INTRODUCTION

Localized RNAs represent a rare class of RNAs in germ cells that are involved in establishing regional cell fates. For example, in Drosophila, both the oocyte’s anterior and posterior poles contain localized RNAs, such as bicoid, nanos and oskar, whose products are required for formation of the embryonic structures characteristic of those regions (reviewed in Nusslein-Volhard, 1991; Lasko, 1992). In Xenopus laevis, nine localized RNAs have been described, four in the animal hemisphere (An1, Linnen et al., 1993; An2, Weeks and Melton, 1987a; An3, Gururajan et al., 1994; xlan4, Reddy et al., 1992) and five in the vegetal cortical region (Vg1, Weeks and Melton, 1987b; Xcat-2, Mosquera et al., 1993; Xcat-3, Elinson et al., 1993; Xlwnt-11, Ku and Melton, 1993; Xlsirts, Kloc et al., 1993).

The vegetally localized RNAs are of particular interest, as this region is known to have important developmental functions in Xenopus, including the specification of mesoderm, the dorsal-ventral axis, and the primordial germ cells (see Slack, 1991). The role of localized RNAs in specifying cell fate in frogs remains elusive, but the capabilities of their proteins are suggestive. Vg1 protein is a member of the TGF-beta family of growth factors and is competent to induce mesoderm when overexpressed (Thomsen and Melton, 1993). Xlwnt-11 has been strongly implicated in dorsal/ventral decisions as it can correct some dorsal defects (Ku and Melton, 1993). Recently, we reported that by sequence homology, Xcat-2 appears to belong to the same family of RNA-binding proteins as nanos (Mosquera et al., 1993). Nanos binds to and suppresses the translation of the RNA for the transcription factor hunchback in Drosophila, thus allowing the specification of the abdominal region and the proper environment for germ cell development (Wang and Lehmann, 1991). Therefore, the activities of these proteins correlate well with the functions of the vegetal region of the oocyte.

There are two known mechanisms by which materials become localized to the vegetal cortex during oogenesis. Germ plasm, the presumptive germ cell determinant, is distributed as “islands” in the cortical region. It is characterized by electron-dense RNA/protein granules surrounded by mitochondria (Holwill et al., 1987; Ikenishi et al., 1987; Thomsen and Melton, 1993; Kloc et al., 1993). The mitochrondrial cloud material, and the germ plasm, fragments and localizes to the cortex at stage II (Heasman et al., 1984). A second mechanism has been described for Vg1 RNA. It becomes localized to the vegetal cortex much later in oogenesis in a two-step process (Melton, 1987; Yisraeli et al., 1990). It is present throughout the oocyte in stages I through II and translocates to the vegetal pole by the end of stage IV in a process requiring intact microtubules.

SUMMARY

In Xenopus, localization of a rare class of mRNAs during oogenesis is believed to initiate pattern formation in the early embryo. We have determined the pattern of RNA localization for one of these RNAs, Xcat-2, which encodes a putative RNA-binding protein related to Drosophila nanos (Mosquera, L., Forristall, C., Zhou, Y. and King, M. L. (1993) Development 117, 377-386). Xcat-2 is exclusively localized to the mitochondrial cloud in stage I oocytes, moves with this body into the vegetal cortex during stage II and, later, partitions into islands consistent with it being a component of the germ plasm. As previously shown, Vg1 is not localized to the vegetal cortex until stage IV and distributes to all vegetal blastomeres during development. We found a direct correlation between the localized condition of these RNAs and their recovery in a detergent-insoluble fraction. We present evidence suggesting that differential RNA binding to a cytoskeletal component(s) in the vegetal cortex determines the pattern of inheritance for that RNA in the embryo.

Key words: Xenopus, localized maternal RNA, mitochondrial cloud, cytoskeleton, vegetal cortex
(Yisraeli et al., 1990). The second step, anchoring of the RNA to the cortex, does not require microtubules, but is sensitive to drugs that depolymerize microfilaments (Yisraeli et al., 1990). Thus, the formation of the vegetal cortex of the stage VI oocyte is a complex process, involving at least two mechanisms of localization that occur at different stages of oogenesis. In this regard, the Xenopus vegetal cortex is similar to the posterior pole plasm of Drosophila, which is also enriched in localized materials and which is formed during oogenesis in a multi-step process (Ephrussi et al., 1991).

