Translational Control of Developmental Decisions

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At fertilization, the calm of oogenesis is broken, 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—relies 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 now clear that messenger RNAs present in the egg before fertilization (so-called maternal mRNAs) have a 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 are controlled and how those controls contribute to proper development are the main focus of this chapter. Translational regulation is vital throughout development in somatic and 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 (3′UTR)—is a key repository for the regulation of cytoplasmic mRNAs.
Other regions of the mRNA will no doubt be found to have critical roles in developmental regulation, but thus far, the 3' UTR is preeminent.

Translational control is defined broadly in this chapter. No mechanism is implied. Ideally, translational control is demonstrated by comparing the level of a specific 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.

In this chapter, we focus on translational controls that are vital for key developmental events. We first describe specific examples drawn from a broad range of biological contexts and organisms. Having presented the facts, we then turn our attention to what generalities may be drawn, to important puzzles that remain, and to speculation.

TRANSLATIONAL CONTROL OF DEVELOPMENTAL EVENTS: SPECIFIC EXAMPLES

Meiotic Maturation and the Early Embryonic Cell Cycle

A dramatic transition from cell cycle arrest to mitotic cleavage occurs upon fertilization. In some species, it is preceded by completion of the meiotic cell cycle, referred to as oocyte maturation. To control cell cycle transitions, eggs of many species contain mRNAs that encode cell cycle regulators, such as cyclin and cyclin-dependent kinases (CDKs). For the purposes of our discussion, it is necessary only to know that cyclin and CDK form a complex that is critical in governing the cell cycle.

Cyclin mRNAs

Frog oocytes contain mRNAs encoding several different cyclins. Translation of cyclin mRNAs appears to be important both for proper post-fertilization mitoses (see, e.g., Dagle et al. 1990) and perhaps for meiotic maturation. The analysis of cyclin function in the oocyte and embryo may be complicated by the presence of multiple cyclins with overlapping roles. Nevertheless, their translational regulation is striking and informative.

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 the signals involved in these controls, chimeric mRNAs were injected that contained each 3' UTR joined to a transla-
tional 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; for review, see Wormington 1994). Thus, 3’ UTRs, by controlling polyadenylation, can impose very different patterns of translation, stimulating translation at different times and to different extents.

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). Similarly, in surf clams and sea urchins, certain cyclin mRNAs are repressed during oogenesis and 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., CDK2, histones, ribonucleotide reductase, and HGPRT; for review, see Standart 1992).

The oocyte and egg have served as model systems in the analysis of cell cycle control, with particular attention given to the regulation of preexisting cyclin and CDK proteins. Control of their synthesis likely also is critical in orchestrating the dramatic transition from quiescence to meiotic maturation and cell division.

c-mos mRNA

The c-mos proto-oncogene encodes a protein kinase that has been strongly implicated in the control of vertebrate meiosis and the early embryonic cell cycle (for review, see Yew et al. 1993; Vande Woude 1994). 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 maturation (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 se-
quence inspection, that *Xenopus* c-mos mRNA contained signals that could cause cytoplasmic polyadenylation and proposed that cytoplasmic polyadenylation of c-mos mRNA might therefore be a critical control point in meiotic maturation, as depicted in Figure 1A. 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

**Figure 1** (See facing page for legend.)
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 (Fig. 1B) (Sheets et al. 1995). The amputated mRNA, lacking its polyadenylation signals, is stable. Maturation can be restored by injection of synthetic c-mos mRNA carrying polyadenylation signals (Fig. 1C, left) or of a prosthetic RNA that brings polyadenylation signals to the amputated endogenous mRNA by base pairing (Fig. 1C, right) (Sheets et al. 1995). These experiments strongly argue that polyadenylation is critical in the activation of c-mos mRNA. They do not, however, demonstrate that polyadenylation is the only process triggered by progesterone that is critical for its activation. For example, repressors might need to be removed from the mRNA as well.

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 the first meiosis as in frogs. Rather, these oocytes complete the first meiotic division but fail to progress normally to meiosis II. This phenotype mirrors 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). Together, these experiments demonstrate that

Figure 1 Polyadenylation of c-mos mRNA as a control point in the maturation of Xenopus oocytes. (A) The hypothesis (Fox et al. 1989) suggests that among the effects of progesterone addition to frog oocytes is polyadenylation of c-mos mRNA, which enhances its translation, thereby causing an increase in the level of c-Mos protein. The elevated level of c-Mos protein is required for the continuation of meiosis (see Yew et al. 1993). Control of c-mos proteolysis (Nishizawa et al. 1992), which may also contribute to the control of c-Mos protein levels, is not depicted. Proposed biochemical roles of the c-Mos protein kinase in frog oocyte maturation (e.g., as an activator of MAPK and MPF) are discussed elsewhere (Yew et al. 1993; Vande Woude 1994). (B) Removal of c-mos polyadenylation signals prevents maturation of frog oocytes in response to progesterone. (Closed box) Polyadenylation signals, including AAUAAA and a cytoplasmic polyadenylation element; (gray box) coding region; (thin lines) 5′ and 3′ UTRs. For details, see Sheets et al. (1995); for analogous experiments in mouse oocytes, see Gebauer et al. (1994). (C) Rescue of maturation by the injection of a synthetic form of c-mos mRNA or of a prosthetic RNA. Oocytes containing the amputated version of c-mos mRNA, generated as diagrammed in B, were injected with the RNAs indicated. For details, see Sheets et al. (1995).
lack of polyadenylation signals creates a functional null for c-mos-encoded protein in mouse oocytes and hence that polyadenylation is indispensable for normal meiosis and early development.

**Specification of Cell Fate**

As development unfolds, cells assume specific fates or paths of differentiation: One cell becomes a neuron, while another becomes a lymphocyte. In this section, we discuss evidence that cell fate can be regulated at the translational level and moreover that the spatial organization of cell fates within a tissue can rely on translational controls.

*The Sperm/Oocyte Decision in the Nematode Caenorhabditis elegans*

The germ line of a C. elegans hermaphrodite makes sperm first and then oocytes, beginning at the proximal end of the tubular gonad. The onset of spermatogenesis depends on translational regulation of one sex-determining gene, tra-2 (Goodwin et al. 1993), whereas the switch from spermatogenesis to oogenesis depends on translational control of a second sex-determining gene, fem-3 (Ahringer and Kimble 1991). The spatial organization of germ-line cell fates therefore depends on the execution of two translational controls.

