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Gene expression pattern

Tissue distribution of *PEBBLE* RNA and Pebble protein during *Drosophila* embryonic development

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Abstract

pebble (pbl) is required for cytokinesis during postblastoderm mitoses (Hime, G., Saint, R., 1992. Zygotic expression of the *pebble* locus is required for cytokinesis during the postblastoderm mitoses of *Drosophila*. Development 114, 165–171; Lehner, C.F., 1992. The *pebble* gene is required for cytokinesis in *Drosophila*. J. Cell Sci. 103, 1021–1030) and encodes a putative guanine nucleotide exchange factor (RhoGEF) for Rho1 GTPase (Prokopenko, S.N., Brumby, A., O'Keefe, L., Prior, L., He, Y., Saint, R., Bellen, H.J., 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. Genes Dev. 13, 2301–2314). Mutations in *pbl* result in the absence of a contractile ring leading to a failure of cytokinesis and formation of polyploid multinucleate cells. Analysis of the subcellular distribution of PBL demonstrated that during mitosis, PBL accumulates at the cleavage furrow at the anaphase to telophase transition when assembly of a contractile ring is initiated (Prokopenko, S.N., Brumby, A., O'Keefe, L., Prior, L., He, Y., Saint, R., Bellen, H.J., 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. Genes Dev. 13, 2301–2314). In addition, levels of PBL protein cycle during each round of cell division with the highest levels of PBL found in telophase and interphase nuclei. Here, we report the expression pattern of *pbl* during embryonic development. We show that *PEBBLE* RNA and PBL protein have a similar tissue distribution and are expressed in a highly dynamic pattern throughout embryogenesis. We show that PBL is strongly enriched in dividing nuclei in syncytial embryos and in pole cells as well as in nuclei of dividing cells in postblastoderm embryos. Our expression data correlate well with the phenotypes observed in pole cells and, particularly, with the absence of cytokinesis after cellular blastoderm formation in *pbl* mutants.

Keywords: *Drosophila*; Embryogenesis; Cell division; Cytokinesis; Cleavage furrow; Postmitotic nuclei; Posterior pole; Cellularization; Pole cells; Gonads; Ectoderm; Mesoderm; Central nervous system; Ventral nerve cord; *pebble*; Exchange factor; RhoGEF

1. Results and discussion

To investigate the developmental expression of *pbl*, we performed Northern and Western analyses, in situ hybridization to whole-mount embryos, and immunohistochemical staining of embryos with anti-PBL immune sera. *PEBBLE* transcripts (4.0 and 5.5 kb, arrows in Fig. 1A) are abundant in adult flies and in embryos, but are not detected in third instar larvae. *PEBBLE* has lower levels of expression in late embryos (12–15 h after egg deposition or stages 15–16) when mitotic divisions are restricted mainly to the central nervous system (CNS). Western analysis using anti-PBL immune sera shows that PBL protein (115–120 kDa, arrow in Fig. 1B) is also abundant in wild-type embryos (lanes 2 and 5). The protein is expressed at elevated levels in embryos ectopically expressing PBL (lanes 3 and 6) suggesting that the immune sera recognize PBL protein on a Western blot. Similarly, anti-PBL immune sera recognize ectopic PBL expressed in embryos (Fig. 3K) in a *paired*-like pattern (Fig. 3L).

In preblastoderm embryos, both *PEBBLE* RNA and PBL protein are much enriched at the posterior pole (Fig. 2A–C, arrows) where the pole cells form during telophase of mitotic cycle 10 (stage 4; Fig. 2D,H,J, arrows). *PEBBLE* RNA (Fig. 2H, arrow in inset) and PBL protein (Fig. 2J, inset) are deposited in cytoplasm and nuclei, respectively, of budding pole cells. *PEBBLE* RNA is expressed ubiquitously in preblastoderm (Fig. 2A) and syncytial blastoderm (Fig. 2D) embryos, but its levels decrease significantly upon cellularization (stage 5; Fig. 2H). PBL protein is expressed

