Joint action of two RNA degradation pathways controls the timing of maternal transcript elimination at the midblastula transition in Drosophila melanogaster

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Maternally synthesized RNAs program early embryonic development in many animals. These RNAs are degraded rapidly by the midblastula transition (MBT), allowing genetic control of development to pass to zygotically synthesized transcripts. Here we show that in the early embryo of Drosophila melanogaster, there are two independent RNA degradation pathways, either of which is sufficient for transcript elimination. However, only the concerted action of both pathways leads to elimination of transcripts with the correct timing, at the MBT. The first pathway is maternally encoded, is targeted to specific classes of mRNAs through cis-acting elements in the 3'-untranslated region and is conserved in Xenopus laevis. The second pathway is activated 2 h after fertilization and functions together with the maternal pathway to ensure that transcripts are degraded by the MBT.

Keywords: Drosophila/midblastula transition (MBT)/localization/stability/Xenopus

Introduction

In animal embryos as diverse as echinoderms, insects, amphibians and mammals, the earliest stages of development are programed by maternally synthesized RNAs and proteins (reviewed in Davidson, 1986). Subsequent phases of embryogenesis require products encoded by zygotically synthesized transcripts. The transition from maternal to zygotic control of development is referred to as the midblastula transition (MBT) which is defined by the first developmental processes that require zygotic products. The MBT should not be confused with the onset of zygotic transcription, which initiates earlier. Prior to the MBT, a subset of the maternally synthesized transcripts is degraded. In mammals, maternal transcript degradation is complete by the two-cell stage, while in echinoderms, amphibians and insects it occurs prior to the MBT, when either several hundred cells (echinoderms), several thousand cells (amphibians) or several thousand syncytial nuclei (insects) are present.

Genetic and molecular studies in Drosophila melanogaster have demonstrated that a subset of the maternal mRNAs encode proteins controlling the cell cycle, positional specification of cells or the morphogenetic movements that drive gastrulation. These transcripts are eliminated during the syncytial blastoderm stage, shortly after zygotic transcription commences (reviewed in Cooperstock and Lipshitz, 1997; Bashirullah et al., 1998).

The subsequent cellularization of the blastoderm is the first developmental process requiring zygotic contributions and thus marks the Drosophila MBT (Edgar et al., 1986; Merrill et al., 1988; Sibon et al., 1997).

During early Drosophila embryogenesis, from egg-laying until the MBT, there are 13 synchronous syncytial nuclear divisions (Zalokar and Erk, 1976; Foe and Alberts, 1983; Campos-Ortega and Hartenstein, 1998). Subsequently, the nuclei enter interphase of nuclear division cycle 14 and the somatic blastoderm cellularizes by concerted invagination of cell membranes from the periphery. Blastoderm cellularization is followed by the first gastrulation movements and the first zygotically programmed mitosis, mitosis 14, which is no longer synchronous (Foe, 1989). It has been shown that this transition from synchronous maternally driven mitoses to asynchronous zygotically programmed mitoses requires degradation of maternal string transcripts (string encodes a homolog of the cell cycle regulator, CDC25) (Edgar and O’Farrell, 1989, 1990; Edgar and Datar, 1996).

While turnover of maternal transcripts was first reported more than 20 years ago, little is known of the degradation machinery or of the mechanisms by which specific classes of transcripts are targeted to it. Here we show that two transcript degradation pathways function in the early Drosophila embryo. The first, ‘maternal’, pathway is driven by maternally encoded factors that are recruited by cis-acting RNA degradation elements independently of whether the transcript is translationally active or translationally repressed. This maternal degradation apparatus is conserved in Xenopus oocytes and early embryos. The second ‘zygotic’ pathway becomes active 2 h after fertilization. Either pathway acting alone is sufficient to eliminate maternal transcripts; however, the joint action of both pathways is necessary for elimination of transcripts prior to the MBT. This dual degradation system is likely
to coordinate developmental events by ensuring the elimination of maternal transcripts prior to the MBT.

