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Mechanisms of Development 108 (2001) 197–201

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Gene expression pattern

Expression pattern of DMAP-85 during *Drosophila* embryonic development

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Received 27 February 2001; received in revised form 2 July 2001; accepted 5 July 2001

Abstract

Microtubule-associated proteins (MAPs) play major regulatory roles on the organization and integrity of the cytoskeletal network. Previously, we identified DMAP-85, a *Drosophila* MAP that promotes tubulin polymerization in vitro. In this work, we examine the distribution of DMAP-85 and its association pattern with microtubules at embryonic stages. Immunoblots revealed that DMAP-85 was present throughout embryogenesis, but it was most abundant in stages 6–9. Immunofluorescence studies showed that DMAP-85 was associated with sub-populations of stable microtubules during embryo cellularization, and after gastrulation with interphase microtubule arrays. At late embryonic stages, it was preferentially found in the ventral nerve cord, co-localizing with axonal microtubules. These observations are in agreement with previous reports on DMAP-85 functions, suggesting that DMAP-85 might be required for the stabilization and organization of cytoplasmic microtubules during embryonic development.

Keywords: Cytoskeleton; Microtubules; Microtubule-associated proteins; DMAP-85; Acetylated-tubulin; Embryogenesis; Cellularization; Ventral nerve cord

1. Results and discussion

We have identified DMAP-85, a novel *Drosophila* microtubule-associated protein (MAP) that initiates and promotes microtubule assembly through its interaction with specific motifs on β-tubulin (Cambiazo et al., 1995; Maccioni and Cambiazo, 1995; Henríquez et al., 1996). In vitro, DMAP-85 can be phosphorylated by the mitotic kinase Polo, affecting its binding to microtubules (Cambiazo et al., 2000). To begin exploring the in vivo roles of DMAP-85, we examined the developmental profile of DMAP-85 expression during embryogenesis. Northern blot analysis shows that the DMAP-85 transcript (2.9 kb in Fig. 1A) has a similar relative abundance in all stages analyzed (12 h embryos, third instar larvae and adults). Western blots of protein extracts made from developmentally staged embryos indicate that DMAP-85 is present throughout embryogenesis (Fig. 1B), the protein is also detected in unfertilized eggs, indicating a maternal contribution (data not shown). The lowest and the highest levels of DMAP-85 were present at stages 1–4 and 6–9, respectively (Fig. 1B). This increase in the relative amount of DMAP-85 might be correlated with main changes in microtubular dynamics at the onset of cellularization (Wolf et al., 1988).

Immunofluorescence microscopy of whole-mount embryos revealed that at stage 4 of development, DMAP-85 is found in a distribution strikingly different from that of microtubules (Foe et al., 1993). It appears as a uniform, grainy network, underlying the cortical cytoplasm that surrounds each nucleus, whereas most of the microtubules are involved in the formation of mitotic spindle structures (data not shown). At stage 5, as embryo cellularization proceeds, two groups of microtubules radiate from the centrosomes (Warn and Warn, 1986; Itoh and Ishikawa, 1989; Calliani, 1989). The first one is composed of short polymers (astral microtubules; Fig. 2A, arrows) that reach toward the cortex. The second one consists of long microtubules surrounding the nuclei in close contact with both the cleavage furrow membranes and the nuclear envelope (Fig. 2A, arrowheads); they contain acetylated α-tubulin isoforms (Wolf et al., 1988) and represent a sub-population of stable polymers that first appears at the time of cellularization. At this stage, DMAP-85 does not co-localize with the centrosomes or with the astral microtubules (Fig. 2A–D). When viewed from the surface of the embryo, DMAP-

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0925-4773/01/$ - see front matter
PII: S0925-4773(01)00481-6