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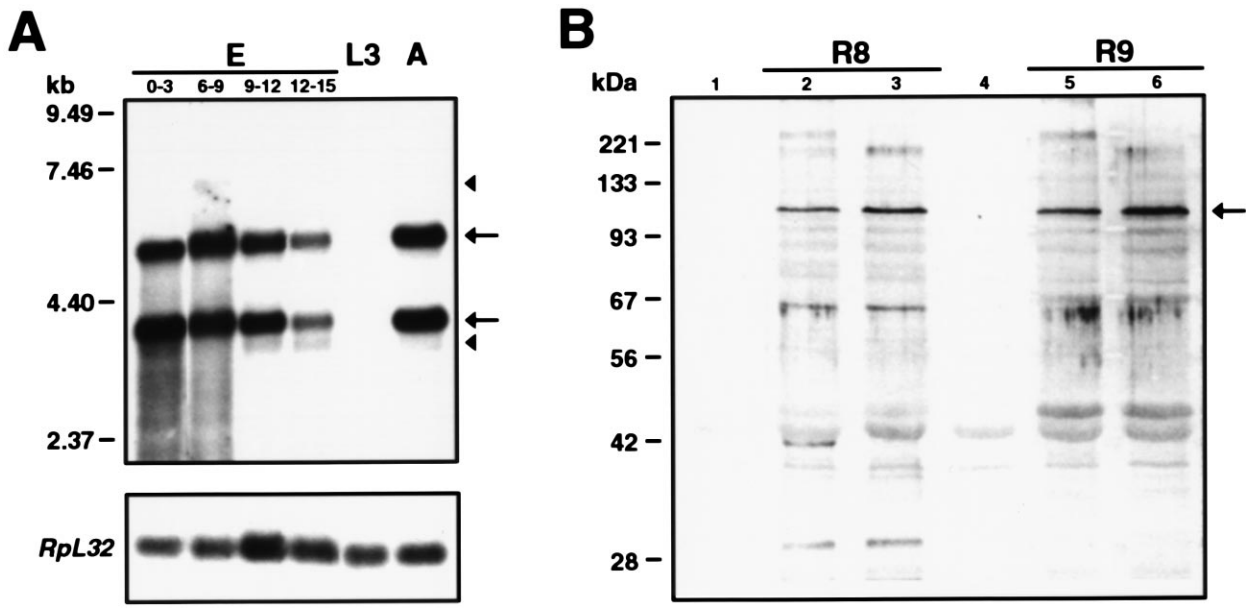

Fig. 1. Northern and Western analyses of *pbl* expression. (A) Northern analysis of *PEBBLE* expression during *Drosophila* development. Each lane contains 5 μg of poly(A)$^+$ RNA isolated from embryos (E, age in hours after egg deposition is indicated), third-instar larvae (L3), and adults (A). The 1A *pbl* cDNA used as a probe (upper panel) detects two major transcripts of about 4.0 and 5.5. kb (arrows) and two faint bands of about 3.7 and 7.0 kb (arrowheads). Expression of the *RpL32* gene was used as a loading control (lower panel). (B) Western analysis of PBL expression in embryos. Each lane contains 30 μg of protein extract from wild-type embryos (lanes 1, 2, 4, and 5) or embryos from a cross between *prd-GAL4* and pbl^{EP3415} flies (lanes 3 and 6; see Materials and methods). Proteins were resolved on a 10% SDS-polyacrylamide gel followed by immunoblotting using R8 (lanes 2 and 3) or R9 (lanes 5 and 6) anti-PBL immune sera or the respective preimmune sera (lanes 1 and 4). Both immune sera recognize a major 115–120 kDa protein band (arrow). Note that only 25% of embryos from a cross between *prd-GAL4* and pbl^{EP3415} flies express ectopic PBL.

in all dividing nuclei in syncytial embryos (Fig. 2C,E,I) beginning from the single nucleus stage (stage 1; Fig. 2B). Levels of PBL protein cycle during each round of nuclear divisions in the syncytium (data not shown), similar to the cycling of PBL during mitoses in the postblastoderm embryo (Prokopenko et al., 1999). PBL is also found in the interphase nuclei of all cells at cellular blastoderm (stage 5; Fig. 2J). The dividing nuclei in syncytial embryos (Fig. 2E, brackets) form dumbbell-shaped structures (Fig. 2E,F) with PBL-positive late telophase nuclei being connected by a thin bridge of PBL staining (red in Fig. 2F, arrows) surrounded by a nuclear envelope (green in Fig. 2F). Nuclear PBL is not distributed homogeneously, but forms an intricate, speckled pattern (red in Fig. 2G).