**Results**

**Degradation of maternally synthesized transcripts begins shortly after fertilization in Drosophila**

We analyzed the dynamics of transcript degradation in the early embryo focusing on three maternally encoded RNAs: Hsp83, string and nanos. Hsp83 transcripts are particularly abundant, representing 1% of the polyadenylated transcripts in the early embryo (Zimmerman et al., 1983). RNA tissue *in situ* analyses demonstrated that maternal Hsp83 and string transcripts are no longer detectable by late stage 5, after completion of blastoderm cellularization (Figure 1A–F); nanos transcripts are undetectable by stage 4 (Figure 1G–I). RNA tissue *in situ* analyses are useful for defining when very low levels of transcripts are reached, but they are not sufficiently quantitative to be used to define the time course of degradation. Consequently, we carried out Northern blot analyses of RNA samples prepared from embryo collections spanning 30 min intervals during the first 5 h after fertilization. These blots were probed for Hsp83, string, nanos and rpA1 transcripts. rpA1 served as an internal control since maternal rpA1 transcripts are stable beyond the MBT (Riedl and Jacobs-Lorena, 1996) (Figure 1J). Maternal Hsp83, string and nanos transcript levels decrease throughout the pre-MBT stages initiating within the first hour after fertilization (Figure 1K). By the MBT (2.5–3.0 h after fertilization), >96% of the maternal Hsp83 transcripts loaded into the embryo have disappeared (Figure 1K). The Hsp83 transcripts present at and after this stage comprise undegraded maternal transcripts in the pole cells and zygotic transcripts. Ninety-five percent of long isoform string transcripts (Figure 1J and K; only the long isoform is strictly maternally synthesized and is shown in K, although quantitation was done on each isoform) and >99% of the nanos transcripts have been eliminated by the MBT (Figure 1J and K). The remaining nanos transcripts represent undegraded maternal transcripts in the pole cells (Figure 1H and I). These results demonstrate that maternal transcript degradation initiates at or shortly after fertilization, and that the vast majority of these transcripts have been eliminated by the MBT.

**Maternally encoded products direct transcript degradation, even in unfertilized eggs**

We used unfertilized eggs to ask whether maternally encoded products are sufficient to direct transcript degradation in the absence of both fertilization and zygotic transcription. In *Drosophila*, egg activation occurs when the mature oocyte passes from the ovary into the uterus. This process does not require fertilization. Egg activation releases the female pronucleus from meiotic arrest at metaphase I, results in translation of various maternally encoded transcripts deposited in the oocyte, and induces...
depolymerization of cortical microtubules and cross-linking of the vitelline membrane (Mahowald et al., 1983).

The transcripts which we analyzed, i.e. maternal Hsp83 (Figure 2A–C), string (Figure 2D–F), nanos (Figure 2G–I) and Polar granule component (Pgc; Figure 7G), are degraded in unfertilized eggs. Northern blot analyses (Figure 2J) indicated that 99% of the Hsp83 transcripts and 90% of the long isoform string transcripts have been degraded by 4–5 h after egg activation (Figure 2K), 1–2 h later than in developing embryos (Figure 2J and K). Over 99% of the nanos transcripts have been degraded by 3 h after egg activation (Figure 2J and K), 0.5 h later than in developing embryos but >1 h earlier than Hsp83 and string transcripts. rpA1 transcripts are stable throughout this period (Figure 2J). We conclude that maternally encoded factors are sufficient for degradation of Hsp83, string, nanos and Pgc transcripts. A second, superimposed, degradation pathway causes the more rapid elimination of transcripts in embryos than in unfertilized eggs (see below).

**Cis-acting elements in the 3'-UTR target transcripts for degradation**

To identify cis-acting sequences essential for transcript degradation, we focused on the 3'-untranslated regions (3'-UTRs) of Hsp83 and nanos transcripts. If an element targets transcripts for degradation, then absence of this element would be expected to stabilize the transcript. Unfertilized eggs were used for the analysis in order to identify those elements necessary for maternally encoded transcript degradation.

For Hsp83, a series of reporter transgenes was constructed that contained a fragment of the *Escherichia coli* lacZ gene and either the full 407 nucleotides of the Hsp83 3'-UTR or deleted versions (see Figure 3A for sequence, Figure 3B for transgenic constructs). By this analysis, a region in the 3'-UTR from nucleotide 253 to 349, which we refer to as the Hsp83 degradation element (HDE), proved necessary for maternally encoded Hsp83 transcript degradation (Figure 3C–F). Transcripts lacking the HDE (252∆350) are stable in unfertilized eggs for at least 6 h (Figure 3F). This contrasts with transcripts that include the HDE (endogenous Hsp83 transcripts or transgenic transcripts that include the HDE) which are fully degraded in unfertilized eggs by 4 h after activation (see Figure 2C, J and K for endogenous transcripts; data not shown for transgenic transcripts). Elimination of either the 5' or the 3' half of the HDE (252A301 or 300A351) results in incomplete degradation (data not shown; see Figure 3B).

