**Resistance and substrate competition**

**PAR-3 and PKC-3**

**PAR-2 to PAR-2**

Evidence from a number of studies suggests that, whilst PAR-2 is highly sensitive to PKC-3 when it’s weakly concentrated, it is able to resist antagonism once in an established pPAR domain. Although PAR-2 requires an initial aPAR asymmetry to establish a domain, stable maintenance of PAR-2 domains does not require this aPAR asymmetry to be maintained. For example, in PAR-1 knockdown/mutant conditions, aPAR and pPAR are initially segregated into domains, but aPARs eventually return to the posterior without displacing pPARs from the posterior cortex (Hao et al., 2006). Similarly, acute targeting of PKC-3 uniformly to the membrane is unable to fully disassemble PAR-2 domains after polarity establishment (Rodriguez et al., 2017). Interestingly, the same study showed that this resistance of PAR-2 to aPARs is acquired only when PAR-2 is concentrated in a domain (i.e. PAR-2 is not resistant to removal by aPARs when uniform).

This phenomenon seems to operate in trans, rather than a cis phenomenon independent to each PAR-2 molecule. PAR-2 mutants that are usually unable to resist aPAR invasion can do so in the presence of endogenous wild-type PAR-2, indicating that PAR-2 is able to provide protection to other molecules against antagonism, possibly via a direct interaction with PKC-3, or possibly via an intermediate. The molecular details of this haven’t been determined.

**PAR-2 to PAR-1**

Part of the role of PAR-2 in recruiting PAR-1 involves interaction with PKC-3. In otherwise wild type systems, PAR-1 is strictly dependent on PAR-2 to bind to the cortex, becoming entirely cytoplasmic when PAR-2 is lost (refs). In contrast, in aPAR mutant backgrounds PAR-1 shows some ability to bind to the cortex without PAR-2, but this is enhanced when PAR-2 is also present (refs). This implies a dual requirement of PAR-2 in localising PAR-1: an aPAR independent mechanism involving direct recruitment of PAR-1 by PAR-2, and a secondary role involving local protection against aPARs.

PAR-2 has been shown to inhibit phosphorylation of PAR-1 by PKC-3 in in vitro assays in a concentration-dependent manner (Ramanujam). This inhibition proceeds even in PAR-1 mutants that are unable to interact with PAR-1, implying that PAR-2 is able to act as a competitive inhibitor. That said, protection is even greater in wild type PAR-1 where an interaction with PAR-2 is permitted, suggesting that the interaction with PAR-2 can additionally block access of PKC-3 to PAR-1. It could be that PAR-2 interaction physically blocks the PKC-3 phosphorylation site on PAR-1, induces a conformational change in PAR-1 that occludes this site, or promotes membrane binding which blocks this site.

Given that competitive inhibition by PAR-2 has been described as a protective mechanism for PAR-1, it is plausible that this might contribute to maintenance of LGL-1 and CHIN-1 in a similar fashion. Indeed, whilst CHIN-1 can localise to the cortex in the absence of PAR-2 in otherwise wild type systems (unlike PAR-1), localisation is severely reduced (Kumfer, Sailer). Alternatively, this may be a secondary consequence of rearwards flows observed in par-2 mutants (discussed later), or the two could be fundamentally linked.