![Diagram A](image)

![Diagram B](image)

*Figure 2 (See facing page for legend.)*
tra-2

The *tra-2* gene normally directs female development (Hodgkin and Brenner 1977). Six regulatory mutations of *tra-2*, 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). Therefore, *tra-2* must normally be repressed to achieve the onset of hermaphrodite spermatogenesis. The *tra-2(gf)* mutants are defective in a cis-acting translational control element located in the *tra-2* 3' UTR (Fig. 2A) (Goodwin et al. 1993). This element consists of two tandem 28-nucleotide repeats and is called a direct repeat element (DRE). The DREs influence translational activity, as judged from Northern blots, polysome analyses, and chimeric reporter experiments (Goodwin et al. 1993). All the criteria thus far indicate that the DREs function as negative cis-acting regulatory elements to mediate translational repression of *tra-2* RNA.

The extent of *tra-2* translation varies with the number of DREs (Goodwin et al. 1993). Thus, the phenotype of a *tra-2(gf)* mutant with no

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**Figure 2** Regulatory elements in *tra-2* and *fem-3* mRNAs. (A) Schematic of *tra-2* 3' UTR regulatory elements. (Gray box) Coding region; (thin line) 5' and 3' UTRs. Each region of the mRNA is drawn to scale (5' UTR, 36 nucleotides; coding region, 4,425 nucleotides; 3' UTR, 218 nucleotides). At the bottom is an expanded view of wild-type and mutant *tra-2* 3' UTRs. Mutants are presented in order of the severity of their phenotype. The wild-type 3' UTR possesses two DREs (arrows) separated by four nucleotides; each DRE has the same 28-nucleotide sequence, UAGAUAUGAGAUAGAAAUAAAAUA; the four-nucleotide spacer is UGAG. The 5'DRE begins 88 nucleotides downstream from the termination codon. The strongest mutant lacks both DREs, an intermediate strength mutant harbors a transposable element (Tc1) in the more 5'DRE, and the weak mutants have one DRE left, due to a deletion or to a Tc1 insertion into the more 3'DRE. The Tc1 mutants have insertions at identical nucleotides in the 5' or 3'DRE. It is intriguing that disruption of the 5'DRE is more severe than disruption of the more 3'DRE; perhaps access to the DREs is limited by a process that moves from 5' to 3', such as transcription or translation. For details, see text and Goodwin et al. (1993). (B) Schematic of *fem-3* 3' UTR regulatory element. (Gray box) Coding region; (thin line) 5' and 3' UTRs. Each region of the mRNA is drawn to scale, the same scale as used in A (5' UTR, 248 nucleotides; coding region, 1164 nucleotides; 3' UTR, 268 nucleotides). At the bottom is an expanded view of wild-type and mutant *fem-3* 3' UTR. Approximately midway in the wild-type *fem-3* 3' UTR (123 nucleotides from the UGA termination codon) is the sequence UCUUG, which is altered in *fem-3* gain-of-function mutants. A deletion of the region has the most severe phenotype. For details, see text and Ahringer and Kimble (1991).
DRE is more severely affected than a mutant with one DRE; furthermore, \textit{tra-2(gf)} mRNA with no DRE is associated with larger polysomes than an mRNA with one DRE, which in turn is associated with larger polysomes than an mRNA with two DREs.

Strong candidates for the translational repressor of \textit{tra-2} have been identified. Each DRE specifically binds a factor, called DRF, in crude extracts (Goodwin et al. 1993). Furthermore, a recently identified gene, \textit{laf-1}, is predicted to either encode DRF or influence its activity (E. Goodwin and J. Kimble, unpubl.). Thus, a decrease in \textit{laf-1} disrupts the translational repression of a chimeric reporter gene by the \textit{tra-2} 3’ UTR (E. Goodwin and J. Kimble, unpubl.).

\textbf{fem-3}

The \textit{fem-3} gene normally directs male development (Hodgkin 1986; Barton et al. 1987). Nineteen \textit{fem-3(gf)} mutations masculinize the hermaphrodite germ line so that it produces a vast excess of sperm and no oocytes (Barton et al. 1987). Therefore, \textit{fem-3} must normally be repressed to achieve the switch in the hermaphrodite germ line from spermatogenesis to oogenesis. Each of 17 \textit{fem-3(gf)} mutant genes carries a single nucleotide change in the middle of the \textit{fem-3} 3’ UTR; the remaining two \textit{fem-3(gf)} mutations possess small deletions within the \textit{fem-3} 3’ UTR (Fig. 2B) (Ahringer 1991; Ahringer and Kimble 1991). Remarkably, all mutations either alter or remove nucleotides in a five-base-pair region, which presumably is part of the regulatory element (Fig. 2B). For simplicity, we refer to it as the point mutation element, or PME, although the regulatory element may comprise more than just these five nucleotides.

The PME appears to be a \textit{cis}-acting translational control element. Thus, the \textit{fem-3(gf)} mutations do not detectably affect transcription, splicing, or stability of \textit{fem-3} RNA, and the \textit{fem-3(gf)} mutant RNAs possess a longer poly(A) tail than their wild-type counterparts (Ahringer and Kimble 1991). Furthermore, in gel-retardation assays, the PME binds specifically to a factor in worm extracts, which we dub here PMF (Ahringer 1991). Finally, animals carrying a transgenic \textit{fem-3} 3’ UTR driven by the \textit{fem-3} promoter exhibit germ-line masculinization (Ahringer and Kimble 1991). One interpretation of this effect is that RNA corresponding to the wild-type \textit{fem-3} 3’ UTR, which is synthesized from the transgene, titrates a negative regulator from endogenous \textit{fem-3} RNA. The endogenous \textit{fem-3} RNA would thereby be activated and direct spermatogenesis inappropriately.
Figure 3 Speculative model for the role of the tra-2 and fem-3 3' UTR elements in controlling the pattern of cell fates in the hermaphrodite germ line of C. elegans. (Top) Translational activity of tra-2 or fem-3; (bottom) pattern of cell fates. Pattern is generated as differentiation proceeds from proximal (left) to distal (right). First, repression of tra-2 is required for the onset of spermatogenesis. DRF is proposed to bind DREs during larval development to repress tra-2 translation and permit spermatogenesis; fem-3, which is translationally active at this time, directs spermatogenesis for a period of hours. Next, repression of fem-3 is required for the switch from spermatogenesis to oogenesis. Thus, PMF is proposed to bind the PME to repress fem-3 translation and permit oogenesis. The translational activity of tra-2 during oogenesis is not known.

The strongest candidates for trans-acting gene products that act through the fem-3 PME are encoded by six mog genes. Among these genes, mog-1 is best characterized (Graham and Kimble 1993). The mog-1 loss-of-function phenotype is a failure to switch from spermatogenesis to oogenesis, much like the fem-3 gain-of-function phenotype. The phenotype of the other mog genes is similarly a masculinization of the hermaphrodite germ line (Graham et al. 1993). Intriguingly, all six mog genes not only are required for control of the sperm/oocyte switch, but also act maternally to influence embryogenesis. Thus, the mog genes may control not only fem-3, but other maternal mRNAs as well.

