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NEGotiating Cell Identity through Regulated Cytoplasmic Polyadenylation

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In this issue of *Developmental Cell*, Elewa et al. (2015) show that combinatorial action of RNA binding proteins modulates poly(A) tail length of maternal mRNAs, leading to asymmetric expression of a cell fate determinant in early *C. elegans* embryos. Genome-wide profiling suggests this mechanism may be widely used to establish cell identities.

Although transcriptional gene regulatory networks direct cell identity and differentiation in embryos (Davidson and Erwin, 2006), the rapid early development of many animals often necessitates a more rapid mode of regulation of gene expression. This demand is met by systems that mediate spatiotemporal differences in expression of previously transcribed mRNAs through the action of regulatory RNA binding proteins (RBPs) (Ivshina et al., 2014). Such mechanisms include modulation of cytoplasmic polyadenylation (reviewed in Weill et al., 2012): mRNAs with longer poly(A) tails generally show increased stability and translatability. In this issue of *Developmental Cell*, Elewa et al. (2015) report that localized RBPs combinatorially modulate expression of a key cell fate regulator, NEG-1, through cytoplasmic polyadenylation of its mRNA. Moreover, poly(A) tail lengths of many other messages are controlled by factors that influence *neg-1* polyadenylation, raising the exciting possibility that combinatorial inhibition and activation of cytoplasmic polyadenylation by RBPs may represent a major mode for establishing differences in gene expression and cell identity in early embryos.

Asymmetric division of the *C. elegans* zygote provides a paradigm for illuminating mechanisms by which maternal factors establish differences in cell fate (Gönczy and Rose, 2005). The larger anterior, AB, and smaller posterior, P_1, blastomeres possess distinct developmental potentials: AB produces most of the ectoderm, whereas P_1 produces endomesoderm, including the gut. This AB/P_1 asymmetry is generated by the evolutionarily conserved PAR machinery. Subsequently, directed transport and spatially specific degradation of maternal factors, as well as localized translation of maternal RNAs, result in unequal distribution of cell fate determinants, including the endomesoderm-activating bZIP transcription factor SKN-1, which is expressed at high levels in the early P_1 lineage. Much of the control of cell type differences in the early embryo occurs post-transcriptionally. Indeed, many mediators that specify cell identity in the AB and P_1 lineages are RBPs, several of which (e.g., POS-1 and MEX-5/6) contain CCCH-type Zn fingers.

POS-1, like SKN-1, is required for endomesoderm development, but not for SKN-1 expression, suggesting that it activates the endomesoderm program through another mechanism. Indeed, in an RNAi screen for *pos-1(-)* suppressors, Elewa et al. (2015) discovered that POS-1 promotes endomesoderm development by blocking, in P_1, expression of a novel protein, NEG-1 (Negative Effect on Gut differentiation), which represses the endomesoderm program in the AB lineage. Loss of POS-1 results in the absence of gut owing to failure to activate *med-1/2*, SKN-1 targets required for endomesoderm development (Maduro et al., 2001). *neg-1* mutations restore *med-1/2* expression and hence gut development in the absence of POS-1. Elimination of NEG-1 function also results in ectopic expression of endomesoderm markers in AB descendants at the expense of ectoderm, explaining the morphogenetic defects of *neg-1* mutant embryos reported in another study (Osborne Nishimura et al., 2015).

While region-specific expression of maternal regulatory proteins often results from localized maternal transcripts, this is largely not the case for *C. elegans* embryos: transcripts of maternal regulators (with exception of *mex-3* and *pos-1*) are generally uniformly distributed. However, in a genome-wide study that profiled differences in transcript abundance between AB and P_1, Osborne Nishimura et al. (2015) identified 281 asymmetrically distributed transcripts. *neg-1* RNA was among the 80 enriched transcripts in the AB lineage. Because zygotic gene expression likely initiates after the two-cell state, control of AB/P_1 asymmetry of *neg-1* expression is likely to occur post-transcriptionally.

Indeed, Elewa et al. found that *neg-1* is post-transcriptionally regulated by two other proteins identified in their screen: the non-canonical poly(A) polymerase GLD-2 and its binding partner GLD-3, a KH-domain RBP that recruits GLD-2, resulting in cytoplasmic extension of poly(A) tails. In wild-type embryos, *neg-1* transcripts (shown by Osborne Nishimura et al., 2015) and NEG-1::GFP are expressed at high levels in the early AB lineage, while in *pos-1(-)* mutants this asymmetry is abolished and NEG-1::GFP appears in the daughters of both AB and P_1. In contrast, NEG-1::GFP expression is eliminated in the absence of GLD-2 and -3, suggesting a role for polyadenylation in regulation of NEG-1 expression (Elewa et al., 2015). Thus, expression of NEG-1 is positively regulated by GLD-2/3 and negatively regulated by POS-1, likely through reciprocal regulation of *neg-1* mRNA stability.

