straightening, but not unfolding, the immunoglobulin segments, and unfolding the PEVK domain. The unfolded PEVK may act more as a leash than a spring, exerting forces greater than 5 pN (the force generated by a single myosin molecule) only near 80% of its maximal extension. It requires extreme force to unfold immunoglobulin domains. Once unfolded, these domains cannot exert a significant force, and must be renatured by other means.

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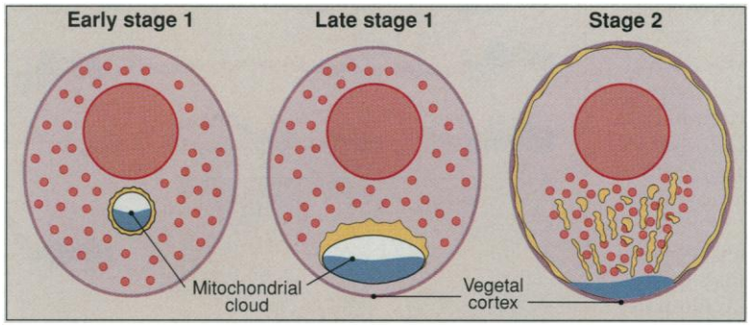
9. A force of 100 pN operating over 0.5 nm corresponds to a free energy of 7 kcal/mol, which is close to the total folding energy of a domain. The implication is that the entire free energy of folding is dissipated by the strong force needed to pull out the first few atoms. In contrast, for refolding, the domain must gather in its full 25 nm or more of polypeptide and compact it into the folded domain. A force of 4 pN operating over 25 nm corresponds to 14 kcal/mol, which is more than sufficient to block refolding. Domain refolding probably cannot occur efficiently until the force is reduced to 1 pN or less.

DEVELOPMENTAL BIOLOGY

A New Face for the Endoplasmic Reticulum: RNA Localization

Laurence D. Etkin

How do you get a perfect sphere to acquire a front and back, right-hand and left-hand sides? Such is the challenge addressed during oogenesis, when the molecular signals that initiate the formation of animal body plan are produced and physically localized in the early oocyte. Much of this information is encoded by localized proteins and RNAs that are organized within distinct regions of the oocyte (*1*). Later on, after fertilization, the developing embryo compartmentalizes and elaborates these early signals to build asymmetries ranging from the simple head versus tail to the complex divergence of right- and left-brain thought processes. How does it all begin?



Vg1 put in its place by the endoplasmic reticulum. In early stage 1 oocytes Vg1 mRNA (red) is distributed homogeneously throughout the cytoplasm. The endoplasmic reticulum (yellow) is first detected surrounding the mitochondrial cloud, which contains the METRO-localized RNAs (blue). During late stage 1 the mitochondrial cloud and associated RNAs move toward the vegetal cortex. The endoplasmic reticulum appears on one side of the cloud. In stage 2 oocytes the wedge-shaped structure consisting of endoplasmic reticulum, the colocalizing Vg1 mRNA, and the Vera protein is fully elaborated. Later, during stages 3 and 4, Vg1 will translocate to the vegetal cortex where it will associate tightly with the cortical layer.

In one well-studied oocyte, that of the frog *Xenopus laevis*, the vegetal end of the oocyte contains RNAs that may control germ cell specification and axial patterning. They arrive there by way of two major pathways: the messenger transport organizer (the METRO or early pathway) and the late pathways (*2*, *3*).

The METRO pathway uses a specialized organelle called the mitochondrial cloud to localize RNAs during a very early stage of oogenesis (stage 1). These RNAs include the noncoding repeat Xlsirts, which serves an anchoring function (*4*); the mRNA Xcat2 (*5*); and Xwnt11, which may function in dorsal-ventral patterning (*6*). The second, or late, pathway localizes molecules such as Vg1 (a transforming growth factor–β family member that controls dorsal-ventral patterning) to the vegetal cortex during stages 2 to 4. In this issue, we learn about a surprising new participant in the mechanism by which these pathways function. Deshler *et al.* (page 1128) (*7*) show that Vg1 mRNA is localized via the late pathway by transport through the endoplasmic reticulum, in association with an endoplasmic reticulum–associated protein called Vera (*V*gLE binding and *e*ndoplasmic *r*eticulum *a*ssociation).

In stage 1 and early stage 2 oocytes, while the METRO RNAs are being localized, Vg1 is distributed homogeneously throughout the cytoplasm (*2*, *8*). Then, later in stage 2, Vg1 begins to appear in a wedge-shaped structure associated with the vegetal cortex, at the same site as METRO RNA localization (*2*). During stages 3 and 4, the rest of the Vg1 mRNA is translocated to the cortex in a microtubule-dependent manner. The anchoring of Vg1 at the cortex relies on microfilaments and the noncoding RNA Xlsirts (*9*).

Both cis elements and trans-acting factors help to localize these RNAs. The cis-acting elements responsible for Xlsirts and Xcat2 localization, through the early pathway, are within the Xlsirt 79–nucleotide (nt) repeat sequences (*3*) and the Xcat2 3′ untranslated region (*10*). The translocation of the Vg1 mRNA, through the late pathway, requires a 340- to 366-nt localization element (VgLE) located in its 3′ untranslated region (*11*). A 69-kD microtubule-associated protein binds to the VgLE and may mediate Vg1 interaction with the microtubules in stage 3 oocytes (*12*). Five other proteins (p78, p60, p40, p36, and p33) bind to specific regions of the Vg1 3′ untranslated region, possibly forming part of the translocation complex (*13*).