The first 186 nucleotides of the nanos 3'-UTR, designated the translation control element (TCE), have also been implicated in control of transcript stability (Dahanukar and Wharton, 1996; Smibert et al., 1996). In unfertilized eggs, this element is shown here to target the maternally encoded degradation machinery to nanos transcripts. Transcripts lacking the first 186 nucleotides (ΔTCE) are not degraded by 2–4 h after egg activation (Figure 4C and D) whereas endogenous nanos transcripts have completely disappeared (Figure 4A and B).

To study whether the Hsp83 HDE and the nanos TCE are functionally interchangeable, two additional transgenic constructs were made. In one, the HDE was added to a nanos transgene lacking the TCE (nos/ΔTCE+HDE)
These data support a model in which specific cis-acting elements recruit a maternally encoded degradation machinery to both the Hsp83 and nanos transcripts.

**Relationship between translation and RNA degradation**

Our next goal was to study whether translational repression is a prerequisite for transcript degradation. Such a requirement had been postulated for nanos transcripts in early embryos since relief of translational repression (by deletion of the TCE) correlated with an increase in transcript stability (Dahanukar and Wharton, 1996; Smibert et al., 1996). Translation of nanos in the head region of the embryo causes repression of bicoid translation and thus head skeleton defects (Dahanukar and Wharton, 1996; Smibert et al., 1996). The severity of the defect serves as a sensitive readout of the Nanos protein level (Wharton and Struhl, 1991; Gavis and Lehmann, 1994) in the absence of a direct assay using anti-Nanos antibodies, which are no longer available.

We examined the head skeleton of transgenic embryos carrying nos[ΔTCE] or nos[ΔTCE+HDE] transcripts. As previously reported (Dahanukar and Wharton, 1996; Smibert et al., 1996), nos[ΔTCE] transcripts are translated and produce head skeleton defects (Figure 4J). Surprisingly, even though nos[ΔTCE+HDE] transcripts are degraded (Figure 4F), they too produce head skeleton defects (Figure 4K). Furthermore, the severity of the head skeleton defects was greater in the case of nos[ΔTCE+HDE] than nos[ΔTCE]. In five independent nos[ΔTCE] transgenic lines, an average of 72 ± 29% of the embryos exhibited defects and these were restricted to the dorsal bridge. In contrast, all four independent nos[ΔTCE+HDE] lines resulted in 100 ± 0% of the embryos exhibiting severe defects (loss of dorsal bridge, dorsal arms, vertical plates and ventral arms).

Thus, not only are the nos[ΔTCE+HDE] transcripts translated, but they are translated at higher levels than the nos[ΔTCE] transcripts, perhaps because the HDE includes a translational enhancer. We conclude that translational repression and transcript degradation are not causally linked; even highly translated transcripts are targeted for degradation. Moreover, non-protein-coding transcripts (e.g. Pgc) are also degraded in unfertilized eggs (Figure 7G). Together, these data indicate that the transcript degradation machinery acts on transcripts that contain a degradation element independently of whether they are untranslatable, translationally repressed or translated.

**Discovery of a second, zygotically encoded or activated degradation pathway**

We next addressed why Hsp83 transcripts are eliminated more rapidly in embryos than in unfertilized eggs. We asked whether embryos contain an additional degradation machinery that acts independently of the HDE, by comparing the stability of transcripts containing or lacking the HDE. Degradation of endogenous Hsp83 transcripts as well as HDE-containing transgenic transcripts is initiated shortly after fertilization, and minimal transcript levels are reached by 2.5 h (Figure 5A, B and F for endogenous transcripts; transgenic transcripts not shown). In unfertilized eggs, transcripts lacking the HDE are stable for well over 5 h (Figures 3E and F, and 5E).
HDE-deleted (252Δ350) transcripts are stable throughout the first 2 h of embryogenesis, after which degradation initiates, and 95% of these transcripts are gone by 4 h after fertilization (Figure 5C, D, F and G). Thus, in addition to the maternally encoded degradation machinery, a zygotically synthesized or zygotically triggered degradation machinery is active starting 2 h after fertilization. Furthermore, since transcript degradation occurs at this stage in the absence of the HDE, this ‘zygotic’ degradation is independent of elements in the 3′-UTR that mediate the maternally encoded degradation. Zygotic transcript degradation occurs for all 3′-UTR reporter constructs listed in Figure 3.

