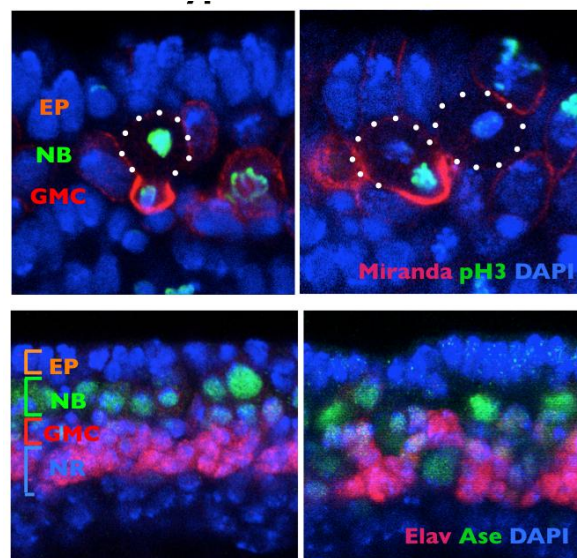


Stem cells show sense of direction

March 20, 2012 – Brains, whether they belong to flies, mice, or humans, are built of numerous types of neurons and supporting cells. This great diversity is the result of asymmetric cell divisions, in which daughter cells often inherit different sets of protein components from their progenitors, and consequently develop different properties. Such activity is highly coordinated, to ensure that the appropriate number and type of cells are produced at just the right times and locations. The *Drosophila* neuroblast, a type of stem cell, provides a good example of this, dividing perpendicular to its overlying epithelium to give rise to a pair of daughter cells of which one is a more highly differentiated intermediate neural progenitor and the other is a neuroblast like its parent cell. The means by which this division is oriented, however, are not well understood. Previous work has indicated that centrosomes and microtubules play a crucial role in larval brains, but other studies suggest that other factors may be important as well in embryonic central nervous systems.



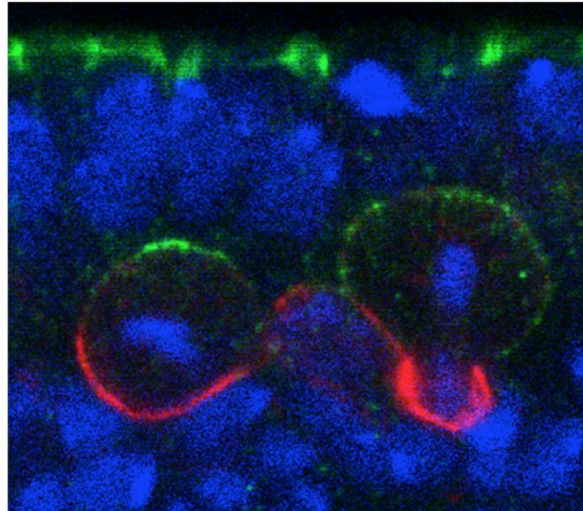
In wild type embryos (left), neuroblasts (NB) asymmetrically divide perpendicular to the epithelial layer (EP) to bud off the daughter ganglion mother cell (GMC) inward, giving rise to a layered organization from the epithelium to neurons (NR). In contrast, in *Tre1* mutants, the orientation of the polarity and division of neuroblasts is randomized (right upper), and the developing nerve tissue is disarranged (bottom right).

Shigeki Yoshiura and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) have now found that the key may lie in molecular signals across the cell membrane that tell neuroblasts how to align. Published in *Developmental Cell*, the group shows that the G protein-coupled receptor (GPCR) *Tre1* is a key factor in a molecular interaction that orients these cells with respect to the epithelium.

The group's study began with a genetic screen for non-cell-autonomous factors involved in orientation, by monitoring the orientation of neuroblasts in embryos lacking genes coding for candidate membrane proteins. They found that mutation of the rhodopsin family orphan GPCR gene *trapped in endoderm 1 (tre1)* was associated with orientation defects, raising the possibility of a causal relationship. Deletion mutants for *tre1* showed significant perturbations of neuroblast orientation with respect to the epithelial ectoderm, resulting in disorganized stratification of the developing tissue in central nervous system (CNS). This phenotype could be rescued by introducing a genomic fragment of the gene, strongly suggesting that it was responsible for the neuroblasts' disorientation.

Interestingly, however, cell polarity was unaffected by *tre1* deletion, and the early-stage orientation defects in such mutants were rectified over the course of development by some unknown compensatory mechanism. Yoshiura et al. speculated that this correction might be due to interactions between neurons and other neighboring cells, such as glia. And indeed when they tested flies in which both *tre1* and *repo*, a critical glial cell gene, were deleted, even later-stage neuronal organization was aberrant, suggesting that neuron-glia interactions are required for the development of the CNS.