In previous studies, we have shown that three RNAs found in the vegetal cortex of stage VI oocytes, Vg1, Xcat-2 and Xcat-3, are also 20- to 60-fold enriched in a detergent-insoluble fraction (DIF) (Pondel and King, 1988; Mosquera et al., 1993; Elison et al., 1993). In the egg, Vg1 is no longer bound to the cortex (Weeks and Melton, 1987b) and is recovered in a soluble fraction (Pondel and King, 1988). In this paper, we show that Vg1 and Xcat-2 have distinctly different patterns of DIF association and localization during oogenesis. Xcat-2 is exclusively found in the mitochondrial cloud at the earliest oogenic stages examined and becomes localized very early with cloud material to the vegetal cortex. Further, we find that Vg1 and Xcat-2 are inherited by different regions of the early embryo. Xcat-2 remains associated with the cortex in the 4-cell embryo and is inherited in a pattern consistent with its association with germ plasm. As previously reported, Vg1 RNA is inherited by all vegetal blastomeres (Weeks and Melton, 1987b). We propose that RNA association with a DIF component(s) determines the pattern of inheritance for that RNA.

MATERIALS AND METHODS

Oocytes and in vitro maturation

Different staged oocytes (Dumont stages I-VI) were collected as previously described (King and Barklis, 1985) except oocytes were defolliculated using 0.15% collagenase B (Boehringer Mannheim) in 100 mM NaH2PO4 (pH 7.4). If oocytes were to be treated with progesterone, calcium-free O-R2 (see below) was substituted for the sodium phosphate buffer during the collagenase treatment as the phosphate buffer inhibits progesterone-induced maturation. For some experiments, stage III and IV oocytes were further divided into early and late stages by hand. Late stage III oocytes were 570-600 μm in diameter; early stage IV were less than 800 μm in diameter.

For in vitro maturation, defolliculated stage VI oocytes were incubated in 30 μg/ml of progesterone (Sigma) in O-R2 (82 mM NaCl, 2.5 mM KCl, 1.0 mM CaCl2, 1.0 mM MgCl2, 1.0 mM Na2HPO4, 5 mM Hepes, pH 7.8, 3.8 mM NaOH) for 10 minutes, rinsed and then incubated in O-R2 without progesterone. Half the progesterone-treated oocytes also received theophylline at a final concentration of 2 mM. Typically, after 5-7 hours, 95% of the progestosterone-treated oocytes had matured as indicated by a white spot at the animal pole while 95% of the theophylline-treated oocytes failed to show a white spot. A probe for ornithine decarboxylase (ODC-2) was used as a control for RNA loading (Bassez et al., 1990).

RESULTS

Localization of Xcat-2 during oogenesis and its recovery in a detergent-insoluble fraction

In previous work, we have shown that Xcat-2 and Vg1 RNAs are both concentrated in the vegetal cortex of fully grown stage VI oocytes (Elison et al., 1993). To determine whether these two RNAs are localized by similar mechanisms, we analyzed oocytes of different stages by in situ hybridization (Fig. 1). The timing and pattern of localization of Xcat-2 was strikingly different from that of Vg1. Whereas Vg1 is uniformly distributed throughout the cytoplasm of stage I and II oocytes, Xcat-2 is already localized in the youngest oocytes, to a perinuclear position corresponding to the location of the mitochondrial cloud (Fig. 1 compare A and B). The pattern of localization of Xcat-2 during stage II follows that of the mitochondrial cloud which distributes to a small patch of the vegetal cortex (Fig. 1D,H). During stage III/early IV, when the animal-vegetal axis is clearly defined by a reduction in pigment at the vegetal pole, both RNAs are now found along the vegetal cortex (Fig. 1E,F). However, whereas Xcat-2 is strictly cortical, Vg1 also appears
within the vegetal yolk mass in small clusters that correspond to Vg1 RNA in the process of translocation (Fig. 1G).

The pattern of localization of these RNAs within the oocyte correlates well with their isolation in a cytoskeletal fraction (Fig. 2). Staged oocytes were extracted in a high salt detergent buffer and the RNA isolated from both the pellet (DIF) and the soluble fraction (SF). Northern blot analysis of these RNAs revealed that when Vg1 RNA was uniformly distributed in the oocyte, it could be recovered in the SF. However, Vg1 RNA became concentrated in the DIF at stage III/IV, corresponding to the time of its translocation and anchoring in the vegetal cortex. In contrast, Xcat-2 RNA was never found in the SF, but was recovered in the DIF at each oogenic stage.