Taken together, our analyses demonstrate that the joint action of both the ‘maternal’ and the ‘zygotic’ degradation pathways is needed to eliminate the bulk of maternal transcripts by the onset of the MBT.

**Elements in the 3′-UTR protect transcripts from degradation in the pole cells**

Certain maternal transcripts fail to degrade in pole cells of developing embryos or in the posterior polar plasm of unfertilized eggs. These include Hsp83 (Figures 1C and 2C), nanos (Figures 1H and I, and 2H and I) and Pgc (Figure 7G) (Wang and Lehmann, 1991; Ding et al., 1993a; Nakamura et al., 1996). Our time course analysis shows that 1% of the total maternally loaded Hsp83 transcripts and <1% of the total maternally loaded nanos transcripts remain in the posterior polar plasm of unfertilized eggs (Figure 2K). Two possible mechanisms could lead to the absence of transcript degradation at the posterior: the degradation machinery might be excluded from the posterior polar plasm and thus also from the pole cells. Alternatively, the machinery could be active in the posterior polar plasm and pole cells, but certain classes of transcripts could be masked from the machinery by components of the polar plasm.

To distinguish between these possibilities, we replaced the Hsp83 3′-UTR in our reporter construct with the 3′-UTR from the Hsp70 transcript (Figure 3B). Transgenic reporter transcripts carrying the Hsp83 3′-UTR are degraded normally in the soma but are not degraded in the pole cells (Figure 6A–C), mimicking the distribution of the endogenous Hsp83 transcripts (Figure 1A–C). The reporter transcripts carrying the Hsp70 3′-UTR are taken up into the pole cells when they bud (Figure 6D and E) but are unstable and are degraded by the cellular blastoderm stage (Figure 6F; see figure legend for details). These results demonstrate that the maternally encoded degradation machinery is present throughout the egg and embryo.

To identify ‘protection’ elements within the Hsp83 3′-UTR, we assayed posterior protection of our reporter transcripts in unfertilized eggs (Figure 3B). Sequences sufficient for protection reside within the 3′-most 107 nucleotides of the Hsp83 3′-UTR since transgenic RNA produced by the 301.407 construct is protected (Figure 3A and B). Since transgenic RNA carrying only nucleotides 351–407 is unstable during oogenesis (Halsell, 1995), it was not possible to determine whether the 3′-most 57 nucleotides alone are sufficient for protection. However, replacement of nucleotides 351–407 with the Hsp70 3′-UTR results in failure of protection (Figure 3B), showing that nucleotides 351–407 are necessary for protection. We refer to nucleotides 351–407 as the Hsp83 protection element or HPE. Thus elements necessary for degradation versus protection map to distinct regions within the Hsp83 3′-UTR.
Identification of mutants that fail to undergo maternally encoded RNA degradation

Since our data demonstrated that maternal factors are sufficient for transcript degradation prior to the MBT, we tested a collection of female sterile mutant lines (Schupbach and Wieschaus, 1989) to identify maternal effect mutants that fail to undergo degradation (see Materials and methods for list). Mutants were assayed for Hsp83 RNA degradation in unfertilized eggs aged well beyond the stage at which transcripts would have disappeared in the wild-type. The presence of protected transcripts at the posterior served as an internal control for the in situ hybridizations (if degradation occurs, then protected Hsp83 transcripts are visible at the posterior). Mutations in two loci, cortex and grauzone, result in failure of Hsp83 transcript degradation (Figure 7B and C). Failure of degradation was not specific to Hsp83 since nanos (Figure 7E and F), Pgc (Figure 7H and I) and string (Figure 7K and L) transcripts also failed to degrade. The cortex and grauzone mutants exhibit defective egg activation and cytoplasmic polyadenylation of transcripts (Lieberfarb et al., 1996; Page and Orr-Weaver, 1996).

