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Cloning by Differential Screening of a *Xenopus* cDNA That Encodes a Kinesin-Related Protein

RENÉ LE GUELLEC, JEANNIE PARIS,† ANNE COUTURIER, CHRISTIAN ROGHI, AND MICHEL PHILIPPE*

Laboratoire de Biologie et Génétique du Développement, Centre National de la Recherche Scientifique, URA 256, Campus de Beaulieu, Université de Rennes I, 35042 Rennes Cédex, France

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By differential screening of a *Xenopus* egg cDNA library, we selected nine clones (Eg1 to Eg9) corresponding to mRNAs which are deadenylated and released from polysomes soon after fertilization. The sequence of one of these clones (Eg5) revealed that the corresponding protein has the characteristic features of a kinesin-related protein. More specifically, Eg5 was found to be nearly 30% identical to a kinesin-related protein encoded by *bimc*, a gene involved in nuclear division in *Aspergillus nidulans*.

For most animals, the developmental period following fertilization is characterized by a state of very rapid cellular proliferation (6). In the case of xenopus, the first cleavage takes place 1.5 h after fertilization and is followed by 11 almost synchronous cell divisions which occur every 30 min (23). The onset of transcription is clearly detected only after cleavage 12 at a stage called the midblastula transition (23). In the presence of dactinomycin, embryos develop up to the midblastula transition, whereas this development is blocked by puromycin or cycloheximide (3, 20). Similar results have been obtained for maturation, which is independent of new transcription but requires de novo translation (15). Qualitative analysis of the proteins synthesized in oocytes (stage VI), in unfertilized eggs, and in embryos has shown that during maturation and after fertilization, new proteins appear but others are no longer synthesized (5, 19, 29). This suggests that the synthesis of specific gene products necessary for maturation, for the metaphase block in unfertilized eggs, and then for rapid proliferation is regulated at the translational level from the bulk of maternal mRNA. By differential screening of a cDNA library prepared with poly(A)$^+$ RNA from unfertilized eggs, we isolated 11 nonoverlapping sequences which undergo either adenylation or deadenylation after fertilization (26). Nine of them which constitute the Eg family correspond to RNAs which are specifically deadenylated (24–26) and released from polysomes after fertilization (24, 26). In a previous work, we have shown that Eg1 has very high homology to p34^{cdc2} but is clearly functionally different from the kinase subunit of the maturation-promoting factor (24). In the present report, we describe the characteristics of Eg5, which clearly belongs to the kinesine-related protein family.

Kinesin was first discovered during studies of organelle transport in giant squid axons (1, 30). Subsequently, kinesin has been found in a variety of organisms and cell types, including avian and mammalian neuronal tissues (4, 30), sea urchin eggs (28), cultured cells (22), and *Drosophila melanogaster* (27). Biochemical studies of kinesin protein revealed that it is a tetramer consisting of two heavy and two light chains (2, 14). The gene that codes for the drosophila heavy chain has been cloned and characterized (33, 34). More recently, genes that encode kinesin-related proteins were identified by genetic criteria in *D. melanogaster* (7, 35) or by molecular genetic critera in *Saccharomyces cerevisiae* (18), *Aspergillus nidulans* (8), *D. melanogaster* (16), and *Schizosaccharomyces pombe* (11).

The *KAR3* mutant of *S. cerevisiae* is defective in karyogamy (18). Mutations in the *bimc* gene of *Aspergillus nidulans* prevent spindle pole body separation and nuclear division (8). In *S. pombe*, *cut7*$^+$ is referred as a gene involved in spindle formation (11). The product of the drosophila *claret* gene is clearly implicated in chromosome segregation and is active in meiosis (7). The *nod* gene is required for distributive segregation of nonexchange chromosomes during meiosis in *D. melanogaster* (35).