Translational Controls of tra-2 and fem-3 in Germ Line and Soma
Figure 3 depicts our current understanding of how translational controls contribute to the spatial pattern of cell fates in the hermaphrodite germ
line. The temporal regulation of two translational controls appears to underlie the spatial pattern of differentiation in the gonad: proximal sperm and distal oocytes (Fig. 3). First, translational repression of tra-2 permits the onset of spermatogenesis at one end of the gonad. Later, translational repression of fem-3 mediates the switch from spermatogenesis to oogenesis in more distal cells. Thus, translational controls of tra-2 and fem-3 are central for determining the spatial distribution of those cell fates in the gonad.

As emphasized above, translational controls of tra-2 and fem-3 are essential for specification of germ-line cell fates. Importantly, translational controls of tra-2 and fem-3 also influence determination of sexual characteristics in somatic tissues (Doniach 1986; Schedl and Kimble 1988). The germ-line and somatic controls depend on the same 3' UTR regulatory elements and may depend on the same trans-acting regulatory machinery (E. Goodwin et al., unpubl.). For example, laf-1 is required for translational repression via the DREs in both germ line and soma (E. Goodwin and J. Kimble, unpubl.).

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 controls 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 have 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 derepression 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 proposes 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 (De Benedetti and Rhoads 1990; Lazaris-Karatzas et al. 1990; Sonenberg, this volume). In this case, as in mesoderm induction, elevation of the levels of a general translation factor has dramatic effects on cell fate.

**Pattern Formation: Embryonic Axes**

During oogenesis and early embryogenesis, asymmetries become evident that foreshadow the anterior-posterior, dorsal-ventral, and left-right axes of the mature organism. Translational controls are critical for establishing body axes (for review, see Wharton 1992; Curtis et al. 1995). In *Drosophila*, each of the maternal patterning systems (St Johnston and Nüsslein-Volhard 1992) requires the translational control of one or more mRNAs (for examples, see Table 1). Recent evidence from *C. elegans* and *Xenopus* suggests that one mechanism of controlling embryonic asymmetry—localized translational repression—may be a very primitive and universal means of laying down the anterior-posterior axis.
### 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)</td>
<td>anterior determinant, activates genes required for head and thorax formation (Frohnhoefer 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>Posterior</td>
<td><em>nanos</em> (Gavis and Lehmann 1994)</td>
<td>posterior determinant (Wang and Lehmann 1991; Wang et al. 1994); collaborates with <em>pumilio</em> to suppress translation of posterior maternal <em>hunchback</em> mRNA (Hulskamp et al. 1989; Irish et al. 1989; Struhl 1989a; Murata and Wharton 1995), which encodes a transcription factor (Hulskamp et al. 1990)</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>Dorsal-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>
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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 may commonly require cytoplasmic polyadenylation. *bicoid*, *torso*, and *toll* mRNAs, encoding key regulatory proteins for the anterior, terminal, and dorsal-ventral patterning systems, respectively (Table 1), undergo polyadenylation concomitant with their activation. The role of polyadenylation in the activation of *bicoid* mRNA has been examined in some detail (Sallés et al. 1994). The poly(A) tail of endogenous *bicoid* mRNA increases from about 50 nucleotides in oocytes, where it is translationally silent, to 150 nucleotides in the early embryo, coincident with its activation. mRNA injection experiments indicate a critical role for cytoplasmic polyadenylation in activation: In-
jected bicoid mRNA possessing its wild-type 3’ UTR receives poly(A) and rescues a bicoid mutant embryo, whereas a truncated transcript lacking part of the 3’ UTR lacks both polyadenylation and rescuing activity. Importantly, the rescuing activity of the truncated mRNA is restored (although not to wild-type levels) by the addition, in vitro, of a 150-nucleotide poly(A) tail. A mere 50 nucleotides, as is present in the oocyte, do not suffice. These data demonstrate that polyadenylation is critical in the activation of bicoid mRNA (and by extrapolation, perhaps to torso and toll mRNAs as well) and indicate that signals required for activation lie, at least in part, in the 3’ UTR.

Unlike bicoid mRNA, translational activation of mRNA encoding nanos, a posterior determinant, does not involve a detectable change in the length of its poly(A) tail upon fertilization (Sallés et al. 1994). Although the mechanism of nanos mRNA activation is not understood, the posterior location of the mRNA in the egg is critical (Gavis and Lehmann 1994). Normally, nanos mRNA is produced in nurse cells, imported into the oocyte at its anterior end, and then transported to its final destination at the posterior pole. nanos mRNA can be activated after fertilization if it resides at the posterior pole; placed in more anterior regions by manipulation of its localization signals, it remains repressed (Gavis and Lehmann 1994). The posterior localization of nanos mRNA may counteract an unlocalized nanos translational repressor. In wild-type embryos, although nanos mRNA is concentrated at the posterior pole, it is present at a low level throughout the embryo (Gavis and Lehmann 1994). Presumably, repression outside the posterior pole is required to avoid deleterious effects of Nanos protein in the anterior, namely, inhibition of head and thorax development (Wharton and Struhl 1989; Gavis and Lehmann 1992; Gavis and Lehmann 1994). The nanos 3’ UTR contains elements that not only cause the localization of mRNA to the posterior, but prevent its translation when it is located elsewhere in the embryo (Gavis and Lehmann 1994).

Regulated Repression
The anterior-posterior axis is established by a set of maternally contributed mRNAs, including bicoid, caudal, nanos, pumilio, and hunchback. Establishing a gradient of Hunchback protein, with high Hunchback in the anterior and low Hunchback in the posterior, is essential. To achieve this gradient, Bicoid protein at the anterior pole activates zygotic transcription of hunchback, whereas Nanos and Pumilio proteins at the posterior pole repress translation of maternal hunchback mRNA.
Repression of maternal *hunchback* mRNA depends on regulatory elements in the *hunchback* 3' UTR, called Nanos Response Elements (NREs; Wharton and Struhl 1991). Both Pumilio and Nanos proteins are necessary for repression of maternal *hunchback* mRNA (Barker et al. 1992); neither protein alone is sufficient. Pumilio protein binds NRE-containing RNAs specifically, and this probably underlies its repressive activity (Murata and Wharton 1995). On the basis of the available evidence, the following model has been proposed to explain how these proteins might collaborate to repress *hunchback* translation: Uniformly distributed Pumilio binds directly to the NREs but is incapable alone of repressing translation. However, in the posterior of the embryo, Nanos protein is recruited to the NREs via protein-protein interactions, enabling suppression of the mRNA. In this elegant model, the asymmetric repression of *hunchback* mRNA function is provided by the asymmetric distribution of Nanos, which may not in fact interact directly with the RNA.

