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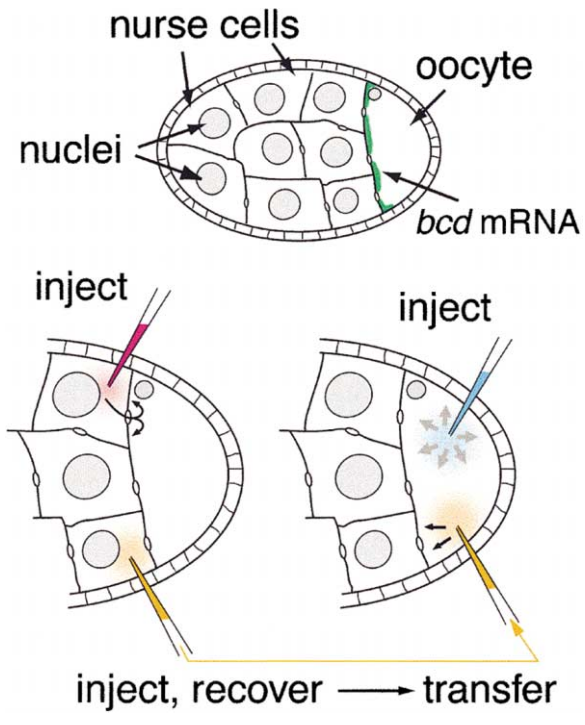
RNA Localization Goes Direct

Direct injection of RNA provides a new view of localization during *Drosophila* oogenesis.

Following nuclear export, certain mRNAs are localized to discrete cytoplasmic sites for translation. Distances traveled can be large, yet specific transcripts are efficiently delivered to specific destinations. How cells accomplish this task has been a subject of substantial interest ever since localized mRNAs were discovered and shown to be crucial for organization of the embryonic body plan in flies and frogs. One prominent example is *bicoid* (*bcd*) mRNA, whose localization to the anterior pole of the *Drosophila* oocyte is required for formation of a concentration gradient of morphogenic Bcd protein in the embryo. An initial mechanistic model of *bcd* mRNA localization was suggested by the structure of the egg chamber, which consists of an oocyte and 15 interconnected nurse cells, all surrounded by a layer of somatic follicle cells (see Figure). Since all connections between the oocyte and nurse cells lie at the anterior of the oocyte and *bcd* mRNA is synthesized in the nurse cells, it could be simply trapped upon entry into the oocyte. Deficiencies in this model soon appeared, and in recent years prevailing explanations for *bcd* localization have invoked anterior-directed movement within the oocyte along strictly polarized microtubules (Cooley and Theurkauf, 1994; Hays and Karess, 2000). Supporting evidence for this model remains indirect, and it has been difficult to prove any specific model or even to define the relevant molecular function of any known localization factor [an exception is the Staufen protein, which acts directly to maintain *bcd* mRNA localization in the early embryo (Ferrandon et al., 1994)].

At least part of the difficulty in defining mechanisms and establishing roles for localization factors can be attributed to the assay typically used to monitor mRNA localization, in situ hybridization to fixed egg chambers. This assay provides beautiful pictures of steady-state mRNA distributions but reveals little about the dynamic processes creating those distributions. It is difficult to quantify accurately and unsuited for distinguishing among populations of a single RNA species that have been localized at different times or by different mechanisms. Since the localization of *bcd* mRNA appears to involve different mechanisms with overlapping but distinct periods of activity (Macdonald and Kerr, 1997), technical advances are clearly required. One solution to the limitations of in situs is provided by real time observation of fluorescently labeled microinjected RNA, which provides advantages analogous to those of classical pulse-chase experiments. This technique has been used to study other mRNA localization events in *Drosophila*, such as the localization of pair-rule and *wingless* transcripts to the apical side of nuclei in blastoderm embryos (Wilkie and Davis, 2001). Upon injection, labeled transcripts incorporate into particles containing several endogenous apically localized mRNAs. These particles travel toward the apical surface along microtubules in a manner dependent on the activity of the motor protein dynein and its receptor dynactin, implicating dynein as potentially driving mRNA translocation in the embryo. In the July 13 issue of *Cell*, Cha et al. (2001) bring such RNA labeling and injection technology to bear on the study of *bcd* mRNA. They are able to focus on a single defined population of RNA to monitor its movement and dynamic association with known localization factors, with impressive and informative results.

