBs: Going Both Ways**

Sequences that control cytoplasmic polyadenylation (CPEs) are located in the 3'UTR; the sequence AAUAAA, located nearby, is also required for the reaction. Most commonly, CPEs have been identified as positive control elements required for polyadenylation and translational activation; injected, mutant mRNAs lacking them are not activated, nor do they receive poly(A) (see Chapter 27).

However, CPEs can also repress, and may mediate masking. This conclusion first emerged in studies of mouse tPA mRNA, in which elements that repress and cause poly(A) removal prior to oocyte maturation overlap with those that activate and cause poly(A) addition once maturation has begun ("ACE" elements; Sallés et al. 1992). More recently, those sequences provided in excess in trans have been shown to cause derepression of endogenous mRNAs, presumably by titrating a repressor (Stutz et al. 1998). CPEs of other mRNAs also can mediate repression before being involved in activation (de Moor and Richter 1999; Minshall et al. 1999; Ralle et al. 1999; Barkoff et al. 2000). The duality of CPEs is not invariant, however, as some 3'UTRs that direct polyadenylation do not repress (Barkoff et al. 2000).

The duality of CPEs complicates predictions of the phenotypes of CPE mutations in endogenous genes. For example, suppose that a single CPE first is required to repress an mRNA, and then later to activate it.
Deletion of the CPE in the endogenous gene would result in premature activation of the mRNA, with no subsequent increase. Thus, such CPEs would appear genetically as negative translational control elements, not as positive-acting signals. This raises the possibility that negative control elements described in a variety of systems might also have later, positive-acting functions.

An RNA-binding protein of the RRM family, CPEB, binds to CPEs and is required for cytoplasmic polyadenylation and translational activation of dormant mRNAs (Hake and Richter 1994; Chapter 27). Consistent with this view, mutants lacking a *Drosophila* CPEB homolog, *orb*, fail to activate *oskar* mRNA (Chang et al. 1999), and *Xenopus* oocytes injected with anti-CPEB antibodies fail to activate or polyadenylate c-mos mRNA (Stebbins-Boaz et al. 1996). However, CPEB homologs can also cause repression. An 82-kD protein that binds to repressive elements in clam ribonucleotide reductase and cyclin A mRNAs is a CPEB homolog (Minshall et al. 1999; Walker et al. 1999). The duality of this protein’s function echoes that of CPEs.

Molecular mechanisms have been proposed for both the repressive and activating activities of CPEBs. CPEB’s repressive role involves a second protein, maskin. *Xenopus* CPEB interacts with maskin, which in turn binds the initiation factor, eIF4E: The three proteins are found in a complex in resting oocytes (Stebbins-Boaz et al. 1999). This interaction may preclude binding of eIF4E with eIF4G and thereby cause repression prior to oocyte maturation. In this model, activation is achieved by disrupting the maskin/eIF4E interaction (Stebbins-Boaz et al. 1999; Chapter 27).

The positive-acting properties of CPEB invoke its facilitation of cytoplasmic polyadenylation, ultimately by recruitment of a cytoplasmic poly(A) polymerase (PAP) (Ballantyne et al. 1995; Gebauer and Richter 1995). This event likely requires binding of a cytoplasmic form of cleavage and polyadenylation specificity factor (CPSF) to the AAUAAA sequence of the mRNA, which in turn binds PAP (Bilger et al. 1994; Dickson et al. 1999). CPSF’s binding preference for CPE-containing RNAs could facilitate such events (Bilger et al. 1994).

Factors other than, or in addition to, canonical CPEB may also be involved in CPE-mediated events. Two new proteins apparently interact with the CPEs of *Xenopus* lamin mRNA (Ralle et al. 1999); the CPEs of mouse tPA mRNA appear to bind non-CPEB factors as well (Stutz et al. 1998). Moreover, in some organisms, multiple CPEB homologs may have distinct activities. Indeed, the *C. elegans* and zebrafish genomes encode multiple CPEB-related proteins.
MECHANISMS

The examples above illustrate the broad biological range of translational regulation of developmental decisions. Interactions between specific mRNA regulatory sequences and single or multiple proteins are established or broken in response to regulatory cues and control the translation of the respective mRNAs. In this section, we discuss how these events alter translational activity. Most of the examples we discuss hinge on the 3′UTR; nevertheless, we begin by discussing one example of regulation through the 5′UTR. We do so because the relatively detailed mechanistic information sets precedent for how mRNAs can be shut off and activated, and how one regulator can control multiple mRNAs.

In principle, translation can be affected at the levels of initiation, elongation, or termination. Most examples that have been investigated appear to be regulated at the level of initiation (see below), although the number examined in detail is small. In at least one case described (lin-4 regulation of lin-14), regulation occurs after initiation.

Two central questions arise. First, how is translational repression exerted? Second, for those mRNAs that first are repressed and later activated, how is derepression accomplished?

Regulation Via the 5′UTR

Perhaps the most intensively characterized example of translational control via a 5′UTR element is that of ferritin mRNA regulation by iron via iron-responsive elements (IRE) and iron regulatory proteins (IRP) (Chapter 21). We discuss this below, emphasizing that the binding of regulatory proteins to 5′UTR sites can act by two distinct mechanisms— inhibition of 43S recruitment or interference with 43S scanning.