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. In the ab-

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**Figure 4** Anterior-posterior gradients generated by translational activation and repression. See text for details.

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1. May occur through direct interaction of bicoid protein with *caudal* mRNA; see text for details.

2. May occur through direct interaction of pumilio protein with *hunchback* mRNA; see text for details.
sence 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 the 3′ UTR of caudal mRNA and this interaction appears to be essential for translational repression (Dubnau 1995; J. Dubnau and G. Struhl, in prep.). 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 (Dubnau 1995; J. Dubnau and G. Struhl, in prep.).

The regulation of axis-forming genes thus involves two parallel cascades of translational control at opposite ends of the embryo (Fig. 4). 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 and ultimately activates zygotic transcription of hunchback; at the posterior, Nanos protein represses hunchback mRNA. The posteriorly localized hunchback mRNA is destroyed (Tautz and Pfeifle 1989). Thus, this web of interactions establishes opposing gradients of Hunchback and Caudal proteins.

A Potentially Primitive Mechanism for Establishing Asymmetry

Translational regulation is critical for polarity along the anterior-posterior axis in C. elegans as well as in Drosophila (Evans et al. 1994; Kimble 1994). This translational regulation effects the asymmetric expression of a membrane receptor, encoded by the glp-1 gene. The Glp-1 protein is required for inductive cell interactions in the early nematode embryo (Priess et al. 1987; Hutter and Schnabel 1994; Mello et al. 1994; Moskowitz et al. 1994). When Glp-1 protein first appears, at the two- to four-cell stage, it is found in anterior but not posterior blastomeres; glp-1 maternal mRNA, in contrast, is distributed uniformly (Evans et al. 1994). The anterior-posterior asymmetry of Glp-1 protein may be crucial for establishment of asymmetric patterns of cell-cell interactions, which in turn specify dorsal-ventral and left-right axes of the C. elegans embryo (Hutter and Schnabel 1994; Mello et al. 1994; Moskowitz et al. 1994).

glp-1 mRNA is subject to two distinct translational controls (Evans et al. 1994). One is temporal: glp-1 is translationally silent in oocytes and one-cell embryos, but it becomes active by the two- to four-cell stage. The second is spatial: glp-1 is translationally silent in posterior blastomeres but active in anterior blastomeres. The elements responsible
for both controls reside in the 3' UTR. Sequences responsible for spatial regulation lie in a 39-nucleotide region in the central region, whereas those required for temporal control lie at the 3' end of the 3' UTR (Evans et al. 1994; T. Evans and J. Kimble, unpubl.). Within the 39-nucleotide sequence is a bipartite sequence with striking similarity to an NRE from *Drosophila* (Fig. 5A). Therefore, asymmetry in early *C. elegans* and *Drosophila* embryos may be established by analogous mechanisms: Translation of uniformly distributed *hunchback* and *glp-1* maternal transcripts is restricted to anterior regions of their embryos, through spatially controlled translational repression (Fig. 5B). The similarity in the sequence elements involved—the NREs of *Drosophila* (Wharton and Struhl 1991) and the NRE-like motif of *C. elegans* (Evans et al. 1994)—suggests that both the molecular components and the overall strategy may be conserved.

The molecular parallels between *hunchback* and *glp-1* regulation suggest the existence of an ancient mechanism for creating asymmetric patterns of gene expression in early embryos (Fig. 5B). A hint that this mechanism may function in vertebrates comes from the identification of...

*Figure 5* A potentially primitive mechanism for establishing asymmetry. (A) Similar regulatory elements. The 3' UTRs of *Drosophila hunchback* and *C. elegans glp-1* contain sequence elements predicted to mediate translational control. The *Drosophila* NRE is a bipartite element: GUUGU separated by five nucleotides from AUUGUA. Two copies of this element reside in the *hunchback* 3' UTR, and 1.5 copies reside in the *bicoid* 3' UTR. The *C. elegans* NRE-like motif has a similar sequence, although the spacer region is larger. The significance of the NRE-like sequences of *C. elegans* remains to be shown by site-directed mutagenesis, although the coincidence of the motifs is striking. A 39-bp deletion that removes the 34-nucleotide sequence shown plus an additional 5 bp disrupts spatial control (T. Evans and J. Kimble, unpubl.). Each mRNA is drawn to the same scale. Two different *hunchback* mRNAs exist, differing in their 5' UTRs (either 510 or 146 nucleotides); the coding region contains 2276 nucleotides, and the 3' UTR contains 562 nucleotides. The first NRE begins 55 nucleotides downstream from the termination codon. For *glp-1* mRNA, the 5' UTR contains 3885 nucleotides, and the 3' UTR 365 nucleotides. The 5'-most AAUGA sequence, part of the *glp-1* NRE-like elements, lies 182 nucleotides downstream from the *glp-1* termination codon. (B) Proposed similarities in posterior translational repression in *Drosophila* and *C. elegans* embryos. *hunchback* and *glp-1* maternal mRNAs are uniformly distributed, whereas the Hunchback and Glp-1 proteins are expressed in the anterior. (*Black dots*) Polar granules in *Drosophila* and P granules in *C. elegans*. Maternal mRNA encoding nanos appears to be associated with polar granules; perhaps a translational repressor is associated with P granules in *C. elegans*.
a maternal *nanos*-like RNA, called Xcat-2, which is localized to the vegetal pole of *Xenopus* embryos (Mosquera et al. 1993). Although the function of Xcat-2 is unknown, its location suggests a role in early pattern formation. If similar molecular machinery regulates polarity in embryos as diverse as worms, flies, and frogs, it seems plausible that this mechanism participates in axis formation in all animal embryos, including mammals. "Molecular tinkering" (Jacob 1982) might then come into play to reinforce or modify this primitive strategy and derive other axes from it.

**Temporal Control of Developmental Events**

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. This progression from L1 to adulthood depends on several "heterochronic" genes, including *lin-14* and *lin-4* (for review, see Ambros and Moss 1994).

![Diagram](image)

**Figure 5** (See facing page for legend.)
Translational regulation of lin-14 is essential for progression through the life cycle. The lin-14 gene directs L1-specific events (Ambros and Horvitz 1984). Normally, L1 larvae possess abundant lin-14-encoded protein, whereas later stages in the life cycle possess little or none (Ruvkun and Giusto 1989). In contrast, lin-14 mRNA is equally abundant throughout larval development (Wightman et al. 1993). Two lin-14(gf) mutants reiterate the L1 larval stage and possess Lin-14 protein throughout larval development (Ambros and Horvitz 1984; Ruvkun and Giusto 1989). The molecular defects in lin-14(gf) mutations reside in the DNA encoding the lin-14 3′ UTR (Ruvkun et al. 1989; Wightman et al. 1991). Furthermore, the lin-14 3′ UTR can confer upon a reporter gene a pattern of expression typical of Lin-14 protein (Wightman et al. 1993). Therefore, the lin-14 3′ UTR is essential for the translational down-regulation of lin-14 activity and progression into the L2 stage.