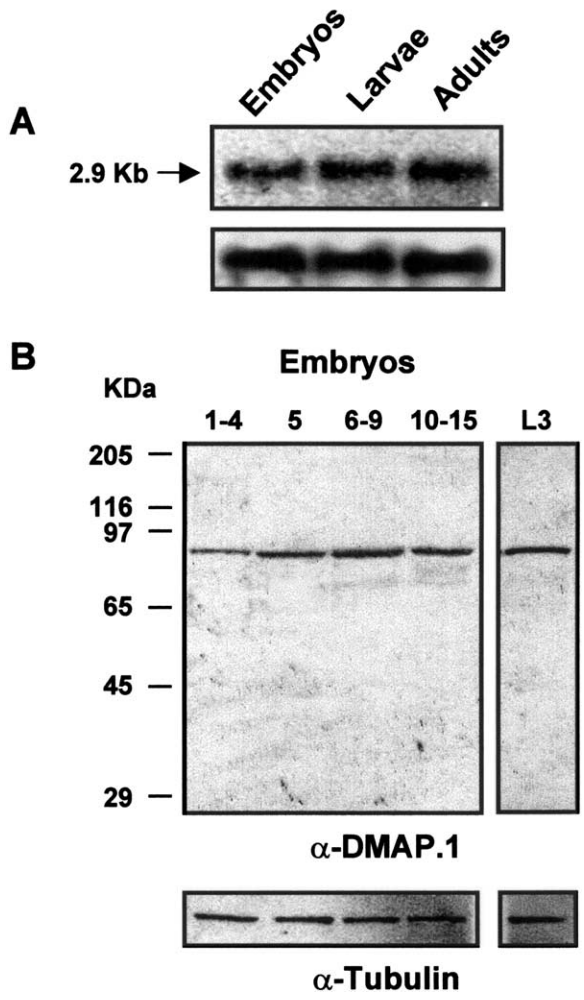

Fig. 1. Northern and Western blot analysis of DMAP-85. (A) Northern blot analysis of DMAP-85 expression during *Drosophila* development. Each lane contains 5 μg of poly(A)$^+$ RNA isolated from embryos, third instar larvae and adults. The DMAP-85 cDNA used as a probe detects one transcript of 2.9 kb. Expression of actin (lower panel) was used as a loading control to calculate the relative abundance of the transcript. (B) Western blot analysis of DMAP-85 expression. Each lane contains 20 μg of protein extracts from embryos at the indicated stages of development (Campos-Ortega and Hartenstein, 1985) and third instar larvae (L3). Proteins were resolved on a 10% SDS–acrylamide gel, blotted to nitrocellulose and probed with the DMAP.1 antibody. Tubulin was detected using an anti-α-tubulin antibody. After quantification of immunoreactive bands, arbitrary densitometric units were used to determine DMAP-85/tubulin ratios.

85 staining reveals ring-shaped structures, suggesting the association of this protein with the long microtubules that envelope the nuclei (Fig. 2A–F). In Fig. 2E,F, a deeper focal plane permits detailed observation of the co-distribution of DMAP-85 with this sub-population of stable microtubules. During the second phase of stage 5, in a view focused on the actin contractile-ring (Foe et al., 1993), DMAP-85 (Fig. 2H) clearly co-localizes with long microtubule bundles (Fig. 2G) in a punctuate circle of filaments.

At stage 6, during embryo gastrulation, DMAP-85 increases its presence in the cellular cytoplasm, where it co-localizes with the microtubule network (Fig. 3A–D). In embryos of stage 8, patches of dividing cells can be distinguished, forming the mitotic domains of the embryo (Fig. 3E,F; arrowheads). DMAP-85 co-distributes with the microtubules exclusively in the interphase cells (Fig. 3E–G). This co-localization can be clearly observed in the extended cytoplasm of amnioserose cells (Fig. 3H,I). In the cells that form a mitotic domain (Fig. 3F), DMAP-85 presents a pattern of homogeneous immunofluorescence that embraces the whole cytoplasm (Fig. 3G). During the final stages of the embryonic development, populations of stable microtubules concentrated at the neuronal projections of the central and peripheral nervous system (Wolf et al., 1988) can be recognized with an anti-acetylated-tubulin antibody (Fig. 3K). DMAP-85 is clearly enriched in the axonal processes of the ventral nerve cord, relative to its overall staining levels in the embryo (Fig. 3J). This observation indicates that the protein appears to be preferentially associated with stable microtubules at the connectives and commissures of the ventral nerve cord (Fig. 3K,L).