**DIF poly(A)+ RNA levels during oogenesis**

One interpretation of these results is that mRNAs in the process of localization become associated with components of the cytoskeleton. However, it is possible that during the course of oogenesis, most mRNAs become enriched in a cytoskeletal compartment and that what is observed for Xcat-2 and Vg1 is not restricted to localized RNAs. To examine this point, the concentration of poly(A)+ RNA in the DIF and SF at each oogenic stage was determined (Table 1). As expected, the concentrations of poly(A)+ RNA in both the DIF and SF decline during oogenesis, as the oocyte stockpiles ribosomal RNA. However, there was no change during oogenesis in either the percentage of total RNA found in the DIF (2%), or in the relative proportion of poly(A)+ RNA in the DIF as compared to the SF. At all stages, the concentration of poly(A)+ RNA in the DIF is consistently 4 times higher than in the SF. This observation was confirmed in two ways. First, isolation of poly(A)+ RNA by oligo(dT) affinity chro-

![Fig. 1. Xcat-2 and Vg1 become localized to the vegetal cortex at different stages of oogenesis.](image)

Alternate sections of albino oocytes of stage I (A,B), II (C,D), III (H), and early IV (E,F,G) were hybridized with either 35S-labeled Vg1 (A,C,E,G) or Xcat-2 (B,D,F,H) antisense RNA probes. Note that Vg1 is distributed uniformly in stages I and II, whereas in the same oocytes, Xcat-2 is restricted to the mitochondrial cloud. In early IV oocytes, Vg1 appears in a punctate pattern between the nucleus and the vegetal cortex as well as being distributed broadly in the cortex (E,G, bar, 50 µm). In contrast, Xcat-2 is found in large clumps aggregated at the vegetal pole (F,H, bar, 50 µm). In situ hybridizations are shown in A-F in reverse image where silver grains appear white.
matography yielded between four and five times more RNA from the DIF than the SF sample. Secondly, when fourfold more SF total RNA was compared by northern blot analysis with DIF RNA, the histone H3 signal is indeed equal for the two fractions. Since the DIF contains 2% of the total RNA, it follows that, at all stages of oogenesis, 8% of an oocyte’s poly(A)+ RNA is in the DIF. Therefore, there is no evidence for a general increase in association of mRNA with the cytoskeleton during oogenesis. After ovulation, the concentration of poly(A)+RNA in the DIF appears to be equal to that of the SF. We conclude that there is a general loss of poly(A)+RNA from the DIF sometime during maturation. This conclusion is in agreement with an observation made by Capco and Jeffery (1982) who reported a general loss of poly(A)+ material from the cortex during maturation as detected by in situ hybridization with poly(U).

**Positive correlation between DIF enrichment and vegetal localization in stage VI oocytes**

Calculation of the concentration of poly(A)+RNA in the DIF relative to that of the SF allows for a reasonable estimate of the percentages of individual RNAs in the DIF based on their relative concentrations on northern blots containing equal amounts of poly(A)+RNA. The degree of enrichment for Vg1 and the Xcats was independently verified by determining the frequency with which these sequences were found in a DIF versus total oocyte cDNA library. We have previously published northern blot analyses of DIF and SF RNA from stage VI oocytes probed for a variety of mRNAs (Mosquera et al., 1993; Elinson et al., 1993). Using densitometric analysis of these northerns, we have calculated the percentage of each RNA found in the DIF in fully grown oocytes, and compared it to the percentage of each RNA found in the vegetal cortex (Elinson et al., 1993) (Fig. 3). RNAs known to be uniformly distributed throughout the oocyte, such as histone H3, cytokeratin, c-mos, and actin, are found primarily in the SF. In contrast, vegetally localized RNAs, Vg1, Xcat-2 and Xcat-3, are all predominantly isolated in the DIF and in the cortex. At most 15% of those RNAs localized to the animal hemisphere (An1-3) were isolated in the DIF. Using northern blot analysis of hand-isolated animal cortices, we have estimated that only 3% of An1 is found in the animal cortex. From these results, we conclude that, in stage VI oocytes, there is a strong correlation between vegetal localization, cortical localization and concentration in the DIF. These results argue that the animal and vegetal pole cortices are not functionally equivalent and further support the view that there is a unique RNA-binding domain in the vegetal cortex.