Both genetic and molecular analyses indicate that lin-4 is required for the translational repression of lin-14 (Ambros 1989; Arasu et al. 1991). Animals lacking lin-4 activity reiterate L1-specific events (Chalfie et al. 1981), as do lin-14(gf) mutants. Furthermore, lin-4 activity is essential for the translational repression of a chimeric reporter gene by the lin-14 3′ UTR (Wightman et al. 1993). Remarkably, the lin-4 gene encodes two short RNAs (22 and 61 nucleotides) with no apparent coding capacity for synthesis of a protein product. Instead, both RNAs have antisense complementarity to each of seven conserved elements present in the lin-14 3′ UTR (Fig. 6) (Lee et al. 1993; Wightman et al. 1993). Therefore, the lin-4 RNAs themselves are likely to be at least part of the trans-acting machinery that regulates lin-14 translation. Proteins may also be critical, however. The secondary structures of each potential hybrid, and the sequence of the "looped-out" regions (Fig. 6B), are quite similar and could serve as protein-binding sites.

Figure 6A presents a simple binary switch, in which lin-14 is translationally active during L1 and repressed in late L1 to effect the transition to L2. However, various lines of evidence suggest that the control may be more complex. Although the data are not yet definitive, a temporal gradient for lin-14 activity has been suggested, with high, intermediate, and low levels of lin-14 activity directing the L1, L2, and L3 stages of the life cycle, respectively (Austin and Kenyon 1994 and references therein). The presence of seven cis-acting elements in the lin-14 3′ UTR provides a plausible molecular mechanism for generating such a temporal gradient: Increasing occupancy of lin-14 sites by lin-4 RNA might result in graded repression of translation.

The identification of the lin-4 repressor is unambiguous, and its likely
Figure 6 lin-14 mRNA and the lin-4 repressor. (Top) Speculative model for the role of lin-4 in translational repression of lin-14 mRNA. (Gray 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 (the arrowhead is at the 3’ end of lin-4 RNA). The lin-14 3’ UTR possesses seven conserved elements (1–7) that are likely to be translational regulatory elements. During the L1 larval stage, lin-14 is translated; the translational repressor, lin-4, then associates with regulatory elements and lin-14 becomes translationally repressed. The mRNA is not drawn to scale. (Bottom) Possible hybrids between lin-14 mRNA and lin-4 RNA. (Open rectangles) Elements in lin-14 mRNA; (closed rectangles) lin-4 RNA. The location of a point mutation in lin-4 which reduces its activity is indicated by an open triangle in hybrids 1, 2, 4, and 6. Note that only a subset of these structures may be needed for repression and that a direct demonstration of the hybrids depicted is not yet available.

binding sites have been identified by sequence inspection. The biochemical mechanism of its action is unknown, however, and could involve collaboration with proteins. Nevertheless, the data hint that the trans-acting regulators of other mRNAs may, like the lin-4 repressor, be RNA, not protein (see Wickens and Takayama 1994).

Terminal Differentiation

Certain genes are expressed late in differentiation, as cells take on their ultimate fates. In the first two examples of terminal differentiation de-
scribed below—spermatogenesis and red blood cell differentiation—the nucleus is effectively silenced: The spermatid pronucleus is highly condensed and inactive, and in most 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. In the third example, involving myogenesis, the nucleus remains active, and transcriptional regulation is in fact critical.

**Spermatogenesis**

The male gamete exhibits translational control of various mRNAs (for listing, see Braun et al. 1989). Mouse protamine-1 (mP1) mRNA is stored in a dormant form as a ribonucleoprotein particle for 1 week in round spermatids before it is activated in elongating spermatids (Balhorn et al. 1984; Kleene et al. 1984). Sequences in the mP1 3’ UTR are necessary and sufficient for proper temporal regulation in transgenic mice (Braun et al. 1989).

Activation of mP1 mRNA is accompanied by shortening of its poly(A) tail (Kleene et al. 1984; Braun et al. 1989). This deadenylation may cause, or be a consequence of, translational activation. *Drosophila* spermatogenesis further complicates the issue, as translational activation of two mRNAs, Mst87D and Mst87F, in postmeiotic spermatids is correlated with an increase in poly(A) tail length (Schaefer et al. 1990; Kuhn et al. 1991).

**15-Lipoxygenase and Red Blood Cell Differentiation**

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 mitochondria. 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).

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). In recent elegant experiments, a 48-kD protein has been purified that binds to this sequence and causes translational repression specifically of LOX mRNA in vitro (Ostareck-Lederer et al. 1994). mRNAs containing as few as two repeats are fully repressed, whereas a single repeat yields only slight repression (B. Thiele, pers. comm.). Important-
ly, repression in vitro appears to be independent of any change in poly(A) length and of the 5′-terminal cap; we return to these points below.

*Myogenesis: A Surprising Feedback Loop*

Myoblasts fuse to form myotubes that express muscle-specific genes at high levels. Myogenic transcription factors, such as myoD, myogenin, and Myf5, are key players in the specification of muscle cell fate (for review, see Weintraub 1993; Olson and Klein 1994). However, a search for genes that influence muscle differentiation has revealed a remarkable regulatory circuit that may operate at the translational level. In cultured myoblasts, overexpression of the tropomyosin 3′ UTR can cause transcriptional activation of muscle-specific genes, such as myosin, actin, and myoD (Rastinejad and Blau 1993; for review, see Wickens 1993). Thus, it appears that a positive feedback loop may reinforce the decision to become muscle. How does this work?

Myogenic differentiation and cell division appear to be mutually antagonistic (for review, see in Halevy et al. 1995; Parker et al. 1995). Remarkably, the tropomyosin 3′ UTR inhibits cell growth in culture (Rastinejad and Blau 1993) and in transplanted tumor cells (Rastinejad et al. 1993). Thus, by inhibiting growth, the commitment to muscle differentiation might be reinforced and cause activation of muscle-specific genes. The mechanism by which the tropomyosin 3′ UTR acts is unclear, but it may involve inhibition of the cell’s translation apparatus. In in vitro translation assays, the tropomyosin 3′ UTR inhibits translation of a reporter mRNA in *trans*, apparently by binding to and activating a cellular kinase, PKR (protein kinase, RNA-activated; Davis and Watson 1996). Active PKR phosphorylates eIF2α, blocking eIF2 function and thereby depressing overall protein synthesis (see Clemens, this volume). In vivo, inhibition of general protein synthesis might depress cell growth and thereby stimulate myogenesis. The translational inhibition presumably would be transient because muscle-specific mRNAs are ultimately translated efficiently. The finding that expression of inactive, dominantly interfering PKR mutants in NIH-3T3 cells elicits a transformed phenotype is consistent with a role for this kinase in growth control (Koromilas et al. 1992; Meurs et al. 1993).

