Letter to the Editor

Interspecific RNA in situ Hybridization Reveals Perinuclear mastermind Transcripts in Drosophila virilis

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Interspecific and intraspecific comparisons of protein coding and noncoding nucleotide sequences have improved our understanding of the function and molecular evolution of numerous genes. In addition to sequence comparisons, for complex developmental genes an interspecific comparison of RNA expression patterns can be informative (Lukowitz et al. 1994). Preliminary data for such comparisons can be obtained from interspecific RNA in situ hybridization experiments. Using probes from Drosophila melanogaster, we examined the embryonic transcription pattern of the neurogenic gene mastermind (mam) in D. virilis. The analysis indicated that mam transcription is highly conserved in the two species and also revealed an unexpected feature of mam RNA localization during very early development.

mam was identified through its role in the formation of the nervous system in the D. melanogaster embryo (Lehmann et al. 1983). Subsequent genetic analyses have demonstrated that mam has numerous roles in embryonic and postembryonic development (Ruohola et al. 1991, Hartenstein et al. 1992). Molecular analyses reveal that mam encodes a nuclear protein and that the protein is highly repetitive, displaying numerous amino acid homopolymers. Nonrepetitive regions of mam show very limited similarity to sequences of known function, but prominent clusters of charged amino acids are evident. Studies of mam expression identified transcripts which accumulate in a spatially and temporally dynamic pattern (Smoller et al. 1990, Bettler, Schmid, and Yedvobnick 1991; unpublished data). Interspecific nucleotide sequence comparison of the protein coding region of mam from D. melanogaster and D. virilis revealed an equilibrium between natural selection and molecular drive; extensive length variation in repetitive regions appears to be balanced by selection to maintain the overall amino acid length between two conserved clusters of charged amino acids (Newfeld, Schmid, and Yedvobnick 1993; Newfeld, Tachida, and Yedvobnick 1994).

Initial interspecific hybridization experiments demonstrated that an RNA probe transcribed from a conserved portion of D. melanogaster mam will recognize mam transcripts from D. virilis embryos on Northern blots (Newfeld, Smoller, and Yedvobnick 1991). A subsequent examination of mam expression, at three stages of development in D. virilis using this probe, revealed a similar pattern of transcript accumulation to that seen in D. melanogaster (Newfeld, Schmid, and Yedvobnick 1993). Figure 1 shows the spatial accumulation of mam transcripts throughout D. virilis development, as measured with the D melanogaster probe. Expression of mam in D. virilis is nearly indistinguishable from that seen in D. melanogaster (Fig. 1f–l) (Bettler, Schmid, and Yedvobnick 1991; unpublished data). However, we note increased accumulation of mam transcript within portions of the peripheral nervous system (PNS) of D. virilis embryos. The concordance of mam expression strongly suggests that the principal functions of mam are highly conserved in the species. Additionally, these data suggest that the interspecific probe was hybridizing with fidelity to D. virilis transcripts. However, considering the high degree of divergence between these species it is conceivable that some nonspecific hybridization contributes to the observed signal. This can only be addressed utilizing homologous D. virilis probes.