The maternally encoded degradation pathway is conserved in Xenopus

Several maternal transcripts are localized during Xenopus oogenesis although it is not yet known whether the type of degradation–protection system described here for Drosophila functions in their localization (reviewed in Bashirullah et al., 1998). Furthermore, as in Drosophila, many maternal transcripts are degraded prior to the Xenopus MBT (reviewed in Davidson, 1986). Thus, it was of interest to determine whether Xenopus oocytes and early embryos have a maternal RNA degradation
machinery similar to that identified here in Drosophila. We injected in vitro synthesized, digoxigenin-labeled transcripts comprising the full-length Drosophila Hsp83 3′-UTR into stage 6 Xenopus oocytes or recently fertilized embryos. These transcripts are unstable and are degraded rapidly after injection (Figure 8A for oocytes; data not shown for embryos). We then injected in vitro transcribed Drosophila Hsp83 3′-UTR ΔHDE transcripts into stage 6 oocytes; these transcripts are extremely stable and are still readily detectable 24 h after injection (Figure 8B). We conclude that the Drosophila HDE functions as a degradation element in Xenopus oocytes and embryos. Thus, Xenopus must contain a transcript degradation activity highly related to the Drosophila maternal activity and capable of recognizing the Drosophila HDE.

**Discussion**

Passage from maternal to zygotic control of development is thought to depend on the elimination of a subset of maternal transcript classes from the early embryo. For example, degradation of ubiquitous maternal string transcripts is necessary to permit zygotically driven, patterned domains of cell division after the MBT (Edgar and Datar, 1996). Here we have shown that the joint action of two RNA degradation pathways controls maternal transcript degradation and its timing in the early Drosophila embryo, and that at least one of these pathways (the 'maternal' pathway) is conserved in Xenopus.

**Two genetic pathways ensure RNA degradation prior to the Drosophila MBT**

The first degradation pathway is maternally encoded and does not require fertilization or zygotic gene transcription for its function. This ‘maternal’ pathway begins to function at or shortly after egg activation, and is sufficient for degradation of even abundant maternally loaded transcripts by 4–5 h after egg activation (Figure 9). The second ‘zygotic’ pathway requires fertilization and becomes active 1.5–2 h after fertilization. Acting alone, the zygotic pathway eliminates transcripts by 4 h after fertilization. Since the zygotic pathway can remove Hsp83 transcripts in 2 h, while the maternal pathway takes 4–5 h, the zygotic machinery is approximately twice as efficient as the maternal machinery. Under normal circumstances, relatively rare transcripts are degraded almost exclusively by the maternal pathway (e.g. nanos). In contrast, more abundant transcripts require the action of both pathways in order to be eliminated by the MBT (e.g. Hsp83, string). The maternal pathway initiates transcript degradation at or shortly after egg activation, while the zygotic pathway ensures degradation of abundant transcripts prior to the MBT.

Our results predict four classes of maternal RNAs in the early embryo. The first class lacks both maternal and zygotic degradation elements and is stable (e.g. rpA1). The second class (if it exists—there are no known examples) would be degraded exclusively by the maternal machinery either because these transcripts lack zygotic degradation elements or because their low abundance enables them to be fully degraded by the maternal machinery prior to activation of the zygotic factors. A third class (if it exists—there are no known examples) would be degraded exclusively by the zygotic machinery, possibly because of a lack of elements that target the maternal factors. The fourth class (e.g. Hsp83, string and nanos) is degraded by the combined action of the maternal and zygotic machinery.

The dual mechanism for transcript degradation discovered here sheds light on two previously reported observations. First, it has been shown that modulation
of the nuclear:cytoplasmic ratio affects the timing of disappearance of maternally synthesized string transcripts (Yasuda et al., 1991). Changing the nuclear:cytoplasmic ratio would not affect the maternal degradation machinery since it would be pre-loaded into the embryo and its activity/concentration should be independent of that ratio. In contrast, changes in the nuclear:cytoplasmic ratio might be expected to alter the synthesis or local concentration of the zygotic degradation machinery, particularly if activation of this machinery is dependent on zygotic transcription. Thus, altering the nuclear:cytoplasmic ratio would alter the exact timing of string transcript disappearance by modulating the zygotic pathway. Secondly, our results explain why α-amanitin injection into embryos prior to nuclear cycle 6 causes a delay in degradation of maternal string transcripts (α-amanitin inhibits RNA polymerase II and thus zygotic transcription) (Edgar and Datar, 1996). If production or activation of the zygotic degradation machinery requires zygotic transcription, α-amanitin would be expected to inhibit it while leaving the maternal degradation machinery unaffected. This would result in a delay in string degradation since only the maternally encoded degradation apparatus would be functional.

