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The *nanos* translational control element represses translation in somatic cells by a Bearded box-like motif

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Abstract

Developmental control of translation is frequently mediated by regulatory elements that reside within 3′ untranslated regions (3′ UTRs). Two stem-loops within the *nanos* 3′ UTR translational control element (TCE) act independently to direct translational repression of maternal *nanos* mRNA in the ovary or embryo. We have previously shown that the *nanos* TCE can also function in select somatic sites. Using an ectopic expression screen, we now identify a new site of TCE function, the dorsal pouch epithelium. Analysis of TCE mutants reveals that TCE activity in the dorsal pouch does not depend on either of the stem-loops required for maternal TCE function, but instead requires a third feature—a sequence that closely matches the Bearded box, a regulatory motif found in the 3′ UTRs of several *Notch* pathway genes. In addition, we identify *pleiohomeotic* mRNA as an endogenous candidate for regulation by Bearded box-like motifs in the dorsal pouch. Together, these results suggest that the TCE has appropriated a conserved regulatory motif to expand its function to somatic tissues.

Keywords: *Nanos*; Translational control; Translational repression; Drosophila; Embryogenesis; 3′ untranslated region; Bearded box; *Pleiohomeotic*

Introduction

Control of mRNA translation plays an important role in the spatial and temporal regulation of gene expression during development. Studies of numerous maternal mRNAs, whose transcription occurs prior to fertilization, have revealed widespread use of translational control mechanisms to limit when and where the proteins they encode are synthesized. Translational regulation is not limited to maternal mRNAs, however. Sex-specific regulation of dosage compensation in *Drosophila*, cell lineage decisions during larval development in *C. elegans*, temporal regulation of organelle destruction during mammalian erythrocyte differentiation, and synaptic plasticity all rely on translational control of zygotically transcribed mRNAs (Banerjee and Slack, 2002; Klann and Dever, 2004; Kuersten and Goodwin, 2003; Steward and Schuman, 2003; Wilkie et al., 2003).

The role of translational control of maternal mRNAs in anterior–posterior patterning of the Drosophila embryo is well established. In the anterior of the embryo, Bicoid (Bcd) directs head and thorax development by activating transcription of genes like *hunchback* (*hb*) and repressing translation of *caudal* mRNA (Ephrussi and St Johnston, 2004). In the posterior, Nanos (Nos) protein represses translation of maternal *hb* mRNA to exclude Hb protein from the posterior, thereby permitting expression of genes required for abdominal development (Hülskamp et al., 1989; Tautz and Pfeifle, 1989). Conversely, synthesis of both Bcd and Hb proteins in the anterior of the embryo requires that Nos be limited to the posterior (Gavis and Lehmann, 1992, 1994; Wharton and Struhl, 1989). This restricted distribution of Nos is generated by selective translation of a subset of *nos* mRNA that is localized to the germ plasm at the posterior of the embryo coupled with translational repression of *nos* mRNA distributed throughout the bulk cytoplasm (Bergsten and Gavis, 1999; Gavis and Lehmann, 1994).

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Both posterior localization and translational repression of *nos* RNA are mediated by the *nos* 3′ untranslated region (3′ UTR) (Gavis and Lehmann, 1992, 1994). A 90-nucleotide translational control element (TCE) within the *nos* 3′ UTR confers repression through formation of two stem-loop structures, whose functions are temporally distinct (Crucs et al., 2000; Forrest et al., 2004). One stem-loop contains the binding site for Smaug (Smg), which represses *nos* translation in the early embryo (Dahanukar et al., 1999; Smibert et al., 1996, 1999). The second stem-loop mediates translational repression during oogenesis, presumably by binding to an ovarian factor (Forrest et al., 2004).

Although *nos* expression was first thought to be restricted to the germline and early embryo, recent work has revealed a role for *nos* in dendritic morphogenesis in the Drosophila peripheral nervous system (PNS). Moreover, the TCE can regulate *nos* translation in Class IV dendritic arborization (da) neurons in the PNS (Ye et al., 2004). The ability of the TCE to function in the PNS, together with evidence that Smg and other translational regulators have multiple RNA targets, suggests that translational control by TCE-like motifs may be used more widely for regulation of other mRNAs in somatic tissues. Using an ectopic expression assay, we have shown that the TCE can repress translation in cells of the central nervous system (CNS) involved in the ecdysis signaling pathway, although the regulated mRNA has not been identified in this case (Clark et al., 2002).