IREs have been identified in the 5′UTR of several different mRNAs that encode proteins involved in iron metabolism (Hentze and Kühn 1996). The IREs are usually located within 40 nucleotides of the cap structure, a feature that is functionally important: Cap-mediated recruitment of the 43S translation preinitiation complex to the mRNA occurs within this region, and is blocked by IRP binding to a cap-proximal IRE (Gray and Hentze 1994). IRP-binding to an IRE still permits assembly of eIF4F on the cap structure in vitro, but the joining of this complex and the small ribosomal subunit is inhibited (Fig. 9) (Muckenthaler et al. 1998). Translation also can be inhibited sterically by high-affinity RNA/protein complexes. Replacement of the IRE by binding sites for RNA-binding proteins that do not play physiological roles in controlling eukaryotic
Figure 9 Translational regulation by the IRE/IRP system. The 5′UTR of ferritin mRNA bearing a cap-proximal iron-responsive element (stem-loop structure in black) is depicted. (Upper panel) Assembly of a 43S translation initiation complex. (Lower panel) Binding of IRP1 (red) to the IRE blocks the recruitment of the 40S ribosomal subunit with its associated translation initiation factors to the preassembled cap-binding complex eIF4F.

translation (the spliceosomal protein U1A or the bacteriophage MS2 coat protein) allows specific translational repression by the respective proteins in vitro and in vivo (Stripecke et al. 1994). Other cis-acting elements for translational repression that are found within the first 40–50 nucleotides of an mRNA may operate through a similar block of 43S preinitiation complex recruitment.

IRE/IRP complexes in an appropriate position can affect scanning. IRP binding to an IRE cloned farther downstream in the 5′UTR of a reporter mRNA fails to inhibit the recruitment of 43S preinitiation complexes, as expected. Such downstream IRE/IRP complexes still cause some degree of translational inhibition in transfected cells (Goossen and Hentze 1992), although substantially less than cap-proximal IREs. In a cell-free translation system from rabbit reticulocyte lysate this effect was
attributed to a kinetic effect on 43S scanning (Paraskeva et al. 1999). While the mammalian initiation machinery is able to eventually overcome cap-distal IRE/IRP complexes, the initiation machinery in wheat germ and yeast translation extracts is not: In these systems, a downstream IRE/IRP complex inhibits efficiently, apparently by stalling the scanning process (Paraskeva et al. 1999). Interestingly, the mRNA encoding one subunit of the *D. melanogaster* succinate dehydrogenase is the only natural example with a cap-distal IRE so far (Kohler et al. 1995; Gray et al. 1996). It should be interesting to explore how the *Drosophila* translation apparatus responds to this IRE/IRP complex.

**Links between the 5′ and 3′ Ends**

Since many mRNAs are regulated via binding sites in their 3′ UTRs or by a combination of 5′ UTR and 3′ UTR sites, it is important to briefly consider the organization of the two mRNA ends during translation (for a more detailed discussion, see Chapter 10). Although mRNAs are commonly drawn as linear molecules with cap structures on the left and poly(A) tails on the right, cellular mRNAs form local secondary and tertiary structures as well as long-range interactions. Moreover, mRNAs in vivo are not naked nucleic acids, but instead are bound by a multitude of cellular RNA-binding proteins with various specificities and functions. Messenger RNAs hence exist as mRNPs with complex folding patterns, which may or may not juxtapose their two ends.

A wealth of biochemical evidence supports the view that the two ends can be placed in proximity through protein-protein interactions. Poly(A) tail-binding protein (Pab1p/PABP) binds to the amino-terminal region of the translation initiation factor eIF4G, which binds through a neighboring region the cap-binding protein eIF4E. Such interactions have been observed using proteins derived from yeast, plants, and mammals (see Chapter 10). Binding of eIF4E and Pab1p/PABP to eIF4G can occur simultaneously, and hence provides a means to effectively circularize the mRNA (Wells et al. 1998). This end-to-end interaction is likely to be important for translation in cell-free systems (see Chapter 10). In living cells, however, the roles of this complex in translation and its regulation in vivo are unclear, and it is possible that the complex has additional functions.

Juxtaposition of the 5′ and the 3′ end of an mRNA is thought to be important for the synergistic positive effect of the cap structure and the poly(A) tail on translation initiation (Preiss and Hentze 1998; Chapter 10). Regulatory proteins that bind to the untranslated regions and stimu-
late or inhibit these interactions would be expected to have profound effects on translation (Fig. 10). Furthermore, the effect of these interactions to bring together the 5′ and 3′ ends could also be important for the function of 3′UTR-binding proteins that target a different step in translation initiation but utilize their topographic effects.

Although the interaction between PABP and eIF4G has been demonstrated in cell-free systems, its role in developing gametes and embryos is not clear. In frog oocytes, although the small quantity of PABP apparently is insufficient to occupy the poly(A) tails of all mRNAs (Zelus et al. 1989), endogenous PABP does interact with eIF4G (Keiper and Rhoads 1999). Moreover, the portion of PABP that interacts with eIF4G, bound to a 3′UTR, stimulates translation of that mRNA in these cells (Gray et al. 2000). Cleavage of eIF4G with viral proteases (Keiper and Rhoads 1999) inhibits oocyte maturation and decreases translation of reporter mRNAs.

*Figure 10* The eIF4E/eIF4G/PABP interaction links the two ends of the mRNA and suggests models for regulation by 3′UTR-bound proteins. Translation initiation factor interactions that contribute to the recruitment of the 40S subunit are depicted. A regulatory element in the 3′UTR (in red) is shown to bind a repressor protein (R) and interfere with any of the depicted biochemical interactions, either directly or indirectly by means of a co-repressor (X).
These data argue that the poly(A) tail may mediate its effects in embryos, at least in part, through interactions with PABP and thence eIF4G; however, portions of PABP that do not interact with eIF4G also stimulate translation in oocytes (Gray et al. 2000).

The mammalian protein PAIP-1 (for PABP interacting protein) may also participate in end-to-end communication (Craig et al. 1998). PAIP-1 displays similarity with the central domain of eIF4G and, like eIF4G, interacts with eIF4A. However, it does not appear to interact with eIF4E or eIF3, and hence it is not yet clear how this intriguing player affects translation or effects its control. Another protein with homology to eIF4G is p97/NAT1/DAP-5, which binds eIF4A and eIF3 but does not bind eIF4E and PABP (Imataka et al. 1997; Levy-Strumpf et al. 1997; Yamanaka et al. 1997). Therefore, it is not a prime candidate for being involved in the formation of interactions between the mRNA ends, and its role in translation remains to be more precisely defined.