An alternative explanation of the ability of tropomyosin 3′ UTR to repress cell growth and stimulate transcription of muscle-specific genes involves effects on the recently discovered stimulation of CDK inhibitors by MyoD (Halevy et al. 1995; Parker et al. 1995; Skapek et al. 1995).
The tropomyosin 3′ UTR could control a specific mRNA in that pathway in *trans*, perhaps by titrating a critical RNA-binding protein. With some of the key players in that circuit now identified, it may be possible to test this hypothesis directly.

A feedback circuit involving the tropomyosin 3′ UTR has not yet been demonstrated in the intact mouse. Nevertheless, the work to date establishes the important principle that a single 3′ UTR, acting in *trans*, can dramatically affect a cell’s properties.

**Masking**

The masking hypothesis, initially proposed by Spirin (1966) nearly 30 years ago, 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. The mRNA itself is unaltered. In its initial formulation, masking was proposed to explain the dramatic increase in protein synthesis observed in sea urchin eggs at fertilization, an effect that is in part due to stimulation of the translation machinery itself. As it is now understood (see, e.g., Standart 1992; Standart and Jackson 1994), masking during early development is operationally defined using extracts derived from oocytes and early embryos. In vivo, a specific mRNA is repressed in the oocyte; upon fertilization, it becomes active. The patterns of protein synthesis are maintained in extracts of oocytes and early embryos; in particular, in the form of mRNPs (mRNA-protein complexes), mRNAs that are repressed in vivo continue to be repressed when translated in vitro. Removal of the proteins 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).

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). An 82-kD protein that may bind to the critical sequences and participate in masking has been identified by UV cross-linking (Standart 1992).
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.

The approaches used to study masking of maternal mRNAs differ substantially from those in which regulatory elements have been identified either genetically or by mRNA injections. Masking of maternal mRNAs is classically defined as repression, which then is followed by activation, or unmasking. Only some of the mRNAs we have discussed in previous sections, bicoid and glp-1, 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; fem-3 and lin-14 mRNAs are initially translationally active and then are shut off, the opposite of the situation in classical "masking." It is uncertain whether these different mRNAs are repressed by the "masking" mechanism exemplified by ribonucleotide reductase mRNA.

**REGULATORY ELEMENTS: GENERAL ISSUES**

Although the regulatory elements discussed here come from many different organisms and control a dramatic array of developmental decisions, they share certain unmistakable similarities.

**3′ UTRs**

All of the elements we have described reside in the 3′ UTR. It is remarkable that methods ranging from classical genetics to biochemistry should all converge on this region of the mRNA. Perhaps the strongest argument that regulatory elements commonly reside in 3′ UTRs arises from genetic analysis: Screens for mutant animals that misregulate key genes have repeatedly yielded mutations in 3′ UTRs, even though the selection schemes had no such bias.

Why the 3′ UTR? The prevalence of regulatory elements in 3′ UTRs may reflect the fact that 3′ UTRs are unconstrained in evolution and so provide fertile ground for the derivation of new regulatory elements (see 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.

**Negative and Positive Control**

By definition, negative elements repress translation, whereas positive elements either activate it or are required for translation per se. With the exception of the signals that control cytoplasmic polyadenylation, all of the other regulatory elements identified thus far in the 3′ UTRs of mRNAs critical for development are negative. Negative control elements might work in either of two modes. Certain negative elements 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., *fem-3* and *lin-14* mRNAs).

Sequences that control cytoplasmic polyadenylation may be both positive and negative. Most commonly, they are identified as positive elements required for polyadenylation and translational activation; mutant mRNAs lacking cytoplasmic polyadenylation elements do not receive poly(A) and their translation is not activated. However, the same elements may also be required to silence the mRNA as it emerges into the cytoplasm, through a process involving poly(A) shortening (Huarte et al. 1992).

Translational control elements in 3′ UTRs may be either on-off switches or rheostats. Many regulatory elements in 3′ UTRs are tandemly repeated. Indeed, the existence of repeated, conserved sequences in 3′ UTRs is reasonable prima facie evidence of a control element. Elimination of some but not all of the regulatory sites in *tra-2* (Goodwin et al. 1993), *lin-14* (Wightman et al. 1993), and LOX mRNAs (B. Thiele et al., pers. comm.) 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 facilitate cooperative binding of regulatory factors and facilitate concerted repression.

The genetic selections that have revealed negative translational control elements in 3′ UTRs, as have been carried out with *tra-2* and *fem-3*, have been exceptionally powerful. As a result, even very rare alleles could be isolated. The frequency of their isolation was one to two orders of magnitude less than that of typical knockout mutations. Negative ele-
ments may well exist in many other genes but have escaped genetic detection.

**Germ Line and Soma**

Translational regulation is vital in both germ-line and somatic tissues. The importance of translational regulation in differentiation of somatic tissues is often disregarded, since the number of well-documented examples is far fewer in somatic cells than in eggs. Moreover, some of the examples that do exist actually undermine the notion that somatic regulation might be general: LOX and protamine mRNAs are translationally regulated in "peculiar" cells in which transcriptional control is impossible. Nevertheless, we suggest that translational control likely has a wider role than previously expected in somatic decisions. The case of lin-14 is exemplary: It must be repressed in somatic cells at the L1 stage for cells to advance to their next developmental fate. Similarly, fem-3 and tra-2 mRNAs are translationally regulated in somatic tissues, as well as in the germ line, and that regulation is critical in determining cell fate. Oocytes and embryos, we suspect, do not exploit mechanisms peculiar unto themselves.

**SPECULATIONS ON MECHANISM**

The examples described earlier demonstrate that translational activity can be negatively controlled by sequences in the 3′ UTR. Two central questions thus arise. First, how is translational repression exerted? Second, for those mRNAs that are first repressed and later activated, how is derepression accomplished? We discuss possible answers to these questions in turn. Both discussions are necessarily speculative as no conclusive answer to either of these problems is available in any system.

**Translational Repression through the 3′ UTR**

Translational repression could be achieved by interfering with initiation or elongation. In several instances, repressed mRNAs appear not to be associated with ribosomes or ribosomal subunits, suggesting that initiation is defective (for review, see Spirin, this volume). We therefore discuss five plausible mechanisms by which initiation may be prevented by elements in the 3′ UTR. The proposed mechanisms of repression likely differ among mRNAs and are not mutually exclusive. For previous discussions of 3′ UTR-mediated repression, presented with varying empha-
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, which hides the mRNA in much the same way as chromatin condensation hides DNA from the transcription apparatus.