In summary, while previous results suggested that turnover of transcripts at the MBT might be controlled by zygotic products (O’Farrell et al., 1989; Yasuda et al., 1991), we have uncovered a dual maternal–zygotic degradation system that is likely to coordinate developmental events by ensuring the elimination of maternal transcripts prior to the MBT.

**Cis-acting elements target transcripts for degradation or for protection**

We have shown that the maternal transcript degradation activity is present throughout the embryo and unfertilized egg. Deletion of specific cis-acting 3′-UTR sequences results in failure of maternally encoded transcript degradation. These elements do not share any obvious primary sequence or secondary structure, nor do they include any previously defined degradation tags (reviewed in Jacobson and Peltz, 1996). Despite the lack of sequence conservation, our data demonstrate that the degradation elements are interchangeable (the Hsp83 HDE can substitute for the nanos TCE, and vice versa). The cis-acting elements defined here are not necessary for targeting transcripts to the zygotic degradation pathway since deletion of these elements has no detectable effect on the zygotic degradation process; in fact, none of our Hsp83 3′-UTR deletions abrogates zygotic transcript degradation. There are three plausible explanations for this result: (i) there are redundant ‘zygotic’ degradation elements in the Hsp83 3′-UTR; (ii) such elements reside in another part of the Hsp83 transcript [given the structure of the reporter transgenes, this could be the 5′-UTR or the first 333 nucleotides of the open reading frame (ORF)]; or (iii) zygotic degradation is not a targeted event but is the default state.

For RNAs such as rpA1, which are stable in the early embryo, we do not yet know whether there are specific stabilization elements or whether transcript stability is a default state. Indirect evidence in support of the latter possibility comes from analyses of a transcript instability element in the ftz 3′-UTR (Riedl and Jacobs-Lorena, 1996). Addition of this element (FIE3) to rpA1 transcripts destabilizes them, suggesting that for rpA1 transcripts, stability is either the default state or that instability elements such as FIE3 are dominant to as yet undefined stability elements.

Our data indicate that it is possible to differentially stabilize transcripts carrying degradation tags in different regions of the cytoplasm and/or in different cell types. Specifically, we have shown that the maternal degradation apparatus is active throughout the egg and embryo, but that transcripts such as Hsp83, nanos and Pgc are protected from degradation in the pole plasm and pole cells. We have also been able to map sequences in the Hsp83 3′-UTR that are necessary for this protection. The most likely candidate organelles for protecting transcripts in the polar plasm and pole cells are the polar granules themselves. First, one of the RNAs we have studied (Pgc) has been shown to be an integral component of the polar granules (Nakamura et al., 1996). Secondly, it has been shown that disruption of the polar granules results in failure of Hsp83, nanos and Pgc transcript protection in the polar plasm and pole cells, while ectopic assembly of polar granules at the anterior of the embryo results in ectopic protection of these transcripts anteriorly (Ephrussi and Lehmann, 1992; Ding et al., 1993a; Nakamura et al., 1996).
Role of transcript degradation and protection in cytoplasmic RNA localization

Differential stabilization of transcripts in specific regions of the cytoplasm represents one of several RNA localization mechanisms (reviewed in Bashirullah et al., 1998). Hsp83 is the only example of a transcript localized by a degradation–protection mechanism (Ding et al., 1993a; St Johnston, 1995). However, we have shown that nanos, Pge and Hsp83 transcript elimination in the somatic region of the embryo is accomplished by the same RNA degradation machinery. Further, our data suggest that 99% of the Hsp83 transcripts and 99% of the nanos transcripts reside in somatic region. This last result is very similar to the 96% value reported recently for somatic nanos transcripts (Bergsten and Gavis, 1999). We speculate that the differences in localization patterns of transcripts that are obviously localized prior to egg activation and those which only appear localized well after egg activation may be largely quantitative.