**Role of 5’-end Modifications during Development**

Methylation of the 2’ position of the second and third ribose moieties of the mRNA (i.e., 7mGpppGmGmGm) may be linked to polyadenylation and hence to translational control of certain mRNAs. Polyadenylation-dependent ribose methylation has been reported using synthetic B4 mRNA injected into *Xenopus* oocytes (Kuge and Richter 1995). Methylation inhibitors prevent both the modification and translational stimulation (Kuge and Richter 1995), and ribose-methylated mRNAs are translated more efficiently in oocytes (Kuge et al. 1998). However, ribose methylation cannot be the universal cause of the effects of poly(A) on translation in oocytes, since translation of injected reporter RNAs that do not undergo efficient ribose methylation can be dramatically enhanced by polyadenylation (Gillian-Daniel et al. 1998). Nevertheless, a model in which polyadenylation in situ causes cap modification has the merit that it explains repression of mRNAs with respectable tail lengths, simply by their lack of a methyl group prior to polyadenylation.

Deadenylation leads to enzymatic cleavage of the cap structure and hence to mRNA decay in yeast (Chapter 28). A comparable deadenylation-dependent decapping reaction could, in principle, provide a simple mechanism by which poly(A) removal results in translational repression. However, RNAs that are completely deadenylated and repressed retain their caps in a methylated form in *Xenopus* oocytes (Gillian-Daniel et al. 1998).
Mechanisms of Repression Via the 3'UTR

Steric blockage mechanisms are more easily imagined from sites in the 5'UTR than 3'UTR: A priori, one might expect 5' and 3'UTR-mediated repression mechanisms to differ fundamentally. However, we discuss at least one mechanism that bears strong resemblance with steric repression of translation.

The physical proximity of the 3'UTR and poly(A) tail immediately raises the question of whether translational control is exerted by affecting the length and/or function of the poly(A) tail, or by mechanisms independent of the poly(A). Biology has made use of both possibilities, as discussed below. Clearly, the mechanisms of repression differ among mRNAs and are not mutually exclusive.

Interfering with the Function of the mRNA Ends in 43S Recruitment

The cap structure and the poly(A) tail exert a positive, synergistic effect on translation, involving the eIF4E/eIF4G/PABP interaction. In principle, 3'UTR-binding proteins could regulate translation through interference with this chain of interactions, either by inhibition of eIF4E binding to the cap structure, blocking the eIF4E/eIF4G interaction, the eIF4G/PABP interaction, or the binding of PABP to the poly(A) tail (Fig. 10). The inhibition could be direct, with the repressor touching a translation factor, or could require interaction between the 3'UTR-bound repressor and an intermediary. Furthermore, the function of other translation factors involved in the recruitment of the 43S preinitiation complex could be affected by a 3'UTR-binding protein.

To determine, to a first approximation, whether repression from the 3'UTR requires a cap, one can ask whether it still occurs on an uncapped mRNA or when translation is initiated by a cap-independent, IRES-driven mechanism. In the case of LOX mRNA regulation by hnRNPs K and E1 via a 3'UTR DICE, translational inhibition persists under these conditions, suggesting that the cap structure and eIF4E are not the primary targets (Ostareck et al. 1997).

Analogously, the effect of the poly(A) tail and PABP can be assessed using an assay system that exhibits strong effects of poly(A). This is unfortunately not the case in the popular rabbit reticulocyte and wheat germ systems, but both Xenopus oocytes and a newly developed cell-free system from Drosophila embryos display this property (Gebauer et al. 1999). Using Xenopus embryos, the TGEs of C. elegans tra-2 mRNA were shown to repress only mRNAs that possessed a poly(A) tail.
(Thompson et al. 2000). This is consistent with their either promoting deadenylation or interfering with poly(A)-dependent enhancement of translation (Thompson et al. 2000).

Certain repressors may interfere with cap function: CPEB, through maskin, may bind eIF4E in such a way that it blocks further assembly of a 48S pre-initiation complex (Stebbins-Boaz et al. 1999). This model has the attractive virtue that it can explain why the preexisting poly(A) tail is insufficient to activate, despite its being sufficiently long to bind PABP.

Keeping Poly(A) Tails Short

A related strategy to inhibit translation would be to keep the poly(A) tail short. Although many studies suggest that polyadenylation is an integral part of translational activation, few address how, or whether, poly(A) length is connected to repression. Do negative elements in the 3′UTR act by keeping the poly(A) tail short? Or do they repress through a mechanism that has nothing to do with having a short tail, but which can be relieved by polyadenylation?

mRNA injection experiments support the hypothesis that the repression of a maternal mRNA can be caused by the shortness of its poly(A) tail. In *Drosophila*, injected bicoid mRNAs with long poly(A) tails rescue bicoid mutant embryos, whereas the same mRNAs with shorter tails do not (Sallés et al. 1994); similarly, injected murine tPA mRNAs are active with long, but not short, poly(A) tails, corresponding to their states before and after oocyte maturation (Huarte et al. 1992).

Whereas short tails lead to less activity than long tails, these differences are not always a sufficient explanation for regulation. For example, poly(A) tails of about 50 nucleotides stimulate translation relative to an mRNA with no tail both in vivo and in vitro, yet repressed mRNAs often have tails longer than this. Furthermore, removal of the poly(A) tail (and 3′UTR) of ribonucleotide reductase turns it on. Translational repression of *msl-2* mRNA by SXL in a poly(A)-responsive extract from *Drosophila* embryos is as efficient when the RNA has a poly(A) tail of 73 nucleotides as when the tail is lacking (Gebauer et al. 1999). Moreover, poly(A) shortening can be a result, rather than a cause, of repression (Muckenthaler et al. 1997), and poly(A) lengthening can occur in the absence of derepression (Culp and Musci 1998).

