**Kemphues, 1988**

Identified by random mutagenesis. Symmetric first cleavage, simultaneous divisions at 2-4 cell stage. Mutations mapped by ‘linkage analysis and meiotic recombination’. Recessive, maternal effect lethal. Discovery of it5 ts. PAR-2 mutations often do not affect localisation of granules in p0 (but strong effects later on).

**Levitan, 1994**

Original sequencing of cDNA, single large open reading frame 628 amino acids. Reveals ATP binding site (of the myosin class, however PAR-2 lacks hallmarks of myosin proteins) and ‘cysteine-rich motif’ (later classed as RING finger, see Lovering 1993?). Mutants lw32 and zu191 characterised (early stop codons). Transcript is germ-line enriched

**Boyd, 1996**

First (?) imaging of PAR-2 (antibody staining). PAR-2 localises to cell periphery in gonads. Cortical localisation in gonad is sensitive to cytochalasin D (actin depolymeriser). First report of PAR-2 asymmetry in P0 and germline. First report of ‘variously sized sports at the cortex’. Polarity dependent on par-3 but not par-1. PAR-1 cortical localisation occurs slightly later than par-2, and is absent in par-2 mutants. In a par-3 mutant background, where par-1 is uniform, par-1 can bind to cortex in absence of par-2, but this is enhanced when par-2 is present. Thus it appears that par-2 plays dual roles in the protection of par-1, one of which is dependent on aPARs (this is still not fully understood). PAR-2 not required for P granule asymmetry at P0, despite disrupted cortical localisation of PAR-1 (imaging insufficient to see cytoplasmic gradient). They talk about ‘cortical localisation’ of P granules, but this seems outdated. Make argument that cortical localisation of PAR-1 may be inessential for its proper functioning, but required to segregate through p lineage.

**Wallenfang, 2000**

Link between par-2 and microtubule localisation.

**Severson, 2003**

LatA treatment causes undetectable PAR-2 at cortex. However, this also causes centrosome defects so may be a triggering defect. As far as I can see there’s no evidence that PAR-2 interacts directly with cortex. Don’t look at par-2 in LatA + par-3 mutant which would be crucial

**Cuenca, 2003**

Detected PAR-2 on centrosomes during pronuclear rotation (how strong is this?). In nmy-2 RNAi embryos PAR-2 detected ‘in foci around the pronuclei of spindle’, as previously reported in Rappleye 2002). They suggest that this may indicate an affinity for microtubules.

PAR-2 domains appear bigger in par-1 mutant and RNAi embryos. Mex-5/6 RNAi embryos show defects in PAR-1/2 localisation, in contrast to previous reports (Schubert 2000). They claim that mex5/6 ‘regulate expansion of the posterior domain by helping to exclude anterior PARs from that region’, however this hasn’t been sufficiently explored since. Looking at the images, it looks like this could be via an effect on flows.

PAR-2 not required for aPAR asymmetry establishment, but essential for maintenance

**Gudgen, 2004**

Performed yeast-2-hybrid on par-2 with various E2 enzymes. Found moderate interaction with ubc-2 (the most promiscuous E2), ubc-18 and uev-2. References unpublished data that ubc-2 and par-2 can cause ubiquitination to occur in vitro. I believe they used C elegans E2s but need to double check

**Munro, 2004**

Found a rearwards flow in par-2 RNAi embryos associated with the appearance of posterior nmy-2 fibres. Suggests that PAR-2 plays a role in inhibiting nmy-2 accumulation. Mechanism unclear, although believed to be independent of aPARs, as shown by looking in par-3 mutants: found uniform nmy-2 which was higher in embryos lacking Par-2 (data not shown).

**Cheeks, 2004** (similar to Munro, inhibits posterior-directed flow?)

**Hao, 2006**

Central portion sufficient for targeting to the cortex and pkc3 dependent exclusion. Does this portion contain the phosphatase site? Don’t think it does, which is interesting. Unclear why this binds relatively ok to the membrane whereas phosphatase site mutant doesn’t. Contains seven putative phosphorylation sites. 7s/a mutant looks uniform, but still shows some level of phosphorylation by pkc in vitro. Interestingly, whilst reduced in level, a 7s/e mutant retains some ability to bind to the cortex, but this is dependent on the presence of endogenous PAR-2. Unclear whether endogenous PAR-2 is directly interacting with the mutant, or if endogenous PAR-2 is able to protect the mutant from phosphorylation of other sites by PKC.

Expression in HEK cells shows similar preference for cortex/PM

C terminal mutants rescues as efficiently as wild type, however membrane affinity not quantified (looks a bit weaker in the image that they show). No defects observed until 8-cell stage (mild).

7c/s looks equivalent to c56s, indicating that c56s is sufficient to completely misfold the domain. N-terminal deletions are more sick, indicating that something else in that region is important, or that cysteine mutations are incomplete nulls. Could be that it’s lacking the phosphatase site, and therefore might have a lower membrane affinity, but not properly quantified.