Relationship of transcript degradation to translation and polyadenylation status

The literature contains numerous examples of a correlation between translational repression of mRNAs and their degradation (reviewed in Jacobson and Peltz, 1996; Cooperstock and Lipshitz, 1997). In the early Drosophila embryo, the best studied example is nanos: nanos transcripts outside of the posterior polar plasm are translationally repressed and are also unstable (Figure 4, and see also Dahanukar and Wharton, 1996; Gavis et al., 1996; Smibert et al., 1996). Deletion or mutation of cis-acting elements that mediate translational repression results in transcript stabilization (Dahanukar and Wharton, 1996; Smibert et al., 1996; this study). Despite this correlation, our data demonstrate that there is no obligatory link between translational repression and transcript degradation. Replacing the element from the nanos 3′-UTR that mediates translational repression and degradation (the TCE) with the Hsp83 degradation element (the HDE) is sufficient to allow maternal degradation of nanos [ΔTCE+HDE] transcripts. However, at the same time, the presence of severe head defects demonstrates that this transgenic RNA is in fact highly translated. Thus, transcript degradation can be unlinked from translational repression.

There are several examples of a correlation between polyadenylation status and transcript stability (reviewed in Jacobson and Peltz, 1996). In particular, long poly(A) tails are associated with translational activation and transcript stabilization, while short poly(A) tails are associated with translational repression and transcript destabilization. In the Drosophila embryo, it has been shown that several classes of maternal transcripts are cytoplasmically polyadenylated after fertilization, correlating with initiation of their translation (Salles et al., 1994). The cortex and grauzone mutations perturb cytoplasmic polyadenylation (Lieberfarb et al., 1996), but transcript stability was not assayed in those studies. We have shown here that maternal transcripts are stabilized by cortex and grauzone mutations. The primary cause of the defects in embryos produced by cortex and grauzone females is unknown; their phenotypes are complex and several aspects of egg activation are abnormal (Lieberfarb et al., 1996; Page and Orr-Weaver, 1996). Thus it is not yet possible to draw firm conclusions from these mutants regarding a possible link between cytoplasmic polyadenylation status and transcript stability.

Evolutionary conservation of transcript degradation pathways

We have shown that Xenopus oocytes and early embryos contain an activity highly related to the maternal transcript degradation activity we have defined in Drosophila. Remarkably, the Drosophila HDE is recognized by this activity, and in vitro synthesized, injected transcripts are degraded rapidly, disappearing within a few hours. Removal of the HDE stabilizes these transcripts for at least 24 h. These results suggest that evolutionarily highly conserved pathways may control transcript degradation and localization throughout metazoan, opening up the possibility of exploiting the particular advantages of different model systems for future mechanistic and functional analysis.

Materials and methods

Drosophila whole-mount RNA in situ hybridization

RNA in situ hybridization with DNA probes was performed as described previously, with minor modifications (Tautz and Pfeifle, 1989; Ding et al., 1993a,b). Digoxigenin-labeled antisense RNA probes were made using the Megascript RNA transcription kit (Ambion, Inc.) according to the manufacturer’s instructions, and included digoxigenin-labeled UTP (Boehringer Mannheim). The RNA probes were subjected to limited alkaline hydrolysis by incubation in an equal volume of 100 mM NaCO3 pH 10.2 for 1 h at 60°C followed by ethanol precipitation. Pre-hybridization and hybridization were carried out at 55°C for RNA probes and at 48°C for DNA probes, and detection of the probe was as previously described (Tautz and Pfeifle, 1989). After hybridization, eggs or embryos were cleared in 50% glycerol followed by 70% glycerol. For exact staging, nuclei were visualized with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in phosphate-buffered saline (PBS) for 5 min prior to clearing of the embryos. Eggs or embryos were then individually mounted in 70% glycerol and images were captured using a Spot cooled-CCD camera (Diagnostic Instruments, Inc.) mounted on a Zeiss Axiosplan microscope. Adobe Photoshop software was used to process the images. For transgenic lines, at least two independently derived lines were analyzed for each construct.