These considerations suggest that the repression of certain mRNAs which show correlations of translational activity with poly(A) length may be due to a poly(A)-independent mechanism. This does not preclude the possibility that poly(A) addition may play an important role in derepres-
sion. Indeed, derepression of mouse tPA (Stutz et al. 1998) and frog cyclin mRNAs (de Moor and Richter 1999) by providing excess CPEs in trans requires that a poly(A) tail be present on the mRNA.

Interfering with the Joining of the 60S Subunit or Elongation

Recruitment of the small ribosomal subunit is considered to constitute the rate-limiting step in translation initiation under many conditions (Sachs et al. 1997). This situation predisposes this early step as a target for translational control, but does not preclude subsequent steps from being target-ed by inhibitory mechanisms. One example of this is the stalling of scanning by cap-distal IRE/IRP complexes (see above). Another point of interference can be envisaged at the joining step between the small and the large ribosomal subunit at the translation initiation codon. At present, no such example has been reported. However, lin-14 mRNA appears to remain polysome-associated when repressed by lin-4 mRNA, implying that repression occurs after initiation (Olsen and Ambros 1999).

Subcellular Localization

Repressors bound to sites in the 3'UTR might move the mRNA into a cellular microenvironment that is translationally compromised or interfere with the movement of an mRNA to a site that is translationally favorable.

Nucleating Assembly of a Repressive Structure

In this model, mRNAs are repressed because they are assembled into a complex that effectively hides them from the translation apparatus. This complex might be an overall structure, that hides the mRNA in much the same way as chromatin condensation hides DNA from the transcription apparatus. As such, this mechanism is an extension of, and quite similar to, a steric interference model. Y-box proteins, such as FRGY2 (also known as mRNP4), may be important in the formation of structures that cause repression (for review, see Wolff 1992, 1994). FRGY-2 is expressed in oocytes and not in somatic cells; homologs are present in somatic cells and may have comparable functions. Y-box proteins, including FRGY-2, are bona fide transcription factors (Tafuri and Wolff 1990, 1992), yet are physically associated with many different maternal mRNAs (see, e.g., Darnbrough and Ford 1981; Dearsly et al. 1985; Murray et al. 1991; Tafuri and Wolff 1993) and can inhibit their translation (Richter and Smith 1984; Kick et al. 1987; Ranjan et al. 1993; Bouvet and Wolff 1994). These data sug-
gest several provocative possibilities. For example, Y-box proteins might assemble with the mRNA to form a structure that effectively hides the mRNA. Dephosphorylation of Y-box proteins appears to enhance translational activation of the mRNA with which they are associated (Kick et al. 1987; Murray et al. 1991) yet may have little effect on the binding of Y-box proteins to RNA (for contrary view, see Kick et al. 1987; Tafuri and Wolffe 1993). Thus, phosphorylation and dephosphorylation may influence Y-box protein activity, and hence translation, without modulating their association with RNA. More speculatively, dephosphorylation might conceivably “decondense” a complex structure and reveal the mRNA.

As yet, little sequence specificity has been demonstrated in either the RNA-binding or repressing activities of the Y-box proteins (Marello et al. 1992; Tafuri and Wolffe 1993). Thus, if the Y-box proteins do cause repression of some mRNAs but not others, some other factor must provide the sequence specificity. Proteins bound to negative elements in the 3′UTR could serve such a function, promoting the assembly of Y-box proteins into a repressive form or structure. Y-box proteins can be found associated with active mRNAs, arguing that their binding is insufficient for repression (Tafuri and Wolffe 1993). However, it may be instructive to bear in mind again the analogy with chromatin: Core histones are present on active and inactive genes, but their positions and higher order structures differ and may play a critical role in regulating transcriptional activity. Perhaps sequence-specific regulatory proteins nucleate or disassemble repressive mRNP structures.

**Interdependent 5′ and 3′UTRs**

In certain instances, translational control requires sites in both UTRs. To repress *msl-2* mRNA in female flies, the protein Sex-Lethal (SXL) must bind to specific sites in the 5′UTR and the 3′UTR of *msl-2* mRNA (see above, Dosage Compensation in *Drosophila*). Localization-dependent translation of *oskar* mRNA involves both its 5′ and 3′UTRs (see above, Pattern Formation in *Drosophila*). In this instance, the 5′UTR element is required for activation rather than repression.

Studies of several mRNAs, particularly plant infectious agents, demonstrate that interactions between 5′ and 3′UTRs can stimulate translation. Some plant viruses harbor positive-acting translational elements in their 3′UTRs: Often, they require the appropriate 5′UTR (Gallie and Walbot 1990). For example, an element in the 3′UTR of the barley yellow dwarf virus 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 and 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 implies that base-pairing between the 5′ and 3′ UTRs, or protein–protein interactions, are critical in activation. In some cases, the 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 (Gallie and Walbot 1990; Timmer et al. 1993; Wang and Miller 1995). Although these examples involve plant viruses rather than germ cells or embryos, they establish a strong precedent for end-to-end communication in translational regulation.

Derepression/Activation of Translation

Conceptually, the simplest way to activate the translation of a repressed mRNA is to remove the repressor. Although this strategy is frequently followed, there are many informative deviations.

Covalent Modification of the Repressor

In several systems, candidate repressors are phosphorylated as repression is relieved (for review, see Standart and Jackson 1994). The temporal coincidence suggests that phosphorylation could negate the repressor and lead to translational activation. For example, phosphorylation of hnRNPs K and E1 affects their binding activity to RNA in vitro (for review, see Ostareck-Lederer et al. 1998) and may provide a basis for translational derepression of the mRNA. An elegant, phosphorylation-independent mechanism explains the activation of ferritin mRNA in iron-loaded cells: The IRE-binding repressor protein IRP-1 is inactivated posttranslationally by the assembly of an iron–sulfur cluster that prevents access to its RNA-binding sites, whereas IRP-2 is degraded by the proteasome following iron-induced oxidation and ubiquitinylation (Hentze and Kühn 1996; Chapter 21).