**Xcat-2 and Vg1 RNA during maturation**

Vg1 RNA is released from the DIF in the ovulated egg (Pondel and King, 1988). To determine when and how quickly Vg1 RNA shifts into the SF during oocyte maturation, we examined Vg1-DIF association at different time points after in vitro progesterone treatment of stage VI oocytes. Approximately 5 hours post-progesterone treatment, oocytes rapidly underwent germinal vesicle breakdown (GVBD) as indicated by a white spot at the animal pole. Within 10 minutes, 50% of the oocytes displayed white spots and these oocytes were quickly removed and frozen (Fig. 4, lane marked W) as were the remaining oocytes not displaying white spots (Fig. 4, lane marked NW). At time points up to and including 2 hours post-progesterone treatment, Vg1-DIF association remained unchanged (Fig. 4, lanes 0, 0.7, 2). Within 10 minutes of GVBD however, Vg1 was no longer in the DIF (Fig. 4, compare lanes W and NW). The process of maturation can be effectively inhibited by the addition of theophylline, a phosphodiesterase inhibitor, to the medium after progesterone treatment. Under these conditions, Vg1 remained associated with the DIF (Fig 4, lane T). From the results, it appears that Vg1 RNA is rapidly released from a cytoskeletal fraction shortly after GVBD and hours before the complete breakdown of the cytokeratin network (Klymkowsky et al., 1991). Furthermore, Vg1 release depends on the cascade of maturation events that requires phosphodiesterase activity. Xcat-2 behaves in a much different fashion from Vg1 as it remains associated with the DIF through the events of maturation and ovulation (Fig. 4). This result confirms our previous finding of a Xcat-2/DIF association (Mosquera et al., 1993).

![Fig. 2. Xcat-2 and Vg1 are isolated with the DIF during the translocation and anchoring steps of vegetal localization. Northern blot analysis of oocyte stages I, II, III, IV and V/VI total DIF (*) and SF (unlabeled) RNA probed sequentially with [32P] labeled Vg1, Xcat-2, and histone H3 DNAs. Lanes were loaded with equal amounts of poly(A)+RNA in the DIF and SF for each stage, as indicated by the equal intensities of the histone H3 response. (The relative amount of RNA between stages cannot be obtained from this figure, which is a composite of several blots.) The percentage of each RNA in the DIF per oocyte was calculated and is shown below each stage. The calculation is based on the ratio of DIF/SF at each stage (determined by densitometry), the percentage of total RNA in the DIF (2% at all stages), and the relative concentration of poly (A)+RNA in the DIF (see Table 1). Xcat-2 is concentrated in the DIF at the earliest stages of oogenesis, while Vg1 becomes enriched in stages III-IV.](#)
Taken together, these observations strongly suggest that Xcat-2 and Vg1 are bound to the vegetal cortex by different components.

**Pattern of Vg1 and Xcat-2 inheritance in the 4-cell embryo**

One consequence of Vg1 RNA release from the DIF at GVBD appears to be that it is no longer cortically localized but takes on a more diffuse pattern throughout the vegetal third of the embryo. By early blastula, Vg1 RNA is found in all vegetal pole cells and is excluded from the marginal zone (Weeks and Melton, 1987b). A prediction that follows is that since Xcat-2 remains with the DIF it likely remains with the vegetal cortex resulting in a different, more restricted pattern of inheritance. To test this prediction, whole-mount in situ hybridization studies were carried out on ovaulated eggs and 4-cell embryos as well as stages I-VI oocytes for Vg1 and Xcat-2 RNA (Fig. 5). The whole mounts of stages I-IV oocytes confirm results from the in situ hybridization analysis in Fig. 1. Xcat-2 is precisely localized to the mitochondrial cloud in stage I/II oocytes, moves with the mitochondrial cloud to the vegetal cortex at stage II and is completely localized by late stage II/early III. Vg1 becomes localized by the end of stage IV. By stage VI, the cortical area containing detectable Vg1 levels appears to be much broader than that for Xcat-2 as revealed by whole-mount in situ hybridization studies.