In a screen for somatic tissues that support TCE function, we have now identified the dorsal pouch epithelium as one such tissue. By analyzing the activity of TCE mutants in the dorsal pouch epithelium, we show that TCE function in these cells does not depend on the same sequence and structural features required for its function in the oocyte and early embryo. Rather, activity of the TCE in the dorsal pouch requires a sequence that closely matches the Bearded (Brd) box, a motif found in the 3′ UTRs of genes involved in the *Notch* signaling pathway (Leviten et al., 1997). Brd boxes have been shown to mediate both RNA degradation and translational control of a heterologous RNA (Lai and Posakony, 1997). A Brd box-like motif embedded within the second TCE stem-loop effects translational control in the dorsal pouch, without significantly affecting RNA stability. We propose that the TCE evolved as a multi-use regulatory element that functions by distinct mechanisms in different tissues.

Since *nos* is not expressed endogenously in the dorsal pouch epithelium, the finding that the TCE is capable of repression in this tissue suggests that one or more endogenous mRNAs are similarly regulated. We identify *pleiohomeotic* (*pho*) mRNA as a candidate for translational repression in the dorsal pouch by Brd box-like motifs. Thus, Brd box-like motifs may be used by a wide range of RNAs for translational regulation in somatic tissues.

Materials and methods

Fly stocks

The following mutants and GAL4 lines were used: *y* w^{67c23} (Lindsley and Zimm, 1992), pum^{680} (Lehmann and Nüsslein-Volhard, 1987), smg^{1} and Df(Scf^{R6}) (Dahanukar et al., 1999; Lindsley and Zimm, 1992), *h-GAL4* (Brand and Perrimon, 1993), and $GAL4^{24B}$ (Luo et al., 1994).

Construction of transgenes and transgenic lines

The *UAS-nos-tub3′UTR*, *UAS-nos-tub:nos+2*, *UAS-nosΔB-tub3′UTR*, and *UAS-luc-tub3′UTR* transgenes have been previously described (Clark et al., 2002). For technical reasons, all of the other transgenes were generated in the pUAST derivative, pUASTβ, in which the *Bam*HI fragment containing the UAS, hsp70 TATA, polylinker and SV40 polyadenylation signal sequences is inverted (R. Ray, personal communication). For consistency, a new *UAS-nos-tub3′UTR* transgene was generated in pUASTβ and used in the experiments shown in Figs. 4–6. For all *UAS-nos-tub3′UTR* derivatives, DNA fragments encoding the relevant 3′ UTR sequences were inserted at a unique, engineered *Nhe*I site within the *α-tubulin* 3′ UTR sequences. The *NR* fragment includes nucleotides 329–438 of the *nos* 3′ UTR, which lack localization and translational regulatory activity. The TCE insert, which includes nucleotides 6–96 of the *nos* 3′ UTR, and the TCEIIA, IIU, IIIA, and SRE^{-} mutants have been described (Crucs et al., 2000; Gavis et al., 1996). Further mutagenesis of TCEIIIA to create TCEIIA/IIIA and mutagenesis of the B1, B2, and B3 motifs were achieved by PCR according to the method of Nelson and Long (1989). Double and triple mutants were generated sequentially. A fragment encompassing nucleotides 42–173 of the *Brd* 3′ UTR was generated by PCR from Oregon-R genomic DNA. The *pho* 3′ UTR fragment (nucleotides 1989–2378) was generated by PCR of the *pho* RE17954 cDNA (Flybase). All 3′ UTR inserts were confirmed by sequencing.

For *UAS-pho*, a 2.1-kb *Bst*N1–*Kpn*I fragment containing the *pho* coding region and 3′ UTR was excised from the *pho* RE17954 cDNA in pFLc-1 and inserted between the *Eco*RI and *Kpn*I sites of pUASTβ after end-filling of the *Bst*NI and *Eco*RI sites. To generate *UAS-pho-tub3′UTR*, sequences downstream of a *Psi*I site in the *pho* 3′ UTR including the Brd box-like motifs were removed by digestion of *UAS-pho* with *Psi*I and *Xba*I and replaced by a *Sca*I–*Hin*dIII fragment from pTα5′3′ that includes the *α-tubulin* 3′ UTR and 3′ genomic sequences.

Transgenes were introduced into *y* w^{67c23} embryos by P element-mediated germline transformation (Spradling, 1986) and multiple transgenic lines were isolated and balanced for each transgene.