Derepression by an Activator Element at the 5′ End

As discussed earlier, relief of Bruno protein’s repression of oskar mRNA requires an activator element in its 5′ UTR. It is possible that the removal of a single component that was initially required to set up a repressed RNP does not suffice to rearrange the active mRNP. The combination of genetic approaches with recently established in vitro assays (Gebauer et al. 1999) may provide the necessary tools to unravel this process.
**Derepression and Polyadenylation**

For many mRNAs, the transition from silence to activity is accompanied by an increase in poly(A) length. The connection between poly(A) and translation has been discussed elsewhere and is not recapitulated here (Gray and Wickens 1998; Chapters 11 and 27). Instead, we discuss only the connection between polyadenylation and relief of repression by 3′UTR regulatory elements and repressors: How does relief of repression increase poly(A) length, and what does that increase in poly(A) length do to translation?

One obvious consequence of cytoplasmic polyadenylation is to provide more potential binding sites for PABP. However, mRNAs that are silent have sufficiently long poly(A) tails to bind one or more PABP molecules. Thus, longer tails would be expected to enhance translational activity, rather than to flip an off/on switch. It is possible that PABP is not present on the repressed mRNAs, however. PABP attached via a tether to the 3′UTR of a reporter stimulates its translation in a resting oocyte; this implies that, in the absence of other influences, bound PABP would stimulate during early development (Gray et al. 2000). Thus, repressors might interfere with either PABP binding or the interaction of PABP with the translational machinery.

We consider three of many possible connections between repressors, translational activation, and cytoplasmic polyadenylation. In the first, the repressor’s primary activity is to keep the tail short; when that activity is lost, the tail gets longer, and that enhances translation. This model accommodates the behavior of certain mRNAs very well, but clearly cannot account for those in which translational activation occurs without polyadenylation. Several instances have been reported of mRNAs that can become active without polyadenylation, even though they normally would undergo it (see above, Keeping Poly(A) Tails Short). In some cases, the presence of a short tail is all that is required to achieve derepression (Stutz et al. 1998); thus, a function of polyadenylation may sometimes be merely to keep a tail there at all, in the face of a competing deadenylation activity (Fox and Wickens 1990; Varnum and Wormington 1990).

In the second pathway, the repressor is inactivated by polyadenylation. For example, bound repressors might be removed or modified by the binding of polyadenylation machinery. This pathway is suggested by experiments in *Xenopus* in which the act of polyadenylation rather than the length of a poly(A) tail appears to be critical for activation (McGrew et al. 1989; Simon et al. 1992; Chapter 27).

In the third pathway, the repressor controls translation and polyadenylation independently. For example, factors bound to the ele-
ments might repress by causing formation of an mRNP structure that hides the mRNA from both the translation and polyadenylation machineries. Once the mRNA is exposed, both act. Polyadenylation would then be required to maintain or enhance translational activity. It could do so, for example, by preventing reassociation of the repressor (Standart and Jackson 1994) or by recruiting PABP.

The third pathway accommodates most of the data. It posits that full derepression requires two experimentally separable steps: an initiation step that is independent of polyadenylation, and a second step that is polyadenylation-dependent. Either process individually would yield incomplete, or improperly controlled, translation. The uncoupling of derepression and polyadenylation in vitro would be due to execution of an initiation step without a contribution by a poly(A) tail; the effect of repression in vitro might be substantial, and poly(A)-independent. In vivo, polyadenylation would be required to complete or sustain the derepression. Conversely, the ability of polyadenylation to stimulate translation of an injected mRNA would reflect only the maintenance step; derepression of endogenous mRNAs in vivo would require a separate initiation step.

REGULATORY CIRCUITRY: EMERGING PRINCIPLES AND PROBLEMS

Networks of transcriptional control are commonplace and play crucial roles in development. A single transcription factor can activate some genes and repress others, including those encoding other transcription factors; the intricate interactions of regulatory proteins at a promoter all provide inputs into a single gene’s expression. How similar might translational controls be? Are there batteries of mRNAs that are interconnected through common factors? Do differences in the interactions among regulators specify different biological outcomes?

Regulatory Elements: General Features

Although the regulatory elements we have discussed come from many different organisms and control a dramatic array of developmental decisions, they share certain unmistakable similarities. Methods ranging from classic genetics to biochemistry have converged on the 3′UTR as a predominant site of regulation. Indeed, highly conserved sequences in 3′UTRs are likely control elements, although not necessarily ones that act at a translational level (Spicher et al. 1998).

Why the 3′UTR? 3′UTRs are relatively unconstrained in evolution and thus provide fertile ground for the derivation of new regulatory ele-
ments (Wickens 1993). In contrast, the 5′UTR must be scanned prior to translation initiation, and alterations in its sequence, structure, or length can affect initiation. The coding region has even more obvious constraints. Although 3′UTRs are in many cases sufficient for regulation, in others, they act in concert with the 5′UTR (see above).

Translational control elements in 3′UTRs may be either on–off switches or adjustable rheostats. Many regulatory elements in 3′UTRs are tandemly repeated. Elimination of some but not all of the regulatory sites in tra-2 (Goodwin et al. 1993) and lin-14 (Wightman et al. 1993) yields an intermediate level of translation. Similarly, mRNAs containing a single NRE, rather than two, appear to be repressed less efficiently in vivo (Wharton and Struhl 1991). In wild-type mRNAs, partial occupancy of multiple sites may allow the level of translation to be modulated incrementally. Alternatively, multiple elements might promote cooperative binding of regulatory factors and facilitate concerted repression.