![Fig. 3. There is a positive correlation between enrichment in the DIF and vegetal localization in stage VI oocytes. Histogram summarizes the results from northern blot analyses of DIF, SF and cortical RNA isolated from stage VI oocytes. Blots were hybridized with a variety of [32P]-cDNAs representing mRNAs uniformly distributed in the oocyte (histone (H3), c-mos (mos), 56 x 10^3 M cytokeratin (CK), cytoplasmic actin (actin)), localized to the vegetal cortex (Vg1, Xcat-2, Xcat-3) or localized to the animal hemisphere (An1, An2, An3).](image)

![Fig. 4. Vg1 is released from the DIF at germinal vesicle breakdown (GVBD) while Xcat-2 is retained. The concentration of Vg1 and Xcat-2 in DIF (*) and SF (unlabeled) RNA isolated from stage VI oocytes at 0 hour (0), 0.7 hour (0.7), 2 hour (2) and 5 hour (5) post-progesterone treatment was assessed by RNase protection assays. An ODC-2 probe was included as a control for RNA loading. At 5 hours, when 50% of the oocytes had undergone GVBD, oocytes were divided into those with white spots (W) and those without (NW). After progesterone treatment, some oocytes were exposed to theophylline, an inhibitor of maturation (T). Controls (C) were not treated with progesterone. Vg1, but not Xcat-2, is lost from the DIF sample at the time of GVBD (compare NW and W lanes). (note: the significance of the shift from a double protected fragment to a single protected fragment at GVBD for Xcat-2 in this assay is not known).](image)
Xcat-2 RNA is found in a speckled pattern composed of some 100 “islands” still within the cortical region. The staining pattern was not identical from embryo to embryo. In some cases, Xcat-2 was partitioned almost exclusively into one blastomere and in others it was evenly distributed between two cells (Fig. 5H). This pattern is entirely consistent with that published for the germ plasm (Savage and Danilchik, 1993). In contrast, Vg1 is uniformly and diffusely localized to the vegetal pole of all four blastomeres (Fig. 5G). These observations support a critical role of the cortical cytoskeleton in determining patterns of RNA inheritance during development as originally proposed by Jeffery (1989) for ascidians.

**DISCUSSION**

**Two localization pathways for vegetal RNAs**

Our results demonstrate two different pathways by which maternal mRNAs become targeted to and incorporated in the vegetal cortex. In the earliest stage I oocytes, Xcat-2 is already restricted to the mitochondrial cloud at a time when Vg1 is uniformly distributed. By stage III, Xcat-2 and the fragmented mitochondrial cloud are localized in identical patterns to the vegetal cortex leading us to propose that Xcat-2 is transported to the vegetal cortex as a component of the mitochondrial cloud (Heasman et al., 1984). Two possible models for mitochondrial cloud localization are that Xcat-2 may exit the nucleus vectorially, directly into the closely apposed mitochondrial cloud, or Xcat-2 may contain cis-acting elements that target it to that body. Current studies in our laboratory are directed at elucidating the mechanism involved. Regardless of how Xcat-2 accumulates in the mitochondrial cloud, it is always recovered in a detergent-insoluble fraction. In contrast, Vg1 RNA is localized after Xcat-2 has reached the cortex during stage III/IV, in what may be multi-message transport complexes (see Fig. 1G). Prior to stage III, when Vg1 is found throughout the ooplasm, it is recovered in a soluble fraction. Seven other vegetally localized RNAs isolated in this lab also have localization patterns virtually identical to either Vg1 or Xcat-2 (Jian Zhang and Mary Lou King, unpublished observations). Therefore, it is likely that the two pathways typified by Vg1 and Xcat-2, are the primary if not the exclusive means for localizing RNAs to the vegetal cortex in Xenopus.

**Fig. 5. Xcat-2 and Vg1 RNA are partitioned differently during oogenesis and development.** Whole-mount in situ hybridization using digoxigenin-labeled Vg1 (A,C,E,G) or Xcat-2 (B,D,F,H) antisense RNA and albino oocytes. (A) Oocyte stages I, II and III. Note that Vg1 begins to localize in the stage III oocyte. (B) Stages I, II, III and IV oocytes. Xcat-2 is localized to one pole from the earliest stage. (C) Stage III and IV. The pattern for Vg1 is much broader. (D) Stage VI oocytes. One oocyte has been cut to show that staining is cortical. (E-F) Eggs. Vg1 (E) stains in a more diffuse pattern than Xcat-2 (F). Egg on left in F is a control probed with the sense strand. (G) 4-cell embryo. Embryo on the right shows Vg1’s diffuse staining pattern in the vegetal hemisphere. Compare with the sense-strand control embryo on the left. (H) 4-cell embryo. Xcat-2 has a different distribution, staining in 80-100 discrete “islands” characteristic of the germ plasm at this stage. Bars, 500 µm except in A; bar, 250 µm.