Using flies in which the distribution of Tre1 could be visualized, the group next sought to determine its mode of function. Yoshiura found that this protein was distributed widely in neuroblasts across the cell cortex, but to a much weaker degree in ganglion mother cells and neurons, indicating that it might function in a neuroblast-autonomous fashion. Disruption of microtubule activity had no effect on orientation in neuroblasts in the embryonic CNS in which Tre1 was normally expressed, suggesting that this protein functions independent of microtubules at embryonic stages.



In wild type neuroblasts, the aPKC-Par complex (green) localizes to the cell membrane facing the epithelium (upper layer of cells), and neuroblasts divide perpendicular to the epithelium to bud off the ganglion mother cell inward, to which cell fate determinants (red) segregate.

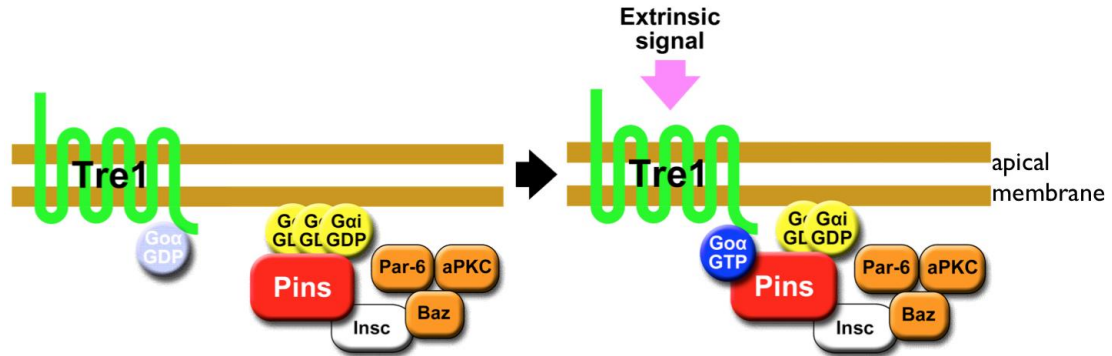
To exclude the possibility that Tre1 helps orient neuroblasts via signals from a tissue other than epithelium (such as mesoderm), the group examined a brain region in which only epithelium, neuroblasts and their progeny are present, and found that the cells remained oriented perpendicular to the epithelium, as expected.

They next used co-immunoprecipitation to find binding partners of Tre1, and found that it interacts with $Go\alpha$, a neuroblast protein of unknown function. Using a toxin that specifically interferes with $Go\alpha$, they found that loss of this protein's function results in a phenotype mirroring that of *tre1* deletion, suggesting that Tre1 may orient neuroblasts in response to signals from the epithelium, by facilitating GDP-GTP hydrolysis by $Go\alpha$.

These findings brought another factor into the picture, as the protein Pins is known to interact with $Go\alpha$ in vitro. Pins is known for its role in mitotic spindle orientation, via binding with another $G\alpha$ protein, $G\alpha i$. In vivo tests using a different assay, however, indicated that only the $Go\alpha$ -Pins interaction depends on Tre1, suggesting that only the GTP form (but not the GDP form) of $Go\alpha$ binds to Pins. Additional testing using $G\alpha i$, in addition to $Go\alpha$ and Pins, in tissue cultures, showed that to be the case – the GTP form of $Go\alpha$ showed much higher interactivity with Pins than the GDP form of $Go\alpha$ or $G\alpha i$. Constructing a cell culture assay system, the group also found that Tre1 could recruit Pins in the presence of $Go\alpha$.

The scenario that emerges is a sort of “handoff” mechanism; binding with the GDP form of $G\alpha i$ is necessary and sufficient for Pins to be recruited to the cell membrane. At the membrane, the GTP form of $Go\alpha$, in turn, is generated from its GDP form only on the apical side proximal to the adjacent epithelium, from which an unidentified signal presumably activates Tre1. $G\alpha i$ is then replaced by the GTP form of $Go\alpha$, due to its more stable binding to Pins, which serves to recruit Pins to the apical membrane. In neuroblasts, Pins associates with the polarity-organizing aPKC-Par complex via interaction with Inscuteable, and consequently recruits this complex to the apical side of neuroblasts. As the aPKC-Par complex determines the polarity and orientation of division in neuroblasts, the Tre1-Pins mechanism ultimately directs the orientation of neuroblasts with respect to the epithelium.

“While cell polarity in *Drosophila* neuroblasts is known to be organized in a cell-autonomous manner, our findings on the Tre1-Pins mechanism show that such self-organized cell polarity can be externally oriented,” says Matsuzaki. “This mechanism may be widely used in the orientation of asymmetric divisions or the polarity of cells relative to adjacent tissues, which may be important for directional tissue growth or stem cell environments.”



In neuroblasts, the aPKC-Par complex binds to the Pins-Gai-GDP complex and localizes asymmetrically at the membrane, while its orientation of polarity relative to the epithelium is not autonomously determined. Tre1 GPCR, activated by a putative extrinsic signal from the epithelium, recruits the aPKC-Par-Pins complex to the apical side facing the epithelium by a “handoff” mechanism.