Most of the regulatory elements identified thus far in 3′UTRs of mRNAs critical for development are negative. Some may repress translation as soon as the mRNA enters the cytoplasm, so that the mRNA begins life silently (e.g., bicoid and LOX mRNAs). Other negative elements may repress translation only after a period of translational activity (e.g., lin-14 mRNAs). There are hints that regulatory elements may also be context-dependent. For example, sequence context may influence which mRNAs are stabilized or translationally repressed, as exemplified by globin and LOX regulation in the red blood cell lineage (see below). Certain CPEs are repressive, and others are not (Barkoff et al. 2000).

The preponderance of negative control of translation appears to differ from the predominance of positive control of transcription in mammalian cells (Struhl 1999). This may reflect differences in the basal states of translation and transcription in higher eukaryotes: In the absence of specific information to the contrary, mRNAs are translated, whereas genes are silent.

Translational Regulators with Multiple mRNA Targets

A key emerging principle is that regulators of key developmental decisions often control multiple mRNAs. The importance of this fact is that modulations of a single factor, or regulation of its cofactors, can cause a range of outcomes. The regulation of multiple mRNAs by IRPs modulates cellular iron levels and exemplifies such coordinate control (Hentze and Kühn 1996; Chapter 21).

The existence of multiple targets for single regulators is often inferred from genetic analysis. The logic is straightforward: The pheno-
type of a mutant that lacks a regulatory site in a single mRNA is a subset of the phenotypes of a mutant that lacks the regulator. For example, consider \textit{fem}-3 and its regulator, FBF. \textit{C. elegans} that lack the regulatory element in the 3′UTR of \textit{fem}-3 mRNA fail in the sperm/oocyte switch, as do animals that lack FBF. However, animals that lack FBF also exhibit defects in proliferation (Zhang et al. 1997). Similarly, mutants in the regulatory elements of \textit{tra}-2 mRNA affect only a single decision in the germ line, whereas mutants that lack its regulator, GLD-1, exhibit a range of germ-line phenotypes (Goodwin et al. 1993; Francis et al. 1995b). \textit{hunchback} mRNA is repressed by Pumilio and Nanos to regulate patterning in the fly embryo, but these proteins regulate germ-line events as well (see below).

Proteins that control poly(A) length during development underlie what appears to be a large network of mRNAs. Many mRNAs undergo polyadenylation as they are activated, or deadenylation as they shut off. A change in the factors responsible (e.g., CPEB, CPSF, PAP, or the deadenylase) could facilitate their coordinate control.

In principle, overexpressing the regulatory signals of a single mRNA might reveal new networks. The feasibility of such an approach has been demonstrated by studies with the negative control element in the 3′UTR of \textit{fem}-3 mRNA; overexpression of this element, on its own, masculinizes the germ line (Ahringer and Kimble 1991). The simplest interpretation of this result is that the regulatory factor that binds to the element has been titrated out and can no longer repress the endogenous \textit{fem}-3 mRNA. Titration experiments of this type could, in principle, yield unexpected phenotypes that would strongly suggest new targets for the regulatory factor.

\textbf{Families of Translational Regulators}

Many of the translational regulators identified to date are members of much larger families of proteins. In some cases, the similarity is trivial: The regulators merely share the ability to bind RNA. On the other hand, some families appear to have related targets and to share other functions. The importance of this point is twofold. First, such families may share common mechanisms of action: Understanding one regulator may illuminate the whole family. Second, if such proteins often act in complexes, as appears to be the case, interactions among them may be critical for regulation.

\textit{ATP-dependent RNA Helicases: Vasa}

ATP-dependent RNA helicases can separate RNA duplexes in an ATP-dependent reaction and are characterized by a constellation of conserved
amino acids. Here we focus on Vasa, a provocative example of the role of such helicases in translational control.

*Drosophila* Vasa protein is a member of the DEAD-box protein family of RNA helicases (Hay et al. 1988; Lasko and Ashburner 1988; Liang et al. 1994). It is required for patterning, assembly of the germ plasm, and germ-cell function (Hay et al. 1988; Lasko and Ashburner 1988; Schupbach and Wieschaus 1991; Liang et al. 1994). *vasa* homologs are expressed in the germ cells of many animal species, including planaria (Shibata et al. 1999), *C. elegans* (Gruidl et al. 1996), zebrafish (Olsen et al. 1997; Yoon et al. 1997; Braat et al. 1999), *Xenopus* (Komiya et al. 1994; Ikenishi et al. 1996), mice (Fujiwara et al. 1994), and rats (Komiya and Tanigawa 1995). In *Drosophila* and *C. elegans*, Vasa proteins are components of granules localized to the presumptive germ line (polar granules and P-granules, respectively)—the putative “mRNA control hubs” discussed earlier.

Genetic evidence suggests that Vasa is required to activate a family of germ-line mRNAs, including *oskar*, *nanos* and *gurken* (Dahanukar and Wharton 1996; Gavis et al. 1996; Styhler et al. 1998; Tinker et al. 1998; Tomancak et al. 1998). Although Vasa binds RNA (Liang et al. 1994), it is unclear that it interacts directly with these putative targets. However, Vasa protein does bind to *Drosophila* IF2 (dIF2; Carrera et al. 2000), a homolog of IF2 of *S. cerevisiae* (yIF2). The dIF2/Vasa complex is likely to be significant in vivo, since dIF2 and vasa mutants interact genetically (Carrera et al. 2000). Two functions in translation have been proposed for IF2: to bring initiator tRNAs to the small subunit of the ribosome (Choi et al. 1998; Lee et al. 1999) and to promote 60S subunit joining (Pestova et al. 2000). Thus, Vasa may facilitate activation of specific mRNAs by regulating IF2 activity. The conserved localization and function of Vasa in the germ line suggests that this mode of regulation may be widespread.