**DIF components and RNA localization**

Although Vg1 and Xcat-2 are localized at different stages and by different mechanisms, at one oogenic stage or another both RNAs are pelleted in the presence of detergent and high salt. Vg1 DIF association is maximal by early stage IV, before complete cortical localization. Thus, it is reasonable to propose that the DIF contains components involved in three aspects of the localization process: (1) anchoring Xcat-2 within the mitochondrial cloud, (2) translocation and (3) anchoring Xcat-2 and Vg1 in the vegetal cortex. Experiments using nocodazole or colchicine have demonstrated a requirement for intact microtubules for both Vg1 translocation (Yisraeli et al., 1990) and for mitochondrial cloud movement to the cortex (Wylie et al., 1985). However, the extraction conditions used in our studies do not preserve microtubules. An intriguing possibility is that RNAs are transported in complexes or translocation particles of sufficient density, size and integrity that they are recovered in the DIF. Interestingly, Ainger et al. (1993) have reported that myelin basic protein mRNA is associated with a DIF in oligodendrocytes and that, most likely, it is transported in particles by microtubules to the periphery of the cell.

It is unclear what DIF components are responsible for anchoring Vg1 or Xcat-2 to the cortex. As much as 70% of Vg1 can be released with cytochalasin B treatment implicating the microfilament cortical network (Yisraeli et al., 1990). But the failure to release all the Vg1 suggests that other components are involved. A direct link between Vg1 and the cytokeratins has been ruled out (Klymkowsky et al., 1991) and disrupting microtubules has no effect. We are currently trying to identify the DIF proteins responsible for cortical binding by north-western and crosslinking experiments.

**Fate of Vg1 and Xcat-2 during maturation and development**

Vg1 and Xcat-2 differ not only in their pattern of localization during oogenesis, but also in their fate during maturation and early development. Xcat-2 remains in the cortex in the ovulated egg. condensed into discrete “islands” by the 4-cell stage and remains associated with the DIF. In contrast, Vg1 loses its cortical position abruptly at GVBD. Thus, our data show that Vg1 and Xcat-2 must be bound in the cortex via different components, and that these components respond differently to maturation signals. The strong correlation between the DIF and localized RNAs in the vegetal cortex, and the loss of poly(A)+ in the DIF after maturation, suggests that the release of Vg1 may be part of a general release of vegetal RNAs, and that Xcat-2 is unusual in that it is retained. In fact, the other mRNA known to be localized to the cortex in stage VI oocytes, Xlwnt-11, is also released during maturation (Ku and Melton, 1993).

The fate of Xcat-2 RNA and the published pattern of germinal granules during oogenesis, through maturation events, and in the 4-cell embryo is very similar if not identical (Heasman et al., 1984; Savage and Danilchik, 1993). However, co-localization of Xcat-2 and the germinal granules at the ultrastructural level will have to be done before we can assign Xcat-2 to these structures. If Xcat-2 is a component of the germinal granules, the mitochondrial cloud could provide a vehicle for physically and functionally segregating the germ cell determinants from somatic determinants, like Vg1, found in the cortex. The mitochondrial cloud may also contribute components...
required for different interactions with the cortex that ultimately dictates patterns of inheritance for RNAs during early development. In fact, recent evidence has implicated other components of the mitochondrial cloud, Xlsirits (short interspersed repeat transcripts), as non-coding docking RNAs (Kloc et al., 1993). Xlsirits anti-sense knock out experiments disrupted Vgl, but not Xcat-2, cortical binding without leading to RNA degradation (Kloc and Etkin, 1994). These results provide additional evidence for differential RNA binding in the cortex and show that the mitochondrial cloud does contain elements that could mediate such differences.

This work was supported by grants from NIH (GM39332 to M. L. K., HD07192 to M. P. and HD07129 to C. F.). We are grateful for the advice and assistance of Yi Zhou and Jian Zhang in providing the DNA probes used in Fig. 4 and 5. Julia Bundschuh provided expert technical assistance.

REFERENCES

(Received 21 September 1994)