Our evidences on the capacity of DMAP-85 to bind and induce tubulin assembly in vitro along with the observations of its distribution during embryonic development, suggest the hypothesis that DMAP-85 functions as a microtubule-stabilizing protein. DMAP-85 does not associate with the highly dynamic spindle microtubules, but it co-distributes with interphase microtubules and with sub-populations of stable microtubules. Therefore, we suggest that DMAP-85 participates in regulating the stability and organization of microtubules during embryo cellularization, and afterwards during the assembly of interphase microtubular arrays, including the stable axonal microtubules. The mechanisms that regulate the association of DMAP-85 with microtubules in vivo remain unknown. However, our data indicate that DMAP-85 is phosphorylated in vitro by Polo kinase, reducing its microtubule-binding capacity (Cambiazo et al., 2000). Therefore, temporal and spatial regulation of DMAP-85 phosphorylation by Polo and other kinases might be responsible for the DMAP-85 expression pattern during *Drosophila* embryogenesis.

Developmental processes that regulate *Drosophila* embryogenesis are closely coordinated with changing patterns of cytoskeletal organization. The complex changes in microtubule assembly and organization during embryogenesis may depend on the combined activity and modulation of several classes of MAPs, including proteins, such as DMAP-85, that promote microtubule stabilization.

2. Materials and methods

2.1. Fly cultures

Canton-S fly cultures and embryo collections were performed by standard procedures. Embryos were individu-