During gastrulation (stage 6; Fig. 3A) and germ band extension (stages 9–11; Fig. 3B and D) when many cells undergo mitotic cycles 14–16, PBL is strongly enriched in the nuclei of most cells, including the pole cells (Fig. 3A, arrow). The highest levels of PBL are found in late telophase nuclei (Prokopenko et al., 1999). This staining persists in early interphase nuclei in the following mitotic cycle resulting in pairs of daughter cells being strongly labeled with nuclear PBL (Fig. 3B, circled and red in inset). We observed no PBL protein during late prophase, metaphase, and early anaphase, suggesting that the levels of the protein cycle during each cycle of cell division (Prokopenko et al., 1999). During maximal germ band extension *PEBBLE* RNA is expressed ubiquitously in the ectoderm and mesoderm (stage 11; Fig. 3C) with higher levels of expression in the brain (Fig. 3C, arrow) and pole cells (Fig. 3C, arrowhead). At the end of germ band retraction (stage 12; Fig. 3E) *PEBBLE* RNA is strongly expressed in the brain (Fig. 3E, arrow), pole cells (Fig. 3E, arrowhead), ventral nerve cord (Fig. 3E, asterisk), and in many ectodermal and mesodermal cells. At this time PBL protein is found ubiquitously with higher levels of expression in the brain (stage 13; arrow in Fig. 3F). By stage 16 when most of the embryonic cells have stopped dividing, *PEBBLE* RNA (Fig. 3G) and especially PBL protein (Fig. 3H) expression is much lower in most tissues than at earlier embryonic stages. However, both RNA and protein continue to be expressed at elevated levels in neuroblasts in the brain (Fig. 3G,H, arrows), ventral nerve cord (Fig. 3I,J), and in gonads (Fig. 3G,H, arrowheads). In late embryos we also observed PBL protein in the hindgut (Fig. 3H) and low levels of *PEBBLE* RNA in ectoderm, cells of the peripheral nervous system, pharynx, and gut (Fig. 3G).

In conclusion, our observations demonstrate that there are high levels of maternally provided *PEBBLE* RNA in preblastoderm and syncytial blastoderm embryos. The persistence of maternal RNA prior to cellularization allows cycling of levels of PBL protein during the fast mitotic

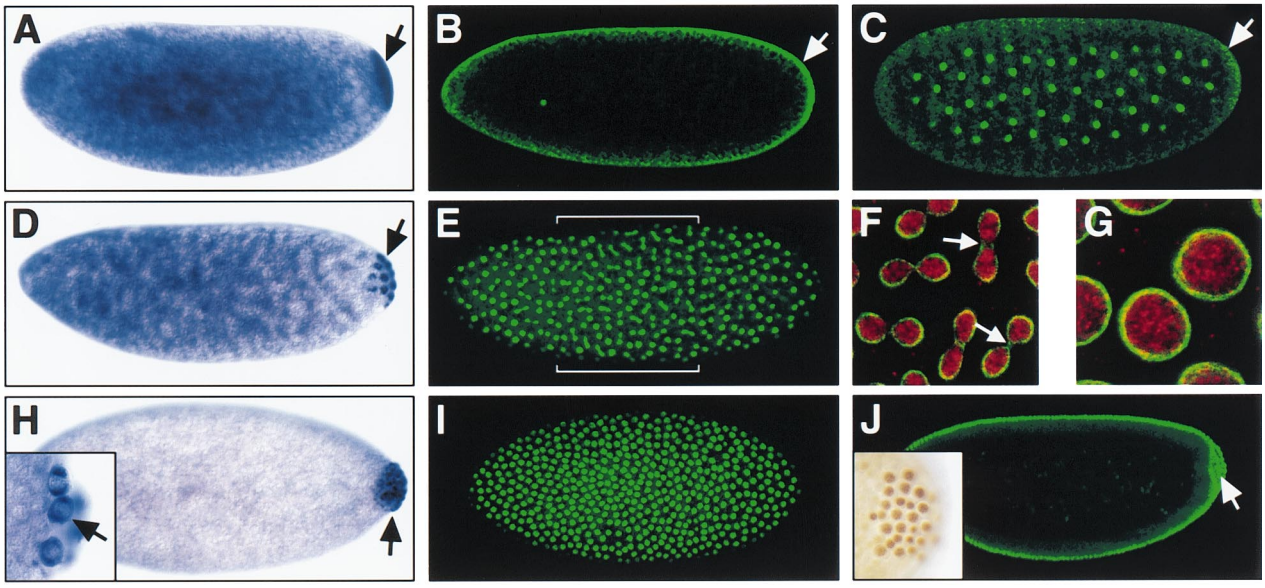

Fig. 2. Expression of *pbl* in preblastoderm and syncytial embryos. Wild-type embryos hybridized with *pbl* antisense RNA (A,D,H) or stained with anti-PBL immune serum (green in B,C,E,I,J; red in F,G). Embryos in (F) and (G) were counterstained with anti-lamin (green) to reveal nuclei. (A–E,J) Lateral views; (H,I) dorsal views.

divisions 1–13. Furthermore, the reduction in the levels of *PEBBLE* RNA at the time of cellularization and the apparent cycling of the protein presumably account for the precise cycle 14 cytokinetic arrest phenotype in *pbl* mutants.