Y-box proteins, such as FRGY2 (also known as mRNP4), may be important in the formation of structures that cause repression (for review, see Wolffe et al. 1992; Wolffe 1994). FRGY2 is expressed in oocytes and not in somatic cells; homologs are present in somatic cells and may have comparable functions (for review, see Spirin, this volume). Y-box proteins, including FRGY2, are bona fide transcription factors (Tafuri and Wolffe 1990, 1992), yet are physically associated with many different maternal mRNAs (see, e.g., Darnbrough and Ford 1981; Dearsley et al. 1985; Murray et al. 1991; Tafuri and Wolffe 1993) and can inhibit their translation (Richter and Smith 1984; Kick et al. 1987; Ranjan et al. 1993; Bouvet and Wolffe 1994). These data suggest 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 (Tafuri and Wolffe 1993; for a contrary view, see Kick et al. 1987). 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 to chromatin: Core histones are present on active and inac-
tive genes, but their positions and higher-order structures differ and may
have a critical role in regulating transcriptional activity.

Interfering with 5' Cap Function

The 5'-terminal cap may be hidden from initiation factors through a
specific RNA-protein interaction. In one form of this model (Standart
and Jackson 1994), a protein bound to a regulatory site in the 3'UTRs
prevents initiation by simultaneously binding the cap. 3'UTR-bound
factors might interact directly with the cap or with proteins that do so.
The finding that LOX mRNA without a cap can be repressed efficiently
in vitro by LOX-BP (Ostareck-Lederer et al. 1994) argues against this
model, at least in this case.

If the cap and poly(A) tail interact (discussed in Decker and Parker
1994; see Richter; Jacobson; both this volume), then repression could be
cau sed by interfering with this interaction. 3'UTR-bound factors could
interfere with the interaction by recognizing the cap, poly(A), or proteins
associated with either element.

Keeping Poly(A) Tails Short

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

Two groups of mRNA injections support the hypothesis that the
repression of a maternal mRNA is caused by the shortness of its poly(A)
tail. In Drosophila, injected bicoid mRNAs with long poly(A) tails res-
cue bicoid mutant embryos, whereas the same mRNAs with shorter tails
do not (Sallés et al. 1994); similarly, injected murine t-PA mRNAs are
active with long, but not short, poly(A) tails, corresponding to their states
before and after oocyte maturation (Huarte et al. 1992). The hypothesis
that short tails cause repression leaves serious problems unresolved,
however. For example, poly(A) tails as short as 25–50 nucleotides stimu-
late translation relative to an mRNA with no tail in several assay sys-
tems, both in vitro and in vivo (Jacobson; Richter; both this volume), yet
repressed mRNAs often have tails longer than this. A further complica-
tion arises from the studies of ribonucleotide reductase mRNA discussed earlier. If its repression is due to its having "too short" a tail, then why does removal of its poly(A) tail (and 3′UTR) turn it on?

These considerations suggest that the repression of certain mRNAs may not be due to their having too short a tail but to a poly(A)-independent mechanism (such as the others discussed in this section). This does not preclude the possibility that poly(A) addition may have an important role in derepression.

**Changing the Cellular Micro-Environment of the mRNA**

Repressors bound to sites in the 3′UTR might move the mRNA into a cellular micro-environment that is translationally compromised. For example, the association of mRNAs or polyribosomes with the cytoskeleton may influence translation in vivo (for review, see Singer 1993a). Factors bound to the 3′UTR might prevent that association. One specific form of this hypothesis suggests that association with the cytoskeleton promotes interactions between translation factors and the mRNA, either because the translation factors themselves are associated with the cytoskeleton ( Howe and Hershey 1984) or because association enhances ribosome recycling (Decker and Parker 1994).

Although mRNA sequences that cause cytoskeletal associations have not been identified, the elements that control mRNA "macrolocalization," such as movement to one end of a cell, often reside in the 3′UTR. These elements, sometimes called mRNA "zip codes," presumably are required for mRNA association with the cytoskeleton, movement and ultimate retention at its destination (for review, see Ding and Lipshitz 1993; Singer 1993b; Wilhelm and Vale 1993). Comparable elements may regulate more subtle movements as well.

**Derepression**

**Changes in Regulatory Components**

Negative cis-acting regulatory elements mediate repression; delete them, and the mRNA is activated aberrantly. If trans-acting factors are required for repression, which seems likely, then derepression presumably involves a change in 3′UTR-associated factors—dissociation from the mRNA, modification, or association with new components. In several systems, apparent regulatory factors are phosphorylated as repression is relieved (for review, see Standart and Jackson 1994). The temporal coin-
Cedence of phosphorylation and derepression suggests that phosphorylation may cause the change in translational activity, although no direct evidence yet demonstrates that this is so.

In the case of positive elements, such as those that cause polyadenylation, it appears that activation entails stimulation of a previously quiescent apparatus, probably through the activation of latent polyadenylation factors (Fox et al. 1992; Bilger et al. 1994; Richter, this volume). CPEB, a positive-acting polyadenylation factor (Hake and Richter 1994), is phosphorylated during maturation concomitant with polyadenylation of an mRNA to which it binds (Paris et al. 1991). Poly(A) polymerase also undergoes phosphorylation during maturation (Ballantyne et al. 1995). However, the role of phosphorylation in the activation of these or other polyadenylation factors, such as CPSF (see, e.g., Bilger et al. 1994) is uncertain.

**Connections between Negative Elements, 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 so will not be recapitulated here (see Munroe and Jacobson 1990; Wickens 1992; Wormington 1993; Standart and Jackson 1994; Richter; Jacobson; both this volume). Instead, we focus explicitly on the connection between polyadenylation and negative translational control elements, so prevalent among critical maternal mRNAs. We consider three of many pathways that can explain how negative elements in the 3′ UTR and polyadenylation might cause translational derepression. The pathways are depicted in Figure 7, in a simplified form intended to prompt consideration of other alternatives not considered here.

**Pathway 1.** Negative elements in the 3′ UTR repress translation by keeping the tail short. The mRNA is derepressed by permitting polyadenylation. For example, a 3′ UTR-bound factor that represses polyadenylation might dissociate, resulting in poly(A) lengthening, and, as a consequence, translational activation. This model accommodates the behavior of certain mRNAs very well, but it clearly does not account for those in which translational activation occurs without polyadenylation.