The sequencing strategy, the nucleotide sequence, and the predicted amino acid sequence of Eg5 are shown in Fig. 1A and B. The cloned Eg5 cDNA is 3,651 nucleotides long and contains 193 nucleotides of 5′-flanking sequence, an open reading frame of 3,180 nucleotides, and a 3′-untranslated region of 278 nucleotides which contains a potential polyadenylation signal (AAUAAA) at nucleotide position 3614 to 3619. The open reading frame codes for a predicted polypeptide 1,060 amino acids long with a relative molecular mass of 119,332 Da. The secondary structure predicted (10, 32) for Eg5 protein is shown in Fig. 1C. The predicted Eg5 protein has two globular domains separated by an α-helical region, which is a characteristic of all known kinesin and kinesin-related proteins. Like all of these proteins, Eg5 also possesses a putative motor domain, which is shown in Fig. 2 (from amino acids 1 to 358). Figure 2 also shows the similarities of the motor domain encoded by Eg5 (defined as amino acids 1 to 358, corresponding to the end of Kar3) to the proteins encoded by *bimc* (8), the drosophila kinesin heavy chain gene (34), *cut7*$^+$ (11), *claret* (7), and *KAR3* (18), which are, respectively, 53.3, 49.7, 38.9, 29.9, and 28.6%. At amino acid positions 92 to 107, Eg5 possesses a putative ATP-binding site (IFAYGQTXXGKTXTM) which is conserved in all five sequences.

As in the drosophila and squid kinesin heavy chains (13, 34) and the *nod* (35)-, *bimc* (8)-, and *cut7*$^+$ (11)-encoded proteins, the proposed motor domain of Eg5 is located at the amino terminus. In contrast, in the *KAR3* (17)- and *claret* (7, 33)-encoded proteins the mechanochemical domain is situated in the carboxy-terminal region. The sizes of the proteins encoded by Eg5, *bimc*, *cut7*$^+$, and the drosophila heavy-chain gene are in the same range, at 1,060, 1,211, 1,073, and

* Corresponding author.
† Present address: Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