In addition, an examination of the earliest stages of D. virilis embryogenesis revealed an unusual transcript distribution. In very early cleavage stage embryos maternally supplied mam RNA displays a perinuclear pattern of localization (Fig. 1b–e). These islands of RNA vary in number and size and in double labeling experiments with the chromatin stain DAPI (4',6-Diamidino-2-phenylindole; data not shown) contain a nucleus at their center. In Drosophila after fertilization, the zygotic nuclei divide synchronously without forming individual cells. Multiple rounds of division (cleavage) lead to a syncytium of nuclei distributed throughout the yolk of the embryo. The nuclei then migrate toward the surface of the embryo and somatic cells are formed as membrane furrows surround each nucleus (Campos-Ortega and Hartenstein 1985). The islands of mam hybridization are most evident during the earliest cleavage stages, growing smaller as the number of nuclei increase. (See fig. 1, panels b–e [b: the zygotic nucleus is undergoing...
Fig. 1.—*mastermind* expression during embryogenesis in *D. virilis*. Interspecific RNA in situ hybridization protocols: embryo preparation, probe generation, hybridization, and colorimetric detection are described in Betterl, Schmid, and Yedovbrick (1991). In all panels, the anterior end of the embryo is to the left. Panel a) a control experiment using a sense strand probe on an early cleavage stage embryo; note the absence of hybridization and the presence of energids with prominent nuclei at their center (arrows). Panels b–e) *mam* hybridization to a series of early cleavage stage embryos reveals a prominent perinuclear pattern of transcript localization which corresponds to the pattern of nuclear divisions in these early embryos. Panel f) a cellularized blastoderm embryo reveals strong *mam* expression in the cytoplasm of somatic cells; apically located nuclei and pole cells (at the far right edge of the embryo) show much weaker hybridization. Panel g) at gastrulation, the cephalic furrow (arrowhead), mesodermal cells of the invaginating ventral furrow (horizontal staining across the center of the embryo, arrow), and gut primordia hybridize strongly. Panel h) at the stage of germ band extension, mesoderm and neural precursor cells show strong expression but epidermal precursors (the outermost cells of the embryo, arrow) do not. Panel i) at the stage of germ band retraction, the condensing ventral nerve cord (dark staining near the bottom of the embryo, arrow) accumulates transcripts in a segmental pattern. The brain, foregut, and hindgut also express *mam*. Panel j) at the surface of the same embryo shows that the peripheral nervous system (segmentally repeated vertical rows of cells) express *mam* at levels greater than the surrounding epidermis. Panel k) *mam* expression remains strong in the central nervous system (brain and ventral cord), foregut, and hindgut (arrows). Panel l) immediately prior to hatching, strong expression in the brain and ventral cord persists while expression in the hindgut is reduced. Persistent expression in an anterior PNS region, possibly the antennal-maxillary complex, is also evident (arrow).

The first cleavage; c. the first division is complete; d. the second division is complete and four nuclei are present; e. the third nuclear cleavage is complete and eight nuclei are present.]

Similar perinuclear distributions of *mam* RNA had not been observed in our previous analyses of *D. melanogaster*. As the developmental rate of *D. melanogaster* is more rapid than that of *D. virilis*, we considered it possible that the very earliest stages of the former species had been overlooked. An examination of *D. melanogaster* embryos enriched for early stages revealed perinuclear localization of *mam* RNA, however the pattern was not as evident as that observed for *D. virilis* (data not shown).

The results suggest that *mam* RNA is associated with structures called energids. Energids are islands of yolk-free cytoplasm surrounding nuclei during the earliest stages of insect embryogenesis (Counce 1972). Structures that we assume correspond to energids are evident in *D. virilis* embryos hybridized with a control sense strand probe (fig. 1a). There are several mechanisms that could account for the concentration of *mam* RNA in such structures. One explanation is that the distribution actually represents newly synthesized transcripts in the process of diffusion from the nucleus, however in the case of *mam* this is unlikely. The perinuclear transcripts observed in the embryo at these early stages are maternal in origin. It is known that a large amount of *mam* transcript is produced in nurse cells and transported into the oocyte during late stages of oogenesis (unpublished data). Additionally, because of the rapid rate of nuclear divisions during early cleavage, zygotic transcripts from large genes such as *mam* (70 kb *D. melanogaster*, 100 kb *D. virilis*) would be aborted.
prior to production of an RNA with sequences contained in the probe (Shermoen and O'Farrell 1992); the probe utilized here derives from near the 3' end of the gene.

An alternative explanation for this RNA distribution derives from the fact that early nuclei are surrounded by a yolk-free zone of cytoplasm. Cytoplasmic RNAs may be enriched around nuclei simply because of a concentration effect derived from yolk exclusion. It is also possible that transcripts are targeted to nuclei by a more specific mechanism, through interactions with cytoskeletal elements. It has been suggested that RNA exhibits an affinity for the cytoskeleton in numerous systems (Jeffrey 1989), and a poly A RNA-microtubule interaction has been demonstrated in cultured neurons (Bassell, Singer, and Kosik 1994). Considering the concentration of cytoskeletal elements around nuclei, a general affinity between these molecules could account for perinuclear distribution. Consistent with this, microtubule-dependent perinuclear localizations have been reported for other transcripts in D. melanogaster (Raff, Whitfield, and Glover 1990; Pokrywka and Stephenson 1991). During early Drosophila development a mechanism that localizes maternal transcripts close to nuclei may be important. This may ensure proper allocation of messenger RNAs within cells at cellularization.

In summary, we have shown that a preliminary interspecific comparison of RNA expression patterns can be performed utilizing heterologous probes for RNA in situ hybridization. The study revealed very similar patterns of mam expression in D. melanogaster and D. virilis, and identified an unusual perinuclear distribution of mam RNA, providing further evidence that comparisons of this type are valuable for addressing evolutionary and developmental questions.

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LITERATURE CITED


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