**Polarity maintenance notes**

Reminder of mutual antagonism

**PKC-3 pathway**

Antagonism from aPARs to pPARs id driven exclusively by PKC-3. <FxR, see Soriano>. Phosphorylation adds negative charge to the proteins, which repels them from the membrane.

In the case of PAR-1 and LGL-1, phosphorylation is at a single site within the membrane association domain (Hoege, Motegi). Phosphorylation of these sites is necessary and sufficient to exclude these proteins from the membrane.

PAR-2 is more complex, with seven predicted phosphorylation sites (Hao), although these have yet to be verified biochemically. Some of these are within the membrane association domain, and likely regulate membrane association in an analogous way to PAR-1 and LGL-1. Phosphorylation by PKC-3 has been shown to disrupt binding of PAR-2 to phospholipids in vitro, which is thought to represent reduced electrostatic attraction via the addition of negatively charged phosphate groups (Motegi). Some of the predicted phosphorylation sites are outside the membrane association region and may play regulatory or gatekeeping roles. Mutation of all seven sites prevents phosphorylation in vivo, leading to uniform PAR-2 (Hao). Whilst individual mutant analysis hasn’t been performed on all of these sites independently, Motegi show that mutation of just one of these sites (S241A), which falls outside of the main membrane localisation region (CHECK), significantly reduces phosphorylation in vitro and achieves the same phenotype as the 7S/E mutant in vivo, indicating that this site may play a key role as a gatekeeper. Phosphomimetic mutation of all seven sites weakens, but doesn’t completely eliminate membrane association, indicating that there may additional sites on the protein, or that these mutations fail to completely mimic phosphorylation (what’s the charge of E compared to a phosphate?).

CHIN-1 doesn’t have an FxR site (check), but is also excluded from the anterior by PKC-3 (Sailer). This is thought to involve direct inhibition of CHIN-1 clustering at the cortex by PKC-3, however the mechanistic basis of this is poorly understood.

**PAR-1 pathway**

PAR-1 can phosphorylate PAR-3, which it does primarily at a single serine (S950) towards the C-terminus of the protein (Motegi). In a wild-type background, depletion of PAR-1, or mutation of the phosphosite on PAR-3, causes PAR-3 to associate with the posterior cortex, although this association is weak (Sailer). It is unclear why a degree of asymmetry is maintained in these conditions, although this may be a remnant of earlier transport by cortical flows and relatively stable cortical association which prevents lateral diffusion and cortical-cytoplasmic exchange that would redistribute the protein. If cortical flows are inhibited, PAR-1 mutation prevents aPARs from polarising at all (Motegi).

**LGL-1 pathway**

LGL-1 has been proposed to antagonise aPARs by forming a complex with PAR-6/PKC-3, the whole of which dissociates from the cortex after LGL-1 is phosphorylated by PKC-3 (Hoege 2010). LGL-1 loss has no observable effects in zygotes in otherwise wild type systems, indicating that this is usually of minor importance, but can enhance phenotypes in PAR-2 mutants (Beatty, 2010). Furthermore, LGL overexpression is able to compensate for absence of PAR-2 (Hoege), indicating that this pathway can be sufficient to take over the roles of the PAR-2/PAR-1 pathway.

**CHIN-1 pathway**

CHIN-1, a GAP for CDC-42 appears on the posterior cortex late in the cell cycle and restricts CDC-42 activity to the anterior (Kumfer, Beatty 2013, Sailer). CHIN-1 loss results in uniform CDC-42 activity, but this doesn’t lead to uniform PAR-6/PKC-3 localisation (Sailer, others), indicating that active CDC-42 isn’t sufficient to recruit PAR-6/PKC-3. When combined with a PAR-1 mutant, however, which leads to a small amount of PAR-3 binding in the posterior, PAR-6/PKC-3 is not recruited to a high level in the posterior. This implies that PAR-3 gates association with CDC-42. Par-3 asymmetry is required to restrict this gating to the anterior, and CDC-42 asymmetry is required to restrict the binding partner of PAR-6/PKC-3 to the anterior, although either one of these behaviours is sufficient.

**Cytoskeleton regulation**

Continued regulation of cortical flow may also play a role in preventing the breakdown of polarity during maintenance phase. PAR-2 plays a role in preventing rearwards cortical flows at maintenance phase. In par-2 mutants, whilst anterior-directed cortical flow proceeds as normal during polarity establishment, these are followed by posterior-directed cortical flows at maintenance phase which leads to significant spread of aPARs (ref). This backwards flow can be prevented by mutation of MRCK-1, <details on this protein>. The precise mechanistic reasons for this misregulation in PAR-2 mutants is unclear. PAR-2 loss leads to higher cortical myosin accumulation independently of PAR-6/PKC-3 presence (Munro, Beatty 2013), suggesting that this isn’t an indirect effect via PKC-3 signalling.

LGL-1 can also regulate flows in a similar way. Dual loss of PAR-2 and LGL leads to stronger rearwards flow (Beatty 2010), and overexpression of LGL-1 is able to rescue rearwards flow in PAR-2 mutants (Hoege). However, unlike PAR-2, LGL-1 has no effect on myosin accumulation in the absence of PAR-6, suggesting that this LGL-1 dependent effect may be indirect via aPARs and the known roles of PKC-3 in cytoskeleton control (i.e. LGL-1 loss weakens antagonism, leading to aPAR invasion, which redistributes flows).

Structure 1:

* aPAR to pPAR antagonism
* pPAR to aPAR antagonism
* Cytoskeleton regulation

Structure 2:

* PKC-3 pathway
* PAR-1 pathway
* LGL-1 pathway
* CHIN-1 pathway
* Cytoskeleton regulation