Deshler *et al.* (*7*) were searching for trans-acting factors that interact with cis-acting elements within the VgLE. They isolated a 75-kD protein, Vera, that interacts specifically with the VgLE region and whose binding to the region is necessary for the proper localization of Vg1 mRNA. Within the VgLE are four elements that, when mutated, interfere with the ability of Vg1 mRNA to localize. Mutations in three of these elements resulted in altered affinity of the 75-kD protein for the VgLE, suggesting that the interaction between the 75-kD protein and these elements is important for the localization of Vg1 mRNA.

The author is in the Department of Molecular Genetics, University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030, USA. E-mail: lde@molgen.mda.uth.tmc.edu

The surprise came when Deshler *et al.* attempted to isolate the 75-kD protein from cytoplasmic extracts and found that the Vg1-binding activity (Vera) cosedimented with a large organelle fraction enriched with TRAPα, an integral protein associated with the protein translocation machinery of the endoplasmic reticulum. In stage 2 oocytes, the TRAPα distribution overlapped with the wedge-shaped, in situ hybridization pattern of Vg1 in stage 2 oocytes (*2*). Because Vg1 is not translated at this time and the endoplasmic reticulum is of the smooth variety, it appears that preferential translation is not the major function of this interaction. Traditionally, the endoplasmic reticulum is viewed as an organelle involved in synthesis and secretion of proteins. The finding of Deshler *et al.* (*7*) demonstrates a novel function for a subdomain of this organelle—in the localization of RNAs during oogenesis.

How does this specialized region of the endoplasmic reticulum originate? The elaboration of this subdomain is a sequential process, first seen when the mitochondrial cloud and the associated METRO RNAs move toward the vegetal cortex (*14*). When the METRO RNAs are anchored, the wedge-shaped endoplasmic reticulum subdomain is fully delineated and Vg1 mRNA becomes associated with this structure. Thus, the function of this specialized region of the endoplasmic reticulum may be in the initial steps of localization of RNAs, such as Vg1, through the late pathway. The morphogenesis of the wedge-shaped endoplasmic reticulum structure is dependent on the functioning of the early pathway via the migration of the mitochondrial cloud. Vera may mediate the interaction between Vg1 mRNA and the endoplasmic reticulum.

Two alternative explanations are possible for the Vg1-endoplasmic reticulum association. (i) The wedge-shaped, endoplasmic reticulum-containing structure may serve as a substrate to orient the microtubule tracks used for Vg1 translocation during stages 3 and 4. (ii) Vg1 remains associated with the endoplasmic reticulum, and endoplasmic reticulum-containing vesicles are translocated to the vegetal cortex along microtubules. A close association between the endoplasmic reticulum and microtubules and the movement of membrane vesicles along microtubules have been observed in a variety of systems, supporting both possibilities (*15*). It is clear, however, that the wedge-shaped region of the endoplasmic reticulum is somehow unique; the Vg1 mRNA associates only with this subdomain of endoplasmic reticulum and not with the endoplasmic reticulum located elsewhere in the cortex. Future studies will focus on understanding the precise mechanism of this interaction and the universality of its use for RNA localization in other systems.

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UPDATE: CIRCADIAN RHYTHMS

As Time PASses: The First Mammalian Clock Gene

Steve A. Kay

Several molecular cogs of the circadian clock have been identified in the fungus *Neurospora* and the fruit fly *Drosophila* (*1*). One of these—the photoresponsive gene *wc-2* from *Neurospora* (*2*)—was recently described in *Science* and discussed in an accompanying Perspective (*3*). Significantly, this protein contains a PAS domain, found in many transcription factors including PER, a *Drosophila* clock component.

Additional support for PAS as a signature motif in clock components now comes from two papers published this week in *Cell* by Takahashi and his colleagues (*4*). (See also the News story on page 1030.) This laboratory has undertaken a truly heroic effort—and succeeded. They have carried out the brute-force screening for mutants of the clock in mice by observing wheel-running behavioral rhythms in the individual progeny of lines of mice treated with the mutagen ethylnitrosourea. Incredibly, mutant mouse number 25 displayed one of the desired phenotypes: lengthening of the period of cyclic behavior observed in constant darkness. The animals homozygous for the mutation eventually exhibited arrhythmicity in constant darkness (*5*). This locus was called *clock*, and further genetic analysis revealed that this allele was both semidominant and antimorphic, implying that the putative mutant protein was interfering with the function of the normal, wild-type *clock* gene (*6*). This notion has now been borne out by cloning of the *clock* gene by positional methods and gene rescue. The predicted CLOCK protein is 855 amino acids long and contains a basic helix-loop-helix–PAS motif, as well as a glutamine-rich carboxyl-terminus.

Thus, another protein that appears to be intimately involved in the circadian clock contains the PAS motif, making it the only conserved signature known so far among widely diverse clock molecules.

The author is in the NSF Center for Biological Timing, Department of Cell Biology, The Scripps Research Institute, La Jolla, CA 92037, USA. E-mail: stevek@scripps.edu

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