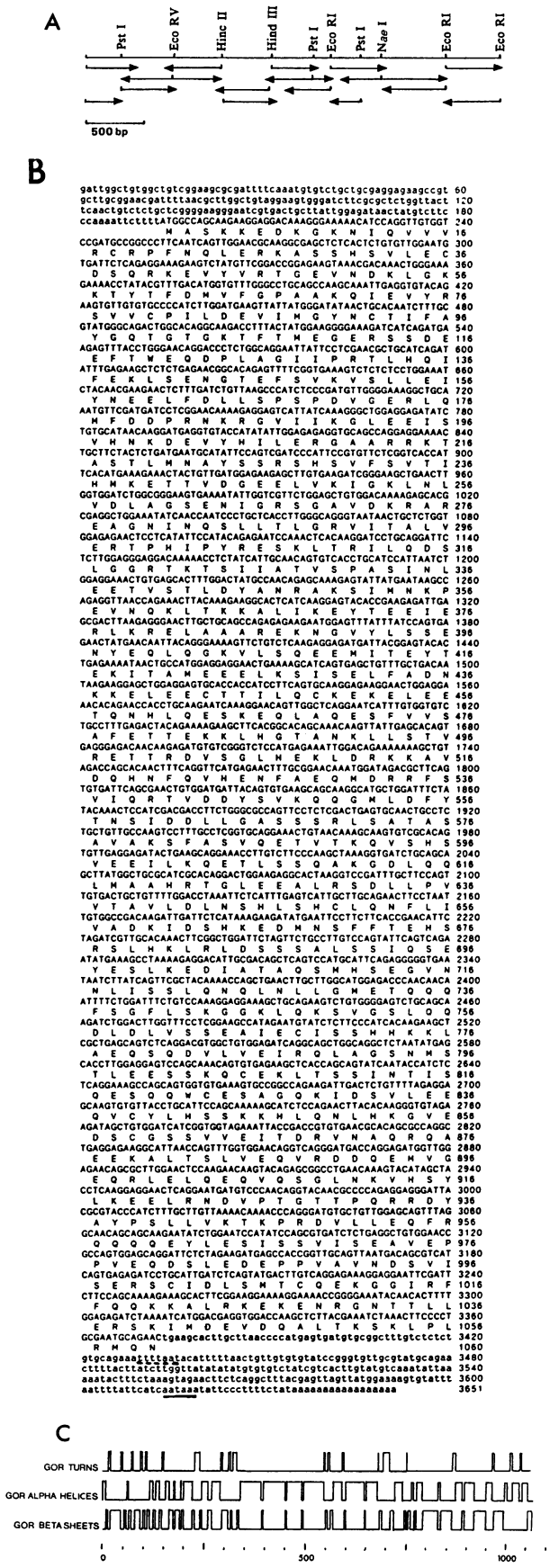

FIG. 1. Nucleotide sequence of Eg1 cDNA and predicted amino acid sequence of the protein. (A) Restriction map and sequencing strategy. The arrows denote the extent and direction of sequence reading of each fragment. (B) Nucleotide and amino acid sequences. The nuclear polyadenylation signal is indicated by solid underlining (nucleotide positions 3614 to 3619). The putative cytoplasmic polyadenylation sequence specific for maturation is indicated by broken underlining (nucleotide positions 3430 to 3436). (C) Secondary structure prediction for Eg5 protein. The secondary structure of Eg5 protein predicted by the method of Garnier et al. (10) is shown. Regions predicted to be β turns (GOR turns), α helices (GOR alpha helices), or β sheets (GOR beta sheets) are indicated by the elevated segments.

975 amino acids, respectively. There is no obvious similarity between the amino acid sequences of Eg5 and the drosophila heavy chain in the carboxy-terminal regions of the molecules. By contrast, as shown in Fig. 2B, significant similarity was found among the proteins encoded by Eg5, *cut7*$^+$, and *bimc*. In particular, from amino acids 911 to 956, more than 35% of the Eg5- and *bimc*-encoded amino acids are identical and more than 50% are of the same family. The similarity extends beyond sequence comparisons; in particular, the structures of the central regions of the proteins encoded by Eg5, *bimc* and *cut7*$^+$ are very closely related, which could suggest similar functions for these gene products. In a previous work (26), we have shown that Eg5 RNA translation is regulated during very early development. In eggs, the RNA is found in both the poly(A)$^+$ fraction and polysomes. After fertilization, Eg5 RNA is deadenylated and concomitantly released from the polysomes. To know when Eg5 RNA appears and when it is adenylated during oogenesis, we performed Northern (RNA) analysis of total RNAs extracted from oocytes (stages I to VI), eggs, and premidblastula transition embryos by using a ^{32}P-labeled Eg5 cDNA probe. Considering that a fixed quantity of total RNA (10 μg) was used and that rRNA constitutes a progressively larger proportion of the total RNA during oogenesis (i.e., 25% in stage I oocytes versus 90% in stage VI oocytes), the decrease in the autoradiogram signals observed in Fig. 3A reflects the dilution of these transcripts in the pool of total RNA. Taking this into account, quantitation of autoradiograms from these Northern analyses confirmed that the number of these transcripts per oocyte or embryo was approximately constant from stage III of oogenesis to the midblastula transition (Fig. 3B). Closer examination of the data (Fig. 3A) shows that Eg5 transcripts appear to be larger in eggs than in either oocytes or embryos, suggesting that the RNA is adenylated only in eggs. When poly(A)$^+$ and poly(A)$^-$ RNAs were separated from the total RNAs extracted from oocytes at different stages and analyzed by Northern blotting with Eg5 as the probe, we never found Eg5 in the poly(A)$^+$ fraction (Fig. 3C). Moreover, in its 3′-untranslated region (at nucleotide positions 3430 to 3436), Eg5 RNA possesses a sequence motif [UUUU(A)AU] similar to those shown to be necessary for maturation-specific adenylation of *Xenopus* mRNA (9, 17). This supports the hypothesis that during maturation Eg5 translation is regulated through adenylation, as recently reviewed (12, 31), and also suggests that the protein is present in eggs but not in oocytes. Antibodies against bovine kinesin reveal a pair of 120-kDa polypeptides in a crude *Xenopus* egg extract (28). After fertilization, Eg5 RNA is no longer detected in polysomes, suggesting that its further translation is not necessary for early development. This is in agreement with the notion that the 12 rapid cell divisions which follow fertilization