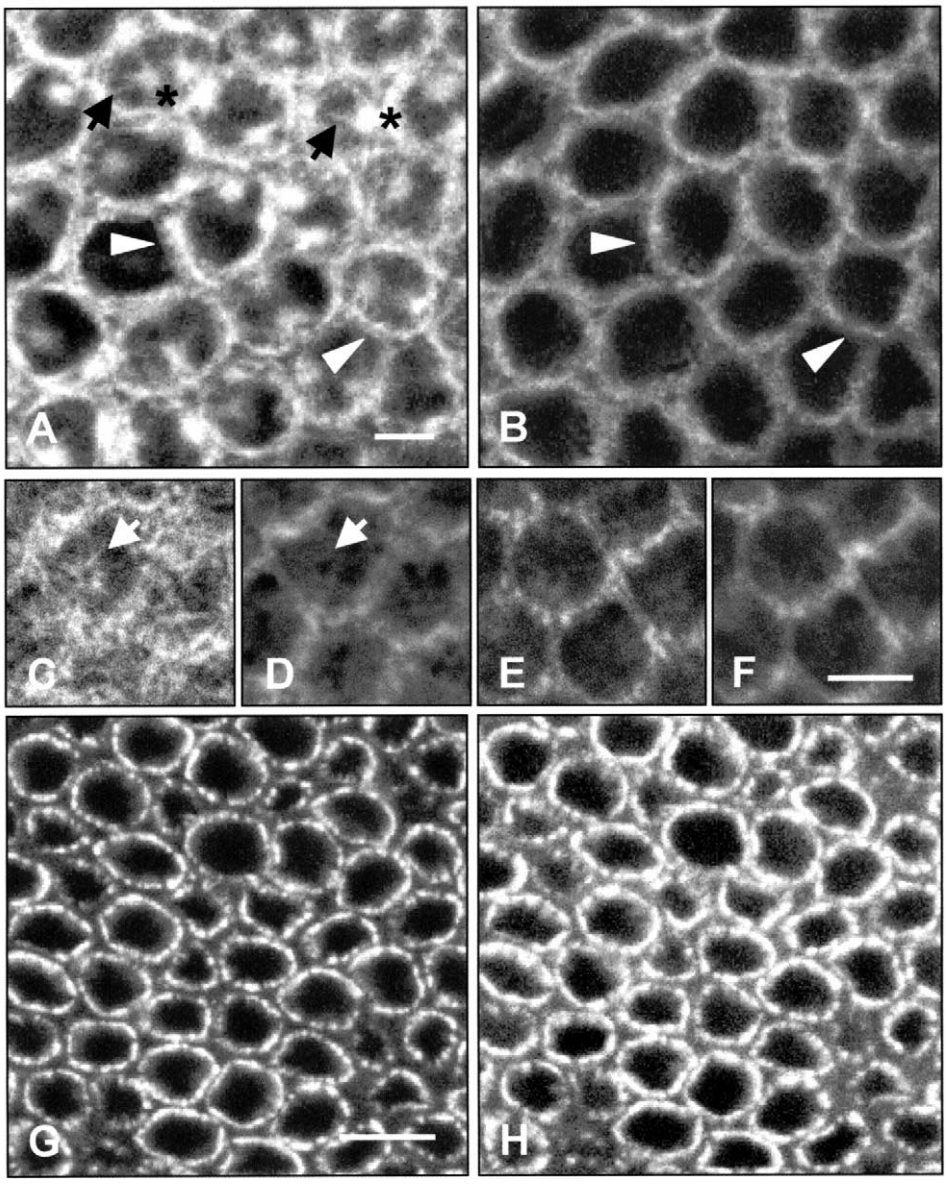

Fig. 2. DMAP-85 distribution in cellularized embryos. Confocal fluorescent images of whole-mount stage 5 embryos double-stained for: (B,D,F,H), DMAP-85; and (A,C,E,G), α-tubulin. (B,D,F) In these transversal focal sections of embryo cells, DMAP-85 staining appears as ring-shaped structures. (A,B) DMAP-85 co-localizes with the long microtubules that envelope the nuclei (arrowheads). Bar, 5 μm. (A,B) The protein does not localize to the astral microtubules (arrows) or to the centrosomal region (asterisks). Higher magnifications show the spatial relationship of DMAP-85 with microtubules at a focal plane: (C,D), 1.2; and (E,F), 1.8 μm below the embryo surface. Arrow indicates astral microtubules. Bar, 5 μm. (G,H) At a focal plane coincident with the localization of the actin–myosin contractile-ring, DMAP-85 and long microtubules co-distribute in a punctuate ring of filament bundles. Bar, 10 μm.

ally staged according to Campos-Ortega and Hartenstein (1985).

2.2. Northern and Western blots

Poly(A)$^+$ RNA was isolated using the PlyATract mRNA isolation system (Promega, Madison, WI). Protein extracts were prepared as described (Cambiazo et al., 2000). Northern and Western blots were performed according to standard procedures. Blots were processed as described in Cambiazo et al. (2000). DMAP.1 antibody (Henríquez et al., 1996) and anti-α-tubulin antibody (clone B-5-1-2, Sigma Chemical Co.) were used at a 1:500 dilution. Positive bands were scanned and quantified using Kodak Digital Science EDAS 120LE software, and the loading was standardized using the tubulin in each lane as an internal control.