The dramatic changes in the levels of PBL during each round of nuclear division, together with our finding that PBL is expressed in all nuclei in preblastoderm embryos suggest that prior to cellular blastoderm formation PBL may play other roles, unrelated to its role in cytokinesis in late embryogenesis. To examine the function of *pbl* prior to cellularization, we generated mitotic clones in the germline (Louise O'Keefe and R.S., pers. commun.). Interestingly, removal of maternally provided PBL results in female sterility suggesting a requirement for *pbl* in cell division during oogenesis. This observation precluded us to assess the role of *pbl* in preblastoderm embryos.

PBL is expressed in pole cells and in gonads throughout embryogenesis. Pole cells are the first mononuclear cells formed in a syncytial embryo in a process mechanistically resembling cytokinesis. Both *PEBBLE* RNA and PBL protein are localized to the posterior pole prior to the formation of the first pole cells and are deposited, respectively, in their cytoplasm and nuclei. Interestingly, mutations in *pbl* affect proliferation of pole cells resulting in formation of few multinucleate cells (Lehner, 1992). This suggests that a 50% reduction in the levels of maternally provided *PEBBLE* RNA causes the pole cell phenotype.

Our results also demonstrate that throughout embryogenesis PBL protein has the highest levels of expression in the nuclei of dividing cells or in young postmitotic cells. PBL expression seems to be specific for proliferating tissues or tissues with proliferative potential (e.g. embryonic neuroblasts). Furthermore, cessation of proliferation and terminal differentiation of cells correlate with the downregulation of *pbl* expression as observed in late embryos. However, *pbl* continues to be expressed in tissues which resume proliferation during larval development - neuroblasts of the CNS and gonads.

2. Materials and methods

Isolation of poly(A)$^+$ RNA from wild-type flies and Northern and Western analyses were performed according to Sambrook et al. (1989). Protein extracts for Western were prepared as described (Bhat et al., 1996). Amount of protein in extracts was quantitated using Bradford assay. For in situ hybridization to whole-mount embryos (Tautz and Pfeifle, 1989) the 1A *pbl* cDNA (Prokopenko et al., 1999) was used to generate antisense riboprobes (DIG RNA Labeling Kit, Roche Molecular Biochemicals).

The following primary antibodies were used: anti-β-galactosidase (1:1000, Promega), anti-lamin Dm0 (611A3A6, 1:20–1:50; Harel et al., 1989), anti-PBL (GP14, R8, and R9; Prokopenko et al., 1999), and anti-α-spectrin (Ab#354, 1:400; Byers et al., 1987). Secondary antibodies used were Cy3 (1:500, Jackson ImmunoResearch Laboratories), Alexa488 (1:400, Molecular Probes) IgG (H+L) conjugates, biotinylated IgG (H+L) (1:200–1:500, Vector Laboratories), and peroxidase-conjugated IgG (H+L) (1:5000, Jackson ImmunoResearch Laboratories). For confocal microscopy, specimens were mounted in Vectashield mounting medium (Vector Laboratories) and analyzed using the MRC-1024 confocal imaging system