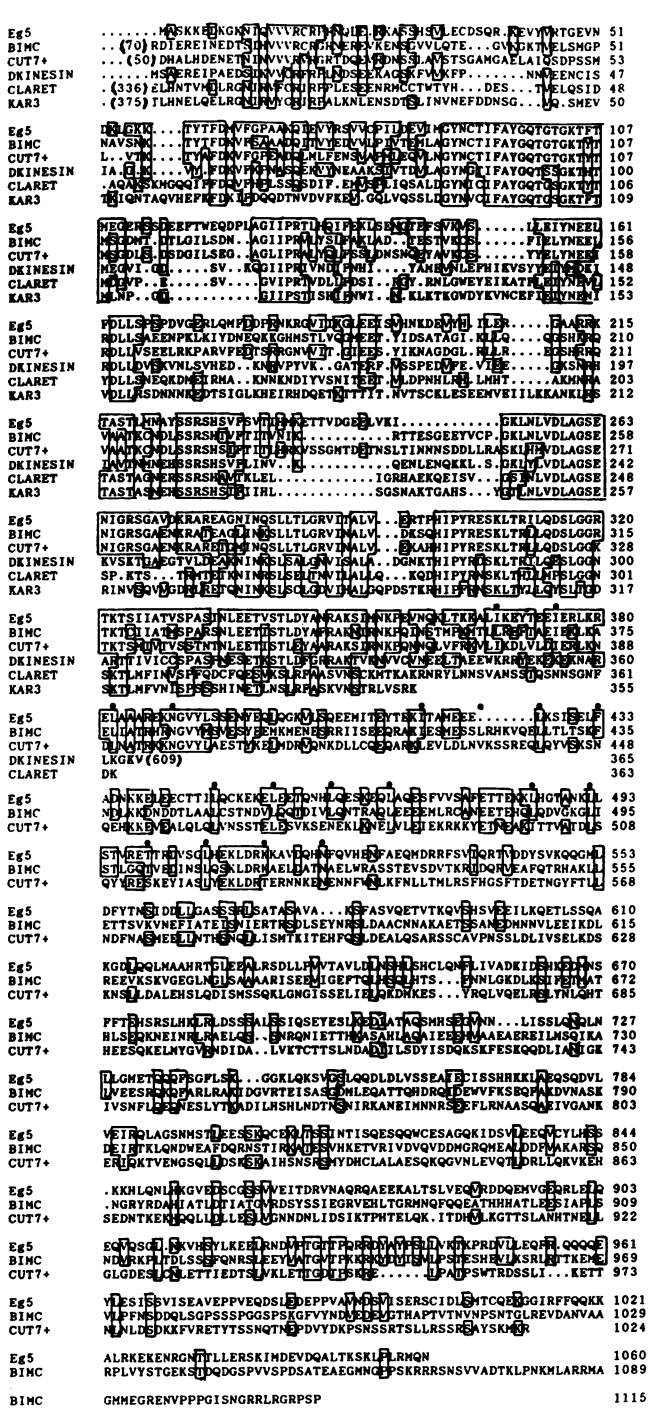

FIG. 2. The predicted amino acid sequence of the motor domain of Eg5 is aligned with those encoded by *Aspergillus bimc*, *S. pombe cut7*$^+$, the drosophila kinesin heavy-chain gene, saccharomyces *KAR3*, and drosophila *claret*. The numbers of the residues at which the *bimc*, *cut7*$^+$, *claret*, and *KAR3*-encoded proteins extend in the amino-terminal direction and the drosophila heavy chain extends in the carboxy-terminal direction are in parentheses. Alignment of the carboxy-terminal parts of the Eg5-, *bimc*- and *cut7*$^+$-encoded molecules is also shown. Identical residues are boxed. The central regions of the Eg5-encoded protein (from amino acids 366 to 535) and the *bimc*-encoded protein (and also the *cut7*$^+$-encoded protein, to a lesser extent) show conserved amino acids (dots) spaced by six amino acids. Most of these conserved amino acids are leucines.

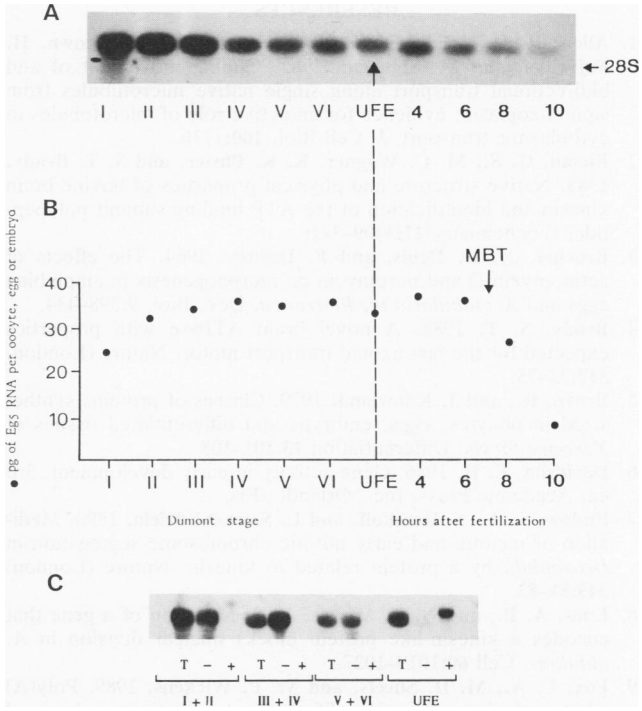

FIG. 3. (A) Northern blot analysis of total RNAs extracted from oocytes (stages I to VI), unfertilized eggs (UFE), and embryos 4, 6, 8, and 10 h after fertilization. Samples of RNA (10 μg) were separated on agarose gel containing 6% formaldehyde and blotted onto nylon membrane (Hybond [Amersham]). Purified inserts were ^{32}P labeled with random primers to a specific activity of 5×10^8 cpm