2.3. Immunohistochemistry of whole-mount embryos

Embryos were fixed in 33% formaldehyde–50 mM EGTA/heptane according to Theurkauf (1992), blocked and stained in PBS with 1% goat serum and 0.1% Triton X-100. Antibodies were used as follows: DMAP.1, 1:500; anti-α-tubulin, 1:1000; and anti-acetylated-tubulin (clone 6-

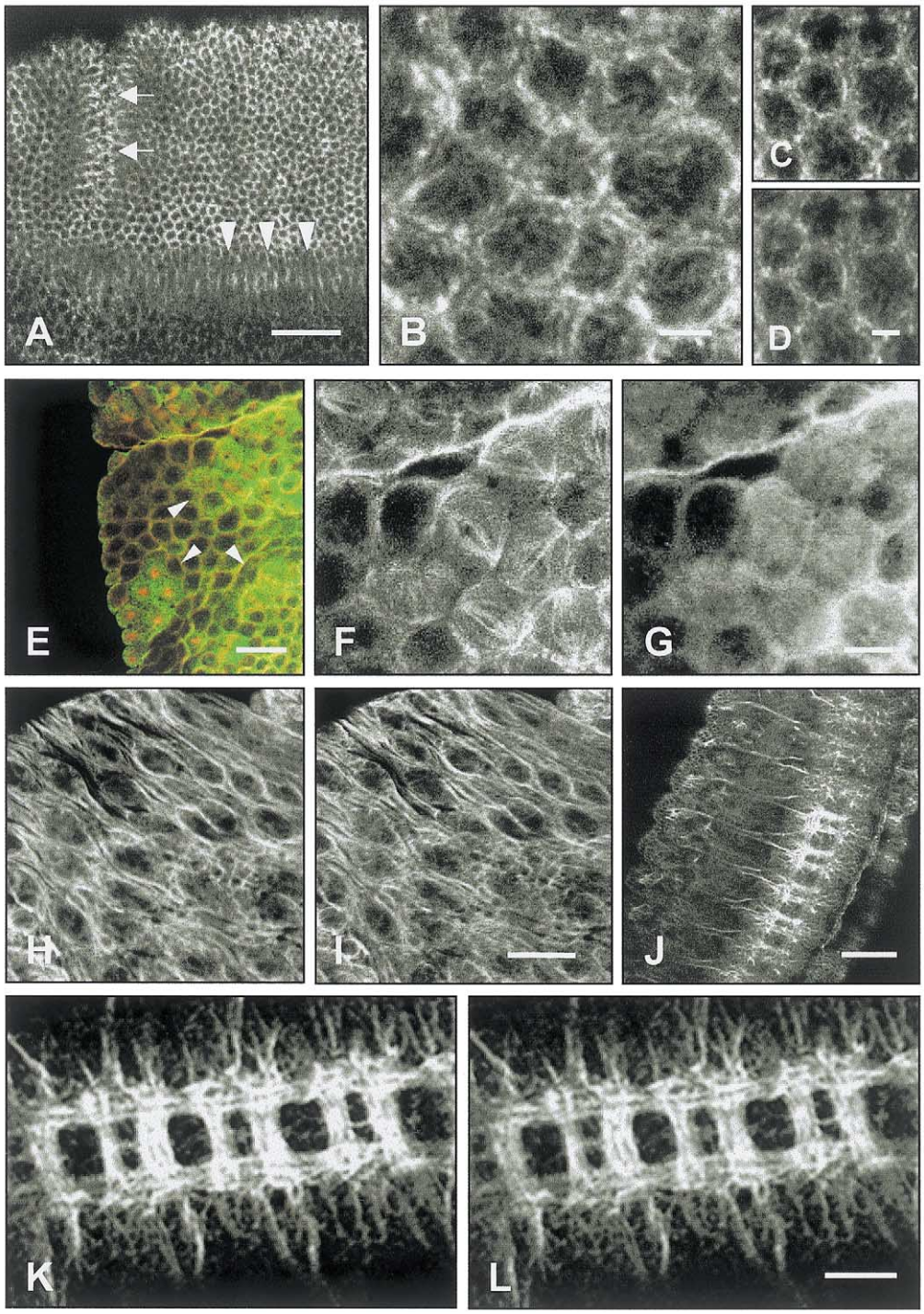

Fig. 3. Expression of DMAP-85 in post-blastoderm embryos. Embryos were labeled for DMAP-85 (A,B,J) or double-labeled for this protein (C,G,I,L) and α-tubulin (D,F,H) or acetylated α-tubulin (K). (A) Lateral view of an embryo during stages 6–7 of development showing DMAP-85 distribution. Arrowheads indicate ventral furrow formation and arrows indicate cephalic furrow formation. Bar, 50 μm. Higher magnification views of similar stage embryos revealing: (B), the DMAP-85 staining pattern; and (C,D), the co-localization of this protein with microtubules. Bar, 5 μm. (E) A general view of a stage 8 embryos showing microtubules (red) and DMAP-85 (green) distribution. Arrowheads indicate mitotic domains. Bar, 10 μm. (F,G) A high magnification view of a mitotic domain reveals DMAP-85 cytoplasm distribution in dividing cells. Bar, 5 μm. (H,I) A detail of the amnioserose membrane to show the striking co-localization of DMAP-85 with the microtubular network of interphase cells. Bar, 10 μm. (J) DMAP-85 preferentially localizes to the central nervous system in stage 15 embryos. Bar, 50 μm. (K,L) DMAP-85 co-localizes with microtubules containing acetylated α-tubulins in the axonal processes that form the ventral nerve cord. Bar, 20 μm.

11B-1, Sigma Chemical Co.), 1:500. Embryos were mounted in Dabco/Mowiol and analyzed using a Zeiss LSM410 confocal microscope.

Acknowledgements

This work was supported by Fondecyt 1990002, and Presidential Chair in Science (to RBM). Marie Curie and Fundación Andes Fellowships and Fondecyt 1980262 (to VC).

References