The authors found that the *neg-1* 3′UTR contains three predicted, overlapping

binding sites, including sites for POS-1 and for MEX-5/6 and MEX-3, other RBPs known to act as embryonic fate determinants (Figure 1). In vitro studies revealed that MEX-5 outcompetes POS-1 for binding *neg-1* RNA. Furthermore, loss of MEX-5/6 from embryos was associated with shorter poly(A) tails on *neg-1* transcripts, whereas loss of POS-1 resulted in longer tails, suggesting that competition between these RBPs determines *neg-1* poly(A) tail length.

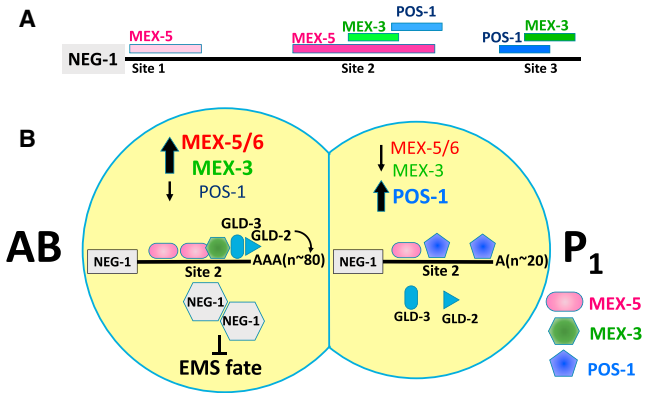

Figure 1. Regulation of *neg-1* by Differential Polyadenylation
Differential expression of NEG-1 depends on the combinatorial action of RNA binding proteins, which regulate recruitment of the GLD-2 poly(A) polymerase. (A) Schematic of *neg-1* 3′UTR. (B) Relative amounts of MEX-5/6 and MEX-3 versus POS-1 determine whether the poly(A) tail of *neg-1* mRNA is extended, resulting in AB/P_1 asymmetric expression. EMS, endomesoderm progenitor.

These results lead to the following model (Figure 1). In anterior blastomeres, high MEX-5/6 and MEX-3 concentrations relative to POS-1 allow recruitment of GLD-2, which extends the *neg-1* poly(A) tail, thereby promoting NEG-1 expression. In posterior blastomeres, higher POS-1 levels lead to its occupancy of the 3′UTR, which precludes GLD-3-mediated recruitment of GLD-2. The resulting shorter poly(A) tails prevent *neg-1* mRNA stabilization and translation, thereby allowing expression of posterior endomesodermal genes.

Interestingly, genome-wide profiling of poly(A) tail lengths identified a large number of POS-1-repressed, GLD-2/3-activated mRNAs, suggesting that regulation of *neg-1* expression by differential polyadenylation may represent the tip of a regulatory iceberg. In addition, many mRNAs were identified whose poly(A) lengths depend only on GLD-2 and not GLD-3, raising the possibility that other RBPs might recruit the GLD-2 poly(A) polymerase to their respective target mRNAs.

Regulation of gene expression through activation or repression of cytoplasmic polyadenylation represents a novel mechanism for establishing differences in cell identity in early embryos before transcription has begun. Combinatorial regulation by RBPs has also been observed in other contexts. For example, the sequential binding of five RBPs restricts translation of the Wnt-type ligand, MOM-2 (Oldenbroek et al., 2013), to particular blastomeres in the early embryo. The 3′UTR of many mRNAs may contain multiple binding sites for both positive and negative regulators, the outcome being determined by the combination of their respective abundance and binding affinities of the RBPs. A recent bioinformatics study predicted that as many as 887 (~4.4% of all) *C. elegans* genes may encode RBPs, of which 250 likely function in a gene-specific manner (Tamburino et al., 2013), providing a wealth of potential regulatory interactions. Thus, the earliest events in embryogenesis might well involve a combinatorial network of RBPs, including the multiplicity of CCCH Zn finger proteins, acting on target mRNA binding sites.

It will be of great interest to learn how widely such combinatorial control systems are used to generate cell type diversity in metazoans (Ivshina et al., 2014). Maturation of *Xenopus* oocytes triggered by progesterone is accompanied by elongation of poly(A) tails of mRNAs by the cytoplasmic polyadenylation element binding (CPEB1) protein and an ortholog of the *C. elegans* GLD-2 poly(A) polymerase. In *Drosophila*, the CPEB proteins Orb1 and Orb2 activate translation of Oskar mRNA at the posterior of the oocyte by recruiting poly(A) polymerase. Post-transcriptional control of mRNA by regulation of poly(A) tail length is also evident in gametogenesis, synaptic plasticity, and immune response associated with acute inflammation (Weill et al., 2012). Thus, modulation of poly(A) tail length may be a general mechanism used widely across metazoans when rapid post-transcriptional deployment of gene expression programs is required.

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