**Cortical association notes**

PAR proteins associate to the cortex in a number of ways. Some display direct and intrinsic cortical localisation activity (PAR-3, PAR-2, CDC-42, CHIN-1, LGL-1). Whilst some were originally thought to interact with the cytoskeleton, these PARs are now known to localise to the cortex by interacting with the plasma membrane through a combination of electrostatics and specific phospholipid interactions. Other PARs lack intrinsic cortical localisation activity (PAR-6, PKC-3), relying instead on other PARs to act as scaffolds and adaptors, or fall somewhere in between (PAR-1), with a mix of direct and scaffold-mediated binding. In this section I will overview some of the key mechanisms.

A picture containing diagram

Description automatically generated

**PAR-3**

PAR-3 acts as one of the major aPAR scaffolds, associating with the membrane via its PDZ2 domain (Li). <MORE, any research on PDZ2 domains>.

The protein contains a CR1 domain at the N-terminus, an oligomerisation domain which assembles into helical filaments in vitro (Feng, Zhang). Oligomerisation of the protein via this domain is essential for stable membrane association in vivo (Dickinson 2017, Li 2010, others: Benton, Feng, Mizuno), although CR1 domain mutants can display transient cortical association (REF). <Comment on this>.

Clustering is negatively regulated by PLK-1 phosphorylation, which conveys cell cycle dependence on PAR-3 cortical association. <MORE, REFS, Dickinson>

The C-terminal portion also plays a role in promoting strong membrane association, although the function of this region isn’t understood (?, I think more is known about it in other species) (Li 2010b).

**CDC-42**

The membrane localisation of CDC-42 is largely due to a c-terminal geranylgeranyl moiety (REF). The protein additionally contains a conserved cluster of positively charged residues directly preceding the geranylgeranyl moiety, including a di-arginine motif which promotes specificity for PIP2 containing membranes (Johnson).

**PAR-6 and PKC-3**

PAR-6 and PKC-3 are stable binding partners, interacting via PB1 domains at the N-terminus of each protein (Hirano), and in normal circumstances are dependent on each other for stable cortical association (refs: Hung, Tabuse).

<Intrinsic lipid binding activity. Pseudosubstrate?>

Proper cortical association of this complex relies on interactions with both PAR-3 and CDC-42. PAR-6 interacts with CDC-42 via its semi-CRIB domain, which is a requirement for proper cortical association (Aceto). It can also interact with PAR-3 via the PDZ1 domain of PAR-3 and the PDZ domain of PAR-6 (Li 2010a). However, this interaction doesn’t appear to play an essential role in vivo, as mutations to this domain which disrupt the interaction in vitro have no effect on PAR-6 localisation in vivo. PKC-3 engages with PAR-3 via its kinase domain. Two sites flanking the phosphosite direct binding to the CR3 domain of PAR-3 (Soriano). Upstream of the phosphosite is an FxR site, a conserved motif found in PKC-3 substrates which provides an anchor point for PKC-3. Downstream is a hook motif which engages pockets within the PKC-3 kinase domain and disrupt an N-lobe required for catalytic activity, keeping PKC-3 in an inactive state.

In zygotes, PAR-6/PKC-3 accumulate at the membrane in two distinct pools: a punctate PAR-3 dependent pool, and a diffuse CDC-42 dependent pool (Aceto, Beers). The punctate pool represents PAR-6/PKC-3 directly associated with PAR-3 (Dickinson), whereas the diffuse pool represents PAR-6/PKC-3 bound to CDC-42.

PAR-3 is usually essential for any PAR-6/PKC-3 localisation, suggesting that the PAR-3 associated state is a prerequisite for assembly into the CDC-42 associated state. Interestingly, however, inhibition of PKC-3 kinase activity allows the complex to bypass this requirement and interact with CDC42 directly in the absence of PAR-3 (Rodriguez). This implies a model where the complex is first recruited into a PAR-3 associated complex <via what interactions?>, PKC-3 is then inactivated by PAR-3 <need details on this: see Soriano>, which permits transfer to a CDC-42 associated state, in which the inhibition of PKC-3 is relieved.

CDC-37 RNAi can have the same outcome (Beers) <comment on this>

**PAR-2**

Cortical localisation of PAR-2 in vivo depends on a central unstructured region of the protein rich in basic amino acids (Hao). Full-length PAR-2 displays an ability to bind to an array of positively charged phospholipids in vitro, suggesting an electrostatics-based interaction rather than specific interaction with any one phospholipid. Given this promiscuous nature, its apparent specificity for the plasma membrane in vivo is poorly understood, but may reflect the increased charge associated with this membrane compared to other membranes (REF). A RING domain at the N-terminus of the protein appears to play a supplementary and poorly understood role in promoting membrane association, which will be discussed in a future section.

**PAR-1**

PAR-1 contains a C-terminal KA domain, a common membrane association domain, which can bind to membranes and interact non-specifically with anionic phospholipids (Moravcevic). This domain has been shown to be both necessary and sufficient for cortical localisation in vivo (Motegi). In addition to this intrinsic membrane binding ability, PAR-1 is able to interact with PAR-2, again via the KA domain, and an unknown region of PAR-2 (Motegi). This leads to local recruitment of PAR-1 by PAR-2, and cortical localisation in regions of PAR-2 enrichment.

**LGL-1**

Cortical association of LGL-1 relies on a region towards the C-terminus of the protein, which is rich in positively charged amino acids and can directly bind to negatively charged membranes (Visco). Independent of overall membrane charge, affinity is strongest for membranes enriched in diphosphoinositides (Visco, NEED TO CHECK), which are most abundant in the inner leaflet of the plasma membrane. Upon membrane binding, the membrane binding domain folds into an alpha-helix, creating a positively charged patch of basic amino acids. Mutations at some, but not all of these basic amino acids, lowers affinity for diphosphoinositides, suggesting that this folded domain is important for membrane binding specificity.