**Pathway 2.** Negative elements in the 3′ UTR repress translation by preventing initiation: This repression is independent of poly(A) length. However, polyadenylation is required to inactivate the repressor. For example, repressors bound to the element might be removed or modified by
Possible pathways of de-repression

1. 3'UTR → poly(A) → translation
2. poly(A) → 3'UTR → translation
3. 3'UTR

Figure 7 Possible pathways of derepression. "3' UTR" designates a negative regulatory element in the 3' UTR. For derepression, the activity of this element (or of the repressor to which it binds) must be shut off. "Poly(A)" indicates an increase in poly(A) length. "Translation" indicates derepression of translation. Thus, in the first pathway, shutting off the activity of the negative element (either by mutating the element or by regulating the repressor to which it binds) leads to an increase in poly(A) length, which in turn causes translational activation. Other possible pathways exist but are not depicted.

the binding of polyadenylation machinery. This pathway is suggested by experiments on G10 and C12 mRNAs 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; Richter, this volume).

**Pathway 3.** Negative elements in the 3' UTR control polyadenylation and translation independently. For example, factors bound to the elements might repress by causing formation of an mRNP structure that hides the mRNA from both the translation apparatus and polyadenylation factors. Once the mRNA is exposed, both the translation and polyadenylation machineries act. Polyadenylation would then be required to maintain or enhance translational activity.

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, as observed with LOX and ribonucleotide reductase mRNAs, would be due to execution of the initiation step alone; 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 re-
quire a separate initiation step. Indeed, synthetic mRNAs injected into the cytoplasm, of the sort commonly used to assay the effect of polyadenylation on translation, may escape a form of repression to which endogenous mRNAs are subject (Bouvet and Wolffe 1994), and so may assay only part of the translational control pathway. Consistent with this view, mRNAs injected directly into the frog oocyte cytoplasm are more active than are the same mRNAs injected into the nucleus and transported to the cytoplasm subsequently (Bouvet and Wolffe 1994; Braddock et al. 1994). Although the mechanism by which "nuclear experience" causes lasting repression is unknown, antibody injection experiments suggest that it requires Y-box proteins, such as FRGY2 (Bouvet and Wolffe 1994; Braddock et al. 1994). Repression due to nuclear experience is relieved during oocyte maturation (Braddock et al. 1994), suggesting that such repression is reversible and may have a role in regulation in vivo.

NETWORKS, CASCADES, AND LOOPS

Networks of transcriptional control are commonplace and have crucial roles in development. A single transcription factor can activate and repress many genes, including those encoding other transcription factors; the intricate interactions of regulatory proteins at a promoter all provide inputs into the expression of a single gene. How similar might translational controls be, particularly during early development? Are there batteries of mRNAs that are interconnected through common factors?

Indications already exist of multiple translational control elements in a single 3′UTR: The glp-I 3′UTR contains separable spatial and temporal control elements; the nanos 3′UTR contains elements that move it to the posterior and permit its activation in the posterior but not elsewhere. In some respects, work on 3′UTRs may be at a stage equivalent to analysis in the early days of developmentally controlled promoters. We see separable elements, and hints of combinatorial regulation, but may not yet appreciate the depth of the complexity.

Cytoplasmic polyadenylation provides one obvious example of a potential network: Many mRNAs apparently require polyadenylation for translational activation or deadenylation for their inactivation. Thus, a change in polyadenylation activities or their regulators could coordinate-ly control many mRNAs (for discussions, see Wickens 1992; Richter; Jacobson; both this volume).

In flies, the translational control circuits involved in formation of the anterior-posterior axis rely not only on the common activation of many
mRNAs, but also on repression by one another's protein products. Do any of those repressors act on other, as yet unidentified, mRNAs? The observation that *pumilio* mutants, in addition to having effects on the anterior/posterior axis, have phenotypes during oogenesis and in somatic cells suggests that *pumilio* may regulate other mRNAs besides *hunchback*. Similarly, the *C. elegans mog* genes, which affect both the sperm/oocyte switch and embryogenesis more generally, may control the translation of numerous maternal mRNAs.

Regulatory components that act on more than one gene might be revealed by examining the phenotypes of animals overexpressing RNA regulatory signals. The feasibility of such an approach has been demonstrated by studies with the negative control element in the 3′ UTR of *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 which binds to the element has been titrated out and can no longer repress the endogenous *fem-3* mRNA, leading to continuous spermatogenesis. Titration experiments of this type could, in principle, yield unexpected phenotypes that would strongly suggest new targets for the regulatory factor. Such was indeed the case with the tropomyosin 3′ UTR and myogenesis.

Two positive feedback loops have been proposed that invoke effects on general translation factors: the "activin-eIF4E loop" in *Xenopus* mesoderm induction and the "tropomyosin 3′ UTR-induced loop" in myogenesis. In both cases, a small incremental signal may be enhanced and reinforced through feedback to the general translation apparatus. Although neither loop has been demonstrated in an intact animal, the data raise the important possibility that other such loops might also exist to reinforce and commit cells to a particular fate.

**WHY TRANSLATIONAL CONTROL?**

It is striking that many key decisions in development rely on translational control. Clearly, during early embryogenesis, when pronuclei or zygotic nuclei are highly condensed and inactive, transcriptional control is not a major option. Why control 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 essential 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 would have a
chance to act in the early embryo. For regulatory proteins such as cyclin or Glp-1, premature translation might disrupt the timing of interaction with downstream factors or ligands. Moreover, in Drosophila, some translationally regulated proteins are themselves translational regulators and must be controlled in time and space. Testing these notions will require translation to be forced at the wrong time or in the wrong place as well as investigation of the biological consequences.

Factors that mediate translational control may be involved in transcription as well. For example, RNA-binding translational regulators, such as the FRGY2 and Bicoid proteins, are also DNA-binding transcription factors. This sort of dual function may be more common than we now realize, as it rarely would have been detected. The existence of translational regulatory factors in the embryo could provide molecular fodder for the subsequent evolution of transcriptional regulatory circuits, and vice versa. There is a sense in which the early embryo seems much like the primitive, prebiotic earth— an RNA world, free of the burden of DNA. Perhaps it is ontogeny’s ultimate recapitulation of phylogeny.

ACKNOWLEDGMENTS

We thank Maria Gallegos, Scott Ballantyne, and Sunnie Thompson for comments on the manuscript and are grateful to Ann Helseley-Marchbanks, Carol Pfeffer, and Toni Daraio for help in its preparation. Laura VanderPloeg and Adam Steinberg of the Biochemistry Media Lab were very helpful preparing the figures. Work in the authors’ laboratories is supported by National Institutes of Health research grants GM-31892 and GM-50942 to M.W., NIH grants GM-31816 and HD-24663 and National Science Foundation grant IBM9205215 to J.K, and NIH grants GM-51584, HD-25922, and HD-17875 to S.S.

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