Drosophila RNA extraction and analysis

RNA samples were isolated from staged egg collections by phenol extraction with Trizol (BRL). Equal amounts of total RNA were electrophoresed in 1.2% agarose/formaldehyde/MOPS gels (Sambrook et al., 1989), and transferred onto nylon membranes (Amersham). Filters were pre-hybridized for at least 1 h at 63°C in hybridization buffer containing 0.5 M Na-phosphate/7% SDS. 32P-Labeled random primed DNA probes were added and hybridization was performed overnight at the same temperature. Filters were rinsed twice at 60°C in 30 mM Na-phosphate/0.1% SDS, and exposed to Kodak XAR5 film at −80°C with an intensifying screen. Films were also exposed to a Molecular Dynamics phosphor screen, and quantitation was performed using ImageQuant software. Hsp83, string and nanos transcript levels were normalized to rp51 mRNA levels to control for variations in loading. The number of repetitions of each experiment was as follows: Hsp83 endogenous transcripts, fertilized = 3; Hsp83 endogenous transcripts, unfertilized = 2; Hsp83 ΔHDE transcripts, fertilized = 2; Hsp83 ΔHDE transcripts, unfertilized = 1; string transcripts, fertilized = 2; string transcripts, unfertilized = 1; nanos transcripts, fertilized = 1; nanos transcripts, unfertilized = 1.

Construction of transgenes for Drosophila germline transformation

Standard protocols were used (Sambrook et al., 1989). Hsp83–lacZ transgenes. The following approach was taken to generate the series of lacZ–Hsp83 3′-UTR constructs that include the 5′-Hsp83 enhancer sequences and promoter (Figure 3B). Previous reports (Kim-Ha et al., 1993) suggested that a full-length lacZ ORF might not
be completely transcribed in vivo. Therefore, we inserted the Hsp83 3′-UTR fragments downstream of a truncated lacZ tag. We accomplished this by constructing pB3Z, a Bluescript subclone that contains all of the Hsp83 5′ upstream region, the first exon, the intron and the first 110 codons of the ORF fused to 603 bp of lacZ sequence (Halsell, 1995). At the 3′ end of the lacZ sequence are AattI, HindIII and KpnI cloning sites for inserting the Hsp83 3′-UTR fragments. The template for the PCR was EcoR9, an 8 kb EcoRI fragment subcloned in Bluescript that contains the full-length Hsp83 transcription unit. 5′ Fragments of the Hsp83 3′ UTR were PCR amplified and were flanked with AattI and HindIII restriction sites, while 3′ fragments of the 3′ UTR were flanked with HindIII and KpnI restriction sites. The position of the HindIII restriction site within the amplified fragments allowed us to generate nested 5′ deletion constructs, the 1.50haa 3′ deletion construct and a series of internal deletions within the 3′-UTR. 3′-UTR constructs with internal deletions (the ‘Δ’ series) were generated by first subcloning an AattI–HindIII 5′ fragment into pB83Z and subsequently adding a HindIII–KpnI 3′ fragment. After subcloning into pB83Z, a NotI–KpnI fragment was isolated and subcloned into NotI–KpnI-digested CaSpeR4 (Thummel and Pirrotta, 1991). As a control, the Hsp70 3′-UTR was also cloned independently of any Hsp83 3′-UTR sequence (Figure 3A, ‘Hsp70’). The Hsp70 fragment was generated by PCR from a CaSpeR-hs template (Thummel and Pirrotta, 1991).

Drosophila hybrid transgenes. (i) nanos [ΔTCE]: a genomic 3′ fragment (BglII–EcoRI) containing a nanos 3′-UTR lacking the TCE was subcloned into pCaSpeR2 (Thummel and Pirrotta, 1991). Subsequently a genomic 5′ fragment (BamHI) extending from the nanos cis-regulatory sequences to the 5′ end of the ORF was inserted. (ii) nanos [ΔTCE + HDE]: a genomic 3′ fragment (BglII–EcoRI) containing a nanos 3′-UTR lacking the TCE was subcloned into pCaSpeR2. The Hsp83 HDE was PCR amplified incorporating 5′-BamHI and 3′-BglII sites. This fragment was subcloned upstream of the nanos 3′ fragment. Subsequently a genomic 5′ fragment (BamHI) extending from the nanos cis-regulatory sequences to the 5′ end of the ORF was inserted. (iii) Hsp83 [ΔHDE + TCE]: the nanos TCE was amplified by PCR incorporating HindIII sites at the ends, and inserted into lacZ-tagged pBS Hsp83 [ΔHDE] (253A350) at the HindIII site.

Drosophila stocks and egg collection

Drosophila melanogaster Oregon-R and w1118 stocks are described in Lindsley and Zimm (1992). The following ‘class 2’ and ‘class 3’ maternal transcript degradation in unfertilized eggs: a hybrid transgene

References


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Received December 22, 1998; revised and accepted March 5, 1999