Calliani, G., 1989. Microtubule distribution reveals superficial metameric patterns in the early *Drosophila* embryo. Development 107, 35–41.

Cambiazo, V., González, M., Maccioni, R.B., 1995. DMAP-85: a tau-like protein from *Drosophila melanogaster* larvae. J. Neurochem. 64, 1288–1297.

Cambiazo, V., Logarinho, E., Pottstock, H., Sunkel, C.E., 2000. Microtubule binding of the *Drosophila* DMAP-85 protein is regulated by phosphorylation in vitro. FEBS Lett. 483, 37–42.

Campos-Ortega, J.A., Hartenstein, V., 1985. The Embryonic Development of *Drosophila melanogaster*, Springer–Verlag, Berlin, pp. 3–84.

Foe, V.E., Odell, G.M., Edgar, B.A., 1993. Mitosis and morphogenesis in the *Drosophila* embryo: point and counterpoint. In: Bate, M., Martinez-Arias, A. (Eds.). The Development of *Drosophila melanogaster*, Cold Spring Harbor Laboratory Press, New York, pp. 149–300.

Henríquez, J.P., Cambiazo, V., Maccioni, R.B., 1996. Tubulin domains for the interaction of microtubule-associated protein DMAP-85 from *Drosophila melanogaster*. Mol. Cell. Biochem. 158, 149–159.

Itoh, K.K., Ishikawa, H., 1989. The cytoskeletal involvement in cellularization of the *Drosophila melanogaster* embryo. Protoplasma 150, 83–95.

Maccioni, R.B., Cambiazo, V., 1995. Roles of microtubule-associated proteins in the control of microtubule assembly. Physiol. Rev. 75, 835–864.

Theurkauf, W.E., 1992. Behavior of structurally divergent α-tubulin isotypes during *Drosophila* embryogenesis: evidence for post-translational regulation of isotype abundance. Dev. Biol. 154, 205–217.

Warn, R.M., Warn, A., 1986. Microtubule arrays present during the syncitial and cellular blastoderm stages of early *Drosophila* embryos. Exp. Cell Res. 163, 201–210.

Wolf, N., Regan, C.L., Fuller, M.T., 1988. Temporal and spatial pattern of differences in microtubule behaviour during *Drosophila* embryogenesis revealed by distribution of a tubulin isoform. Development 102, 311–324.