Antibody identification, chromosome map assignment, and sequence analysis of a Rab escort protein homolog in Drosophila

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Abstract

Using a polyclonal antiserum a cDNA encoding a Rab escort protein (REP) homolog in Drosophila has been identified and sequenced. The gene encodes a 511 residue protein with a predicted molecular mass of 56855 Da. Antibody labeling demonstrates that Drosophila REP protein is present in the early embryo and that it is being apportioned uniformly throughout the embryo in a process likely to be linked to the syncytial nuclear divisions. In situ hybridization to polytene chromosomes reveals that the Drosophila REP gene is located in the 56E region on the second chromosome. Drosophila REP is the first invertebrate REP homolog to be identified and characterized. © 1999 Elsevier Science B.V. All rights reserved.

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been characterized, only two related mammalian REP proteins, REP1 and REP2, and one *Saccharomyces cerevisiae* homolog have been identified to date. REP1 in human is encoded by the X-linked choroideremia (CHM) gene [9], while REP2 or CHM-like gene [10] is located on chromosome 1 [11]. Deletion of the REP1 gene causes a progressive retinal dystrophy leading to complete degeneration and blindness by middle age. In yeast, one gene of REP (MRS6/MSI4) has been identified [12]. This gene is required for cell viability [13], suggesting that there may be only one REP gene in yeast. Here we report on a polyclonal antiserum which we have used to clone and characterize the full length sequence of a *Drosophila* REP homolog.

We have been interested in protein localization and distribution during syncytial nuclear divisions in *Drosophila* embryos [14]. Embryos in *Drosophila* begin with a single zygotic nucleus which undergoes eight synchronous mitotic divisions before the majority of the somatic nuclei migrate to the embryonic peripheral cortex. Only after four more rounds of mitotic cycles do membranes form between the nuclei converting the syncytium into a cellularized blastoderm. During attempts to generate polyclonal antibodies to a GST-fusion protein of a novel nuclear protein kinase, JIL-1 [14], that localizes to the nucleus and chromosomes throughout the cell cycle, we obtained a rabbit serum which does not recognize the nucleus or the JIL-1 protein but rather discrete punctate structures surrounding the nuclei of the syncytial embryos (Fig. 1A). On immunoblots the antiserum recognizes the antigen as a single band with an apparent molecular mass of 60 kDa (Fig. 2A). This is in contrast to the nuclear protein JIL-1 which has a predicted molecular mass of 158 kDa suggesting that...
the 60 kDa band represents a completely different protein. Interestingly, the distribution of the antibody-positive structures during nuclear division in the syncytial embryos appears to be linked to the mitotic cycle. During prophase the punctate antibody-positive structures congregate around the nuclei (Fig. 1B) and during metaphase they are apportioned equally to the spindle poles (Fig. 1C) colocalizing with the asters of the mitotic spindle. At telophase the nuclei reform with each of the duplicated nuclei associated with their half of the punctate structures (Fig. 1D). This redistribution probably reflects a mechanism of apportioning maternally supplied proteins uniformly during the syncytial divisions in preparation for cellularization.

Since the localization and redistribution of the 60 kDa antigen clearly is coordinated with the mitotic cycle we decided to determine its molecular identity. To this end we screened $5 \times 10^5$ plaques of a *Drosophila* oligo-dT primed expression vector library with the antiserum and a single partial positive cDNA clone which included the poly A tail was identified. Subsequently, another cDNA library was screened with a radiolabeled nucleotide probe generated from the 5' end of the original cDNA clone. From this screen several overlapping cDNA clones were isolated which are likely to encompass the entire coding sequence since it has a 5' ATG initiation codon just downstream from an in-frame TAG stop codon. The predicted sequence is for a protein containing...
511 residues (Fig. 3) with a calculated molecular mass of 56,855 Da which is close to the 60 kDa estimate for the antigen based on SDS-PAGE analysis (Fig. 2A). To further verify that the cloned protein indeed corresponded to the 60 kDa antigen and the punctate staining in the syncytial embryos we generated a GST-fusion protein containing the coding sequence of the original cDNA isolate identified by the antiserum. Affinity purified GST-fusion protein was then used for preabsorption of the antiserum. As shown in Fig. 2B preabsorption with GST-fusion protein of the antiserum completely abolished staining on immunoblots whereas preabsorption with GST protein was without effect as compared with control lanes. Likewise, immunocytochemical staining of syncytial embryos were eliminated by GST-fusion protein preabsorption but not by GST protein alone (data not shown).

The complete sequence of the identified protein is shown in Fig. 3 and it shares three highly sequence conserved regions (SCRs) with other members of the REP and Rab GDI families which have been shown by site directed mutagenesis to be involved in the binding of Rab proteins [8]. Fig. 4A shows the rela-
tive location of the SCRs in schematic diagrams of the molecules to their N-terminal and central portions. Residues and sequence motifs in these regions are particularly diagnostic of REP and GDI family members owing to the composition of invariant di- and tripeptides in evolutionarily divergent species [8,15]. In contrast to GDIs and yeast REP the mammalian REPs are distinguished by containing a large insert (138 amino acids) that separates SCR1 and SCR2 [8]. Fig. 4B shows a sequence comparison of the protein for SCR1 and 3 with the most homologous sequences in the data banks all of which belong to the mammalian REP representatives suggesting that we have identified a *Drosophila* REP homolog. Thus, to further determine the evolutionary relationship between the identified protein and members of the REP and GDI gene families we constructed phylogenetic trees based on maximum parsimony using the PAUP computer program. Fig. 4C shows a consensus tree based on REP and GDI sequences from the SCRs from different organisms. The tree is rooted using sequences from yeast GDI as an outgroup; however, the same topology was obtained for unrooted trees. The identified protein is clearly a member of a monophyletic clade comprising mammalian and yeast REPs that is supported by a bootstrap value of 100% confirming its designation as a *Drosophila* REP homolog.

In situ hybridization of polytene chromosomes revealed that the *Drosophila* REP gene maps to the right arm of the second chromosome in the 56E region (Fig. 5). This region is relatively poorly characterized and we have not been able to identify any previously reported mutations likely to correspond to the *Drosophila* REP gene. However, the future isolation and characterization of mutants defective in REP in *Drosophila*, an animal amenable to genetic manipulation, promises to provide further insights into the function of this protein.

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**References**