In the simplest form of their assay, Cha et al. inject labeled *bcd* RNA directly into nurse cells or oocytes. When introduced into nurse cells, injected transcripts form cytoplasmic particles that are transported into the oocyte, leading to accumulation of fluorescence at the anterior cortex in a pattern identical to that of endogenous *bcd* mRNA. Injection of *bcd* RNAs into the oocyte cytoplasm also leads to a cortical accumulation, but all specificity for the anterior is lost. Thus, trafficking



Tracing Movements of Injected *bicoid* mRNAs

A *Drosophila* egg chamber, the basic unit of development in oogenesis, is diagrammed at top. The oocyte is oriented with anterior to the left, with the site of *bcd* mRNA localization indicated in green. Below are schematic representations of experiments performed by Cha et al. Separate experiments, in different colors, involve simple injection into either nurse cells or oocytes, or the more elaborate transfer protocol outlined at bottom. For all experiments, narrow black arrows indicate anterior-directed movements and wide gray arrows indicated nonspecific cortex-directed movements.

through the nurse cells seems to be essential for specific anterior localization in the oocyte. Nurse cell injections provide a robust and reproducible localization assay, with control experiments revealing dependence on known components of the localization system such as sequences from the *bcd* mRNA 3′ UTR, the Exu protein, and intact microtubules. The involvement of the latter two components is addressed in detail by Cha et al., and, among a wealth of information that will be savored by enthusiasts, two sets of observations stand out.

First, experiments involving injection of *bcd* RNA into oocytes lead to a new view of microtubule organization. The nonspecific cortical accumulation of these transcripts is at odds with a highly polarized microtubule scaffold placing minus ends at the anterior and plus ends at the posterior pole. Additional evidence for more complex cytoskeletal organization in the oocyte is provided by a newly refined immunolabeling and confocal imaging technique. Cha et al. observe microtubules terminating at all cortical regions outside of the posterior pole, with very few oriented along the anterior-posterior axis. Thus, some presently unknown form of locational information must be recognized by the RNA localization machinery. Cha et al. propose the existence of an as yet unidentified subset of functionally distinct microtubules that are polarized toward the anterior of the oocyte. This model is appealing and is consistent with the discovery that Swallow protein, which contributes to *bcd* mRNA localization and is itself anteriorly localized in the oocyte, associates with a subunit of a retrograde microtubule motor (Schnorrer et al., 2000). Nevertheless, contrarians can still point to the lack of direct evidence for movement of *bcd* mRNA along microtubules in the oocyte.

Second, *bcd* mRNA-containing particles also contain Exuperatia (Exu) protein, demonstrating an association long suspected but not proven (Theurkauf and Hazelrigg, 1998). Inclusion of Exu into these particles seems to be microtubule dependent, since colcemid can inhibit the coassembly of Exu with *bcd* mRNA. Exu proves to be required for both specific and nonspecific forms of localization in the oocyte. RNA injected into *exu*$^-$ nurse cells moves into the oocyte with apparently normal kinetics but fails to stably localize to the anterior while RNA injected into *exu*$^-$ oocytes fails to display even nonspecific localization to the cortex. The interpretation of these results is not simple, and various views can be entertained. However, in a remarkable extension of their assay, Cha et al. inject labeled RNA into a nurse cell and then remove the resulting particles via the injection needle and inject them into an oocyte. Now the RNA can move specifically within the oocyte to the anterior. This directed movement requires that the injected nurse cells be *exu*$^+$ (and have intact microtubules), but the injected oocyte can be *exu*$^-$ (dependence on oocyte microtubules has not been tested). Thus, Exu in concert with other nurse cell factor(s) confers the property of directed movement on the *bcd* mRNA-containing particles.

The results presented by Cha et al. represent a significant advance in research into *bcd* mRNA localization. Introduction of the direct injection assay provides a powerful new method to pose questions about the roles played by different localization components and is likely to help overcome some of the obstacles inherent in studying a process where multiple mechanisms contribute to the observed patterns of mRNA localization. The new insights into specific requirements for Exu and microtubules represent only the first fruits of this approach, and we may expect to learn a great deal more in the future.

Eric A. Arn and Paul M. Macdonald
Institute for Cellular and Molecular Biology
Section of Molecular Cell and Developmental Biology
University of Texas
Austin, Texas 78712

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Sex and the Neighboring Cell

Bizarre sexual abnormalities attract attention, even in the scientific world. Recent studies of the *Drosophila doublesex* gene have produced a more accurate description of the origin, growth, and differentiation of the male and female genitalia. The big surprise is that the neighbors have more influence than previously recognized.

In *Drosophila* the sex of the soma is determined by an X chromosome signal that ultimately leads to the production of male- or female-specific versions of the zinc finger transcription factors encoded by the *doublesex* (*dsx*) gene. Null mutations in *dsx* cause a number of sexual alterations, the most dramatic of which is the production of distinct male and female genitalia in the same individuals. This "double sex" phenotype suggests that, in males, the Dsx^m protein inhibits the formation of female structures by blocking the growth and differentiation of the female primordia within the genital imaginal disc. Conversely, in females, Dsx^f inhibits male genital development by preventing the growth and differentiation of the male genital primordium.

This conventional model for genital disc development is not without problems. While *dsx* null mutants can form