μg^{-1}. Hybridization was carried out in 50% formamide–1% sodium dodecyl sulfate–10× Denhardt's solution–10% dextran sulfate–1% PP_i–1 M NaCl–0.05 M Tris-HCl (pH 8) at 42°C overnight. Filters were extensively washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate at 65°C. (B) Quantitative analysis of Eg5 RNA during oogenesis and very early embryogenesis. MBT, midblastula transition. (C) Total RNAs were extracted from oocytes (stages I and II, III and IV, and V and VI) and unfertilized eggs (UFE). Poly(A)$^+$ was separated from poly(A)$^-$ by oligo(dT) chromatography. Samples [10 μg of total RNA, 10 μg of poly(A)$^-$ RNA, and 200 ng of poly(A)$^+$ RNA] were analyzed by Northern blotting as described for panel A.

could only be under the translation control of cyclins, as suggested by in vitro experiments using egg extracts (21). Moreover, the fact that Eg5 RNA was not detected in adult tissues (26) suggests that it is involved in cell proliferation. Taking these points into account, and also the similarity among Eg5, *cut7*$^+$, and *bimc*, one can postulate that Eg5-encoded protein is involved in nuclear division and may be the counterpart of *bimc*-encoded protein in xenopus.

Nucleotide sequence accession number. The sequence described here has been assigned EMBL accession no. X 54002.

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