Genetic analysis of the role of *pebble* during cytokinesis in *Drosophila*

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by

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ABSTRACT

The *pebble (pbl)* gene is required for cytokinesis in *Drosophila,* and encodes a guanine nucleotide exchange factor that activates Rho small GTPase family members. PBL is located in the nucleus of cells that are not dividing, and at cortical regions of dividing cells. The role of nuclear localised PBL is unknown, while it is likely that cortical PBL acts to reorganise the actin cytoskeleton during cytokinesis. Real-time imaging of *pbl* mutant cells, in this study, has revealed the formation of transient, partial cleavage furrows, suggesting a possible later role for PBL in contractile ring function than was initially thought.

Ectopic expression of PBL had no effect during embryogenesis, suggesting that PBL is not rate-limiting during cytokinesis. However, ectopic expression of PBLΔDH (unable to catalyse GDP/GTP exchange) resulted in an inhibition of cytokinesis, consistent with it functioning as a dominant negative form of PBL. Distinct rough eye phenotypes were observed when either PBL or PBLΔDH were ectopically expressed during eye development. Extra cells observed with *GMR>pbl* resulted from an inhibition of apoptosis, while *GMR>pblΔDH* inhibited cytokinesis, resulting in fewer cells in the eye.

Genetic interactions using the *GMR>pbl* and *GMR>pblΔDH* rough eye phenotypes, showed that PBL is acting predominantly through Rho1. Furthermore, downstream signalling of PBLRhoGEF activity could involve the activation of Diaphanous to reorganise the actin cytoskeleton, and Rho-kinase, for the activation of myosin. Genetic interactions with regulators of mitosis suggested that CDK1, together with CYC-B and/or CYC-B3, could also regulate PBL activity. This would provide a link between exit from mitosis and the onset of cytokinesis. However, *in vivo* studies showed that PBL is not regulated by phosphorylation at consensus CDK1 sites.

The genetic systems established here have also been used in an initial screen to identify components of PBL signalling pathways. Interactors identified to date include PP2A and WUN, phosphatases that could act in opposition to kinases downstream of PBL-activated Rho1. Further screening, using these systems, should enable a more comprehensive analysis of PBL signalling pathways during cytokinesis.