*Puf and Nanos Families*

*Drosophila* Pumilio and *C. elegans* FBF share eight repeats of approximately 40 amino acids, with distinctive sequences in each repeat; these repeats plus short flanking sequences are necessary and sufficient to bind their specific RNA targets (Zamore et al. 1997; Zhang et al. 1997). These structural features are shared among a large family of proteins, termed Puf proteins. Remarkably, both FBF and Pumilio bind to specific sequences in the 3'UTRs of their targets and cause repression, and both combine with Nanos-related proteins to mediate their effects. Because Pumilio and FBF are distant relatives among Puf proteins, this suggests
that other Puf proteins may also be repressors that act through the 3’UTRs of their targets.

The Nanos proteins themselves are weakly conserved, sharing a domain that contains two distinctive CCHC-containing, RNA-binding motifs. This domain is required for all the known functions of *Drosophila nanos* (Arrizabalaga and Lehmann 1999). NOS homologs have been identified in a range of species, including vertebrates, and some are expressed in the germ line (Mosquera et al. 1993; Pilon and Weisblat 1997).

Although *Drosophila nanos* and *pumilio* are best known for their roles in patterning the early embryo, they are also required for various aspects of germ-line development, including the maintenance of germ-line stem cells (Kobayashi et al. 1996; Lin and Spradling 1997; Forbes and Lehmann 1998; Asaoki-Taguchi et al. 1999; Bhat 1999; Deshpande et al. 1999; Parisi and Lin 1999). Similarly, *C. elegans nanos* homologs and several Puf proteins are required redundantly for multiple germ-line functions, including germ-line survival (Kraemer et al. 1999; Subramaniam and Seydoux 1999). In the absence of *nanos*, germ cells in fly embryos ectopically express Sxl as well as the somatic segmentation genes *fis* and *eve* (Deshpande et al. 1999). Both these effects are at the transcriptional level, suggesting that *nanos* may regulate translation of a transcription factor; alternatively, Nanos might control transcription directly, as do other RNA-binding proteins, even including translational repressors (e.g., Bicoid). Thus, the ancestral function(s) of the Puf/Nanos system may have been specific to the germ line. In this view, the specialized roles of the system, such as the sperm/oocyte switch in nematodes and axis formation in *Drosophila*, are later evolutionary accretions (Forbes and Lehmann 1998).

**STAR Proteins**

*C. elegans* GLD-1 is a member of the STAR protein family. Members of this family of RNA-binding proteins share a KH-type RNA-binding domain, plus two conserved domains that flank the KH homology region (for review, see Vernet and Artzt 1997). STAR family members are widespread, and include murine Quaking (Ebersole et al. 1996), mammalian SAM-68 (Fumagalli et al. 1994; Taylor and Shalloway 1994) and SF-1 (Kramer 1992; Arning et al. 1996), frog Xqua (Zorn and Krieg 1997), and *Drosophila* HOW proteins (Sidman et al. 1964; Hardy et al. 1996; Baehrecke 1997; Zaffran et al. 1997).

One mouse STAR protein, QKI-6, binds to TGEs and can repress translation of TGE-containing mRNAs in vitro and in *C. elegans* in vivo (Saccomanno et al. 1999). These activities mimic those of *C. elegans*
GLD-1 and suggest that STAR family proteins may commonly mediate translational repression. SF1/BBP, a mammalian STAR protein, is involved in splicing (Abovich and Rosbash 1997; Berglund et al. 1997), suggesting that STAR proteins may have diverse, or multiple, functions. The ability of STAR proteins to hetero- and homodimerize may modulate their activities or the targets they recognize (Chen et al. 1997; Zorn and Krieg 1997).

**Multiprotein Complexes**

The emerging principle that translational control often involves protein complexes has broad implications. The nature of the complexes may identify which targets are regulated, and what happens to them.

Puf proteins provide an example of the importance of protein–protein interactions among regulators. In both *C. elegans* and *Drosophila*, Puf and Nanos proteins form complexes that regulate target mRNAs. As discussed earlier, FBF and NOS-3 regulate *fem-3* in *C. elegans*, whereas Pumilio and Nanos regulate *hunchback* mRNA in flies. The details of the interactions differ in two respects. First, distinct portions of the *C. elegans* and *Drosophila* Nanos proteins are critical for interaction with their Puf partner (Kraemer et al. 1999; Sonoda and Wharton 1999). Second, the *C. elegans* interaction is RNA-independent, whereas the *Drosophila* interaction requires the RNA and only forms in its presence. Thus, the relative contributions of protein–protein and protein–RNA interactions differ in these two complexes. Nevertheless, the common Puf/Nanos partnership in flies and worms suggests that these protein families may often act in functional pairs. However, this is unlikely to be the only way Puf proteins can function, since *S. cerevisiae*, which possesses five different Puf proteins (Zamore et al. 1997; Zhang et al. 1997), lacks Nanos homologs.

Each member of a regulatory complex on one mRNA may have alternative partners and targets. For example, although FBF and NOS are both required for the sperm/oocyte switch, FBF’s role in other *nos*-mediated effects is distinct, and redundant with other Puf proteins (Subramaniam and Seydoux 1999). Moreover, although the other *C. elegans* NOS proteins are required to regulate the sperm/oocyte switch, they do not interact directly with FBF; instead, they may collaborate with other *C. elegans* Puf proteins. The combinatorial nature of regulation by these NOS and Puf proteins prompts an analogy to well-documented principles of transcriptional regulation, in which distinct protein–protein interactions between transcriptional regulators discriminate among various target DNAs and yield specific biological outcomes.
hnRNP E1 (αCP-1) and hnRNP K act together to repress LOX mRNA, but each has additional roles as well. 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). 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 may also function in the transcriptional activation of c-myc, which contains a CT-rich promoter (Takimoto 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.

**Linked Processes**

As in the film *Rashomon*, a single event—the binding of a protein to a 3′ UTR, for example—may be seen quite differently depending on the biological lens through which it is filtered. Translation, stability, and mRNA localization are interconnected.