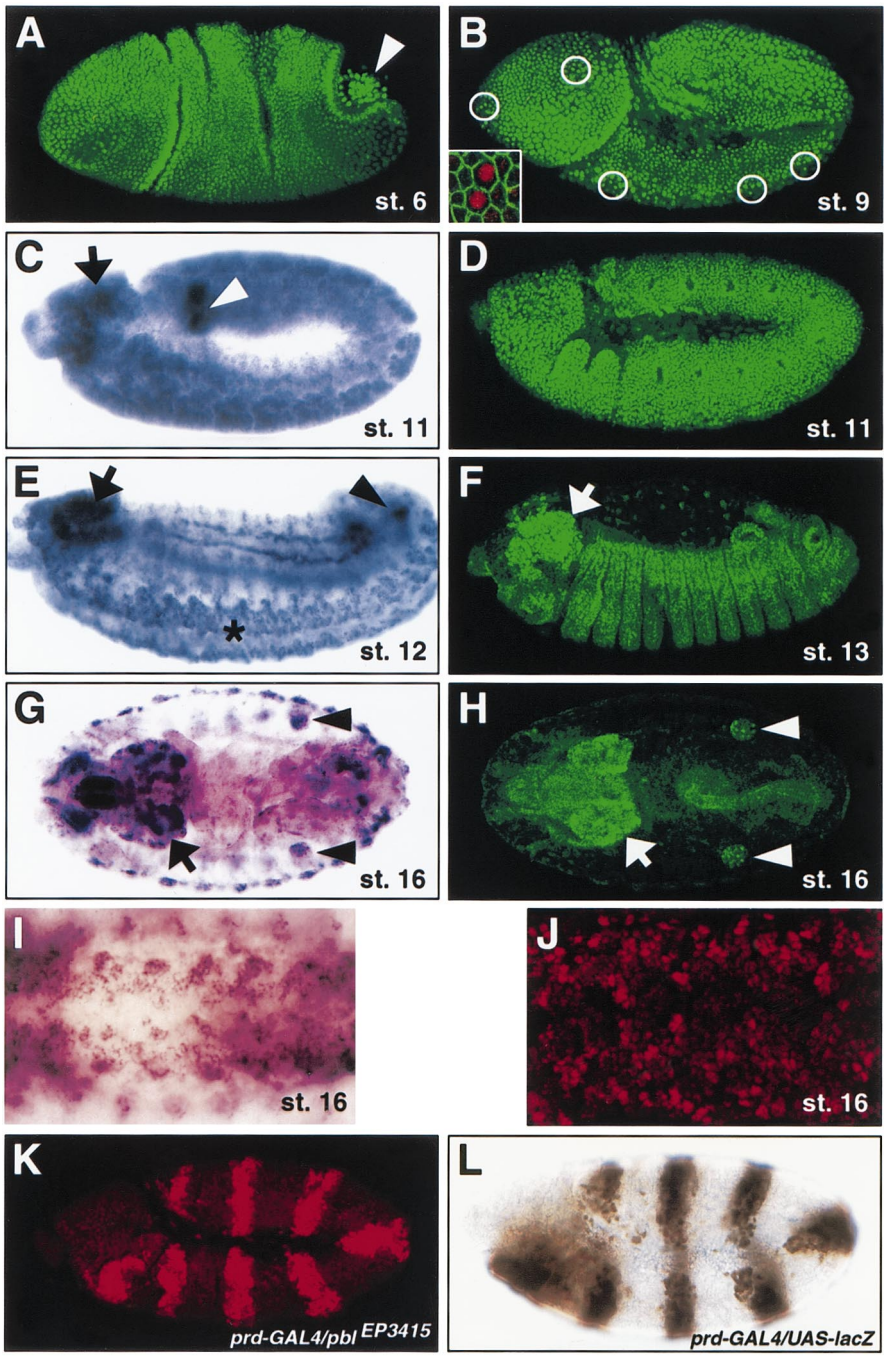

Fig. 3. Expression of *pbl* in postblastoderm embryos. (A–J) Wild-type embryos hybridized with *pbl* antisense RNA (C,E,G,I) or stained with anti-PBL immune serum (A,B,D,F,H,J). (B, inset) Cycle 15 ectodermal cells stained with anti-PBL (red) and anti-α-spectrin (green) to mark plasma membrane. (K) Embryo expressing ectopic PBL in a *paired*-like pattern (*prd-GAL4/pbl*^{EP3415}, stage 11) stained with anti-PBL. (L) A similar embryo expressing β-galactosidase using the same driver (*prd-GAL4/UAS-lacZ*) stained with anti-β-galactosidase. (A–D,F,K,L) Lateral views; (E) ventrolateral view; (G,H) dorsal views; (I,J) ventral views.

(Bio-Rad). Images were processed using LaserSharp 3.0 software (Bio-Rad).

To ectopically express PBL protein in stage 8–11 embryos, we used the *pbl*^{EP3415} line (Berkeley *Drosophila* Genome Project, unpublished; Prokopenko et al., 1999) and the RG1 *paired-GAL4* (*prd-GAL4*) driver. Canton-S was used as the wild-type strain.

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