**CHIN-1**

CHIN-1 localises to the cortex in discrete puncta (Kumfer). Little is known about how it associates to the cortex (CHECK LITERATURE), although it only appears during late maintenance phase, indicating that this association may be under strict regulatory control.

Themes:

* Association with lipids
  + Direct binding vs charge based
  + PAR-3
  + CDC42
  + PAR-2
  + PAR-1
  + LGL-1
* Interaction with scaffolds
  + PAR-6/PKC-3
  + PAR-1
* Clustering/self-association
  + PAR-3, PAR-2, CHIN-1

ASSOCIATION WITH LIPIDS

Some PARs display direct and intrinsic cortical localisation activity. Whilst originally thought to interact with the cytoskeleton, these PARs are now understood to localise to the cortex by interacting with the plasma membrane, through a combination of electrostatics and specific phospholipid interactions.

PAR-3, associates with the membrane via its PDZ2 domain (Li). <MORE, any research on PDZ2 domains, what is this binding to?>. The C-terminal portion also plays a role in promoting strong membrane association, although the function of this region isn’t understood (?, I think more is known about it in other species) (Li 2010b).

The membrane localisation of CDC-42 is largely due to a c-terminal geranylgeranyl moiety (REF). The protein additionally contains a conserved cluster of positively charged residues directly preceding the geranylgeranyl moiety, including a di-arginine motif which promotes specificity for PIP2 containing membranes (Johnson).

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PAR-1 contains a C-terminal KA domain, a common membrane association domain, which can bind to membranes and (similarly to PAR-2) interact non-specifically with anionic phospholipids (Moravcevic). This domain has been shown to be both necessary and sufficient for cortical localisation in vivo (Motegi).

Cortical association of LGL-1 relies on a region towards the C-terminus of the protein, which is rich in positively charged amino acids and can directly bind to negatively charged membranes (Visco). Independent of overall membrane charge, affinity is strongest for membranes enriched in diphosphoinositides (Visco, NEED TO CHECK), which are most abundant in the inner leaflet of the plasma membrane. Upon membrane binding, the membrane binding domain folds into an alpha-helix, creating a positively charged patch of basic amino acids. Mutations at some, but not all of these basic amino acids, lowers affinity for diphosphoinositides, suggesting that this folded domain is important for membrane binding specificity.

INTERACTION WITH SCAFFOLDS

Other PARs lack intrinsic cortical localisation activity, relying instead on other PARs to act as scaffolds and adaptors. PAR-6 and PKC-3 are stable binding partners, interacting via PB1 domains at the N-terminus of each protein (Hirano), and in normal circumstances are dependent on each other for stable cortical association (refs: Hung, Tabuse). Proper cortical association of this complex relies on interactions with both PAR-3 and CDC-42. PAR-6 interacts with CDC-42 via its semi-CRIB domain, which is a requirement for proper cortical association (Aceto). It can also interact with PAR-3 via the PDZ1 domain of PAR-3 and the PDZ domain of PAR-6 (Li 2010a). However, this interaction doesn’t appear to play an essential role in vivo, as mutations to this domain which disrupt the interaction in vitro have no effect on PAR-6 localisation in vivo. PKC-3 engages with PAR-3 via its kinase domain. Two sites flanking the phosphosite direct binding to the CR3 domain of PAR-3 (Soriano). Upstream of the phosphosite is an FxR site, a conserved motif found in PKC-3 substrates which provides an anchor point for PKC-3. Downstream is a hook motif which engages pockets within the PKC-3 kinase domain and disrupt an N-lobe required for catalytic activity, keeping PKC-3 in an inactive state.

In zygotes, PAR-6/PKC-3 accumulate at the membrane in two distinct pools: a punctate PAR-3 dependent pool, and a diffuse CDC-42 dependent pool (Aceto, Beers). The punctate pool represents PAR-6/PKC-3 directly associated with PAR-3 (Dickinson), whereas the diffuse pool represents PAR-6/PKC-3 bound to CDC-42. PAR-3 is usually essential for any PAR-6/PKC-3 localisation, suggesting that the PAR-3 associated state is a prerequisite for assembly into the CDC-42 associated state. Interestingly, however, inhibition of PKC-3 kinase activity allows the complex to bypass this requirement and interact with CDC42 directly in the absence of PAR-3 (Rodriguez). This implies a model where the complex is first recruited into a PAR-3 associated complex <via what interactions?>, PKC-3 is then inactivated by PAR-3 <need details on this: see Soriano>, which permits transfer to a CDC-42 associated state, in which the inhibition of PKC-3 is relieved.

Whilst PAR-1 displays some intrinsic lipid binding activity, it’s cortical localisation is largely mediated by interaction with PAR-2. Again, this interaction is via the KA domain of PAR-2, which interacts with an unknown region of PAR-2 (Motegi). This reaction leads to local recruitment of PAR-1 by PAR-2, and cortical localisation in regions of PAR-2 enrichment.

SELF-ASSOCIATION AND CLUSTERING

For some PAR proteins, a key determinant for stable association is the ability to self-associate into oligomers, in some cases forming large clusters.

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Whilst little is known about the mechanisms of CHIN-1 cortical association, it has also been observed to localise in discrete puncta (Kumfer). These punca only appears during late maintenance phase. It’s therefore plausible that, similar to PAR-3, self-association might be under regulatory control.

PAR-2 has also been suggested to self-associate and form clusters on the cortex (Arata). <leave main discussion until later>