**PAR-2: ROLES AND MECHANISMS OF ACTION**

**Roles**

To summarise some of the information in the previous sections, the roles for PAR-2 are as follows:

* **Recruitment of PAR-1**. This plays a key role in preventing PAR-3 from associating to the cortex in the posterior, although other mechanisms contribute to keeping PAR-3 in the anterior, which isn’t understood (refs). This acts redundantly with the CHIN-1 pathway to keep the kinase PKC-3 out of the posterior (Sailer, others), and LGL-1 may also contribute to this (Hoege, Beatty, others). Whilst recruitment of PAR-1 doesn’t play an essential role in patterning of fate determinants at P0, this is clearly important at later stages in the embryo, which may reflect an essential role in segregating PAR-1 down the P-lineage (refs).
* **Protection of PKC-3 substrates**. PAR-2 has been demonstrated to protect both PAR-1 and itself from the antagonistic action of PKC-3 (Motegi, others). In the case of PAR-1, this involves direct shielding of the phosphorylation site on PAR-1 as well as competitive inhibition (Rumunanjan). It’s plausible that a similar competitive inhibition mechanism may operate to protect other PKC-3 substrates, namely LGL-1 and CHIN-1, although this hasn’t been formally tested. In the case of cortical PAR-2 being able to protect other PAR-2 molecules, the mechanism isn’t clear. Competitive inhibition may contribute, and the RING domain of the protein has also been implicated in self-recognition, as described below.
* **Restriction of cortical flows at maintenance phase**. PAR-2 appears to play a direct role in preventing rearwards flows at maintenance phase which would otherwise redistribute aPARs to the posterior. The mechanistic basis of this is not understood.
* **Signalling to the mitotic spindle**. May contribute to placement of the division boundary, although the mechanism is unclear.

**Oligomerisation**

By TIRF imaging, the authors were able to resolve distinct particles of varying size at the cortex, which showed a slight asymmetry towards larger oligomers at the posterior. Based on fluorescence intensity, the largest particles were estimated to be at least tetrameric. Membrane lifetime was found to vary across the cell according to oligomer size and local PKC-3 concentration, indicating that oligomerisation can increase, and phosphorylation decrease, stability of membrane binding.

They also found an asymmetry in the membrane association rate, highest in the posterior of polarised cells, which they suggest could be due to direct recruitment of cytoplasmic PAR-2 into cortical oligomers.

This ability to self-associate is supported by in vitro pull-down studies, which show that tagged PAR-2 is able to pull down untagged PAR-2 (Motegi, Arata). These reports show that the amount pulled down is very small, indicating a weak, non-constitutive interaction. However, quantitative measurements of dimer affinity and oligomer size using biophysical methods have not been performed.

Taken together, Arata et al propose that an initial PKC-3 asymmetry leads to a PAR-2 oligomer size asymmetry, either as a direct effect of phosphorylation disrupting oligomerisation, or as a result of concentration-dependent oligomer growth/dissociation. Oligomer size asymmetry would in turn stabilise concentration asymmetries, through on and off rate effects, leading to a degree of positive feedback and polarity stabilisation.

Whilst the core-concepts of their model (namely concentration/phosphorylation dependent oligomerisation, oligomer-size dependent membrane stability, and self-recruitment) are attractive and intuitive, they have yet to be formalised by mechanistic computer models in the context of PAR-2 polarity. Furthermore, the mechanistic basis of the putative PAR-2 oligomerisation reaction remains elusive, although an attractive hypothesis involving the RING domain of the protein has been proposed (Lang review), as discussed in the following section.

**Roles for the RING domain**

Studies of mutant PAR-2 alleles have demonstrated an important role for the N-terminal RING domain in establishing strong PAR-2 domains. Mutations to the putative zinc coordinating residues in the RING domain, designed to misfold the domain and render it non-functional, weaken the strength of PAR-2 cortical localisation (Hao). This is accompanied by faster cortical dynamics as revealed by FRAP, indicative of a shorter membrane lifetime (Motegi). Whilst these mutants can form posterior domains at establishment phase (albeit weakly concentrated), they are rapidly cleared by invading aPARs at maintenance phase (Hao et al., 2006).

The mechanistic basis of this mutant phenotype is poorly understood, and perhaps surprising given that the RING domain is distinct from the cortical localisation domain of the protein and shows no cortical binding activity in isolation (Hao). RING mutants show reduced membrane affinity even in the absence of aPARs, indicating that the phenotype is, at least partially, intrinsic to PAR-2, rather than through increased sensitivity to aPARs. It is also unlikely that reduced membrane association is entirely a secondary consequence of the unfolded domain in cysteine mutants, as truncation mutants lacking the domain show a qualitatively similar phenotype, although a quantitative comparison hasn’t been performed.

Studies on RING mutants also shed potential light on the mechanisms of PAR-2 protection. Hao showed that RING mutants are unable to be protected by endogenous PAR-2 in PAR-1 mutant conditions in which aPARs become uniform, suggesting that the RING may be required to react to endogenous PAR-2. Whilst unclear at the time of the study, more recent work showing that PAR-2 can oligomerise, as described above, along with a characteristic role of RING domains in oligomerisation, as described below, may shed potential light on this. However, this hypothesis is yet to be formally tested. Intriguingly, however, Motegi showed that in near identical experimental conditions RING mutant PAR-2 *can* (seemingly) be protected by endogenous PAR-2. The reason behind this discrepancy is unclear.

**RING domains across the proteome**

*See RING notes*