Unable to maintain cortical localisation in par-1 RNAi, even when endogenous PAR-2 is around. Indicating that ‘protection’ requires the RING domain. Note that this conflicts with the Motegi paper. Overall they conclude that the primary function of the RING is to ‘protect cortical PAR-2 from exclusion by PKC-3, perhaps by increasing PAR-2’s affinity for the cortex of by making PAR-2 a poorer substrate to PKC-3’. Unclear which of these is dominant based on their data.

Claim that RING mutants have elevated protein levels, despite unchanged RNA levels, indicating that the RING plays a role in accelerating PAR-2 turnover. Unclear why, but may be specific to later stages as they look at embryonic extracts.

Ability of PAR-2 to exclude PAR-3 depends on PAR-1 and doesn’t depend on the RING domain. At this point it’s still unclear how PAR-2 recruits PAR-1.

They mention RNF41 as a protein possibly similar to PAR-2 in humans (interacts with PAR-1, has a RING domain)

**Petrasek, 2008** (evidence for uniform cytoplasm?)

Measure diffusion coefficients in the cytoplasm (=1.5um2s-1), significantly slower than PH in the cytoplasm. Three times slower than CDC-37, which is similar in size. Could be compatible with larger complexes. Cytoplasm looks very uniform. Nothing quantitative here, just an image. Two-photon laser scanning microscope. I imagine that autofluorescence is contributing here.

Their FCS measurements are compatible with a multicomponent diffusion model at the cortex. Whilst nmy-2 is required for PAR-2 to bind to the cortex, their measurements imply that ‘although there might be minor fractions that bind to each other’, most PAR-2 does not bind to nmy-2 directly. (got a bit lost in the details). Raises the question of PAR-2 binding directly to the membrane. Main justification is the fact that PAR-2 doesn’t appear to colocalise with nmy-2, and its diffusion is faster than nmy-2 (/different diffusion characteristics). First (?) observation of PAR-2 on the cortex, which shows heterogeneity.

**Hoege, 2010** (protection of LGL?)

LGL overexpression is able to compensate for some of the problems of PAR-2 loss. Requires that LGL be phosphorylatable by PKC. Need to look at the details a bit more, but might imply that some of the functions of PAR-2 are generic to PKC substrates, i.e. you just need to have lots of PKC-3 substrate around.

No effect of LGL on PAR-1 localisation (still low in par-2 RNAi).

Unclear how LGL might rescue the flow-dependent effects of PAR-2

**Beatty, 2010**

LGL shows considerable cortical localisation prior to symmetry breaking, indicating weaker sensitivity to PKC than PAR-2.

LGL mutant accentuates some of the PAR-2 phenotypes (e.g. increased pkc localisation in the posterior), but has no strong phenotypes on its own

Overexpression of lgl can rescue PAR-2 loss of function (restored viability, prevents pkc accumulation in posterior). Interesting that this is the case, even though par-1 localisation is not restored (not shown here but shown/suggested(?) in Hoege). Indicates that the primary role of PAR-2 isn’t PAR-1 recruitment. Additionally we have to consider that enhanced LGL localisation will be able to antagonise aPARs. So, as long as the ability to antagonise and restrict backwards flows are restored (whatever the mechanism), then par-1 localisation isn’t actually required.

Phosphomimetic mutants do not rescue PAR-2, and show uniform but low(ish) cortical levels

Stronger effect on nmy-2 backwards spread compared to par-2 mutant alone. Unclear if this is direct or via aPARs or even via PAR-2. Is PAR-2 localisation dependent on LGL? Seems to depend on let-502 and mrck-1 (which is a downstream effector of cdc-42). Could this be an indirect effect through cdc-42?

**Motegi, 2011**

Faster cortical dynamics for C56S than wild type by FRAP. Indicates higher off rate

RING mutant (and mt mutant) can be protected from aPARs by the presence of endogenous PAR-2. Indicates ‘local protection’ which is independent of the RING domain. Conflicts with Hao paper, where endogenous PAR-2 cannot protect mutant par-2. Their evidence that PAR-2 self-recruits.

In no flow regimes, PAR-2 localisation correlates spatially and temporally with the site of MTOC-cortex contact. Reduced localisation in treatments that interfere with mt nucleation. Interacts with microtubules in vitro.

Microtobules inhibit phosphorylation of par-2 by pkc in vitro.

Found that par-2 interacts with phospholipids. Strong binding to the following: PS, PI(4)P, PI(4, 5)P2, PI(3,4,5)P3. Moderate binding to PA and phosphatidylinositol. No binding to triglycerol, dag, pe, pc, cholesterol, sphingomyelin. All of which were disrupted by phosphorylation. Prevented by preincubation with microtubules. Need to look at charge of these lipids to see how compatible this is with charge model. Also need to compare to par-1 and par-3 which have also been shown to bind to phospholipids.

Show a direct interaction between PAR-2 and PAR-1, which depends on the c terminus of par-1.

Show that GFP-tagged par-2 is able to very weakly associate with untagged par-2. However, I’m not totally convinced by this

**Griffin, 2011**

PAR-1 is still able to form a cytoplasmic gradient in PAR-2