**Translation and Localization**

mRNA localization impinges on translational regulation in two modes. In one, the movement of mRNAs to specific but large regions of the cell is critical: *oskar* mRNA is specifically directed to the posterior pole of *Drosophila* oocytes but is not translated until that destination has been reached (Kim-Ha et al. 1995). In the other, more subtle, movements, such as regulated associations with the cytoskeleton, may be targets of regulation. Clearly, these two modes of control may overlap.

As the numbers of examples of localized mRNAs increase, it should become clear whether mRNAs that are mis-localized or still in transit are commonly less active. At this early stage, this seems to 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 may include the formation of transport particles in which the mRNAs are trafficked but translation does not occur (for review, see Bassell and Singer 1997).

Although many lines of evidence argue that cytoskeletal associations enhance translation, it is unclear that these associations are regulated in a
sequence-specific fashion, independent of the large-scale movement of
the mRNA. The reconstitution of repression in in vitro systems tends to
argue that, at least in those cases, an intact cytoskeleton is not critical.

**Translation and Stability**

The connections between translation and stability are numerous, and will
not be recapitulated here (see, e.g., Jacobson and Peltz 1996; Wickens et
al. 1997; Chapters 28 and 29). However, a few comments focused on the
early embryo may be useful.

During oogenesis and early embryogenesis, many transcripts are sta-
ble, even those that lack a poly(A) tail; presumably this allows mRNAs to
accumulate over long periods in the growing oocyte, and to persist until
the stage at which their translation is first required. In frog oocytes,
mRNAs typically are stable until the so-called mid-blastula transition.
Turnover at that stage appears to require deadenylation (see, e.g., Audic
et al. 1997; Voeltz and Steitz 1998). It is possible that the same events that
would render an mRNA subject to decapping and turnover in yeast cause
translational repression in early embryos because the next step (e.g.,
decapping) simply does not occur. In particular, disruption of the end-to-
end complex might cause turnover in yeast, but repression in an oocyte.
In this context, the finding that certain sequences that cause instability in
mammalian cells cause translational repression in oocytes is provocative,
because it suggests that the same event can lead to either outcome. This
line of reasoning strongly suggests that understanding modes of decay in
yeast may directly shed light on translational control in oocytes and
embryos (Wickens et al. 1997).

In some instances, translational repression in an embryo appears to
lead to decay, whereas activation avoids that fate. For example, maternal
*hunchback* mRNA localized in the anterior is activated and persists,
whereas posteriorly localized *hunchback* mRNA never is activated and is
rapidly destroyed. Repression places the mRNA in a state that is tolerable
until the embryo’s decay apparatus has been activated; then the mRNA is
destroyed. Translational activation makes the mRNA resistant to that
turnover machinery.

**Translational Regulators with Other Functions**

An mRNA regulator can not only act with different signs—activating or
repressing—but can also affect different processes. Sex lethal regulates
both splicing in the nucleus and translation in the cytoplasm. *Drosophila*
Bicoid is a transcription factor, but it also represses the translation of caudal mRNA by binding to sites within its 3'UTR (Dubnau and Struhl 1996; Rivera-Pomar et al. 1996). hnRNP E1 increases expression of globin, in that it helps stabilize the mRNA, but decreases expression of LOX mRNA in the same cells as a member of a different complex. Thus, a given regulator may act through multiple mechanisms, only one of which is translational, to regulate an mRNA's expression. Modulating its activity, or changing its partners, may have wide and varied repercussions.

WHY TRANSLATIONAL CONTROL?

It is striking that many key decisions in development rely on translational control. Why should this be so? Clearly, during early embryogenesis, when pronuclei or zygotic nuclei are highly condensed and inactive, transcriptional control is not a major option. Controls of protein activities—regulated ligand/receptor interactions, for example—are widely exploited. What are the advantages of controlling maternal mRNA rather than maternal protein? Questions of this type are in one sense futile, as patterns of development evolve and so are restricted by contingency and history. However, within the constraints of a given developmental strategy, translational control can offer unique advantages. For example, activities involved in the earliest stages of pattern formation must be controlled in space and time. The Bicoid protein gradient cannot be established during oogenesis, because diffusion would collapse the gradient before it had a chance to act in the early embryo. For regulatory proteins such as cyclin or glp-1, premature translation would clearly disrupt the spatial localization of the regulator, and it also might disrupt the timing of interaction with downstream factors or ligands.

A related and commonly invoked rationale for translational regulation is the quickness and magnitude of the response. Although transcriptional responses can be very rapid, they do not yield high amounts of product as rapidly as translational activation. As a result, one might expect translational regulation in situations requiring a large and instantaneous change in the pattern of protein synthesis. Neuronal plasticity might be such a case, as it appears to require rapid changes at specific, newly stimulated terminals. Indeed, homologs of some of the regulatory proteins discussed here—CPEB and Staufen, for example—are present in mammalian neurons (Wu et al. 1998; Kiebler et al. 1999). Similarly, mouse Quaking protein, the founder of the STAR protein family of which GLD-1 is a member, has neurological functions (Sidman et al. 1964), consistent with the presence of Quaking proteins in oligodendrocytes and Schwann cells.
(Hardy et al. 1996). Whether the regulatory circuitry first evolved in the embryo or nervous system is unclear.

Enormous progress recently has been made in identifying regulatory proteins and elements that bind to one another. The stage is now set for delineating how these proteins interact and communicate, and how those events are controlled during development. Who does what to whom, and when? And how do the regulators, wrapped in their own liaisons, communicate with the translational machinery? These events, requiring specific mRNA–protein interactions, must be overlaid on the control of cell growth by modification of the translational apparatus. From the perspective of any one regulator, the end result of these analyses will be a local plexus of interactions and controls; collectively, it will reveal a large, dynamic web of proteins and RNAs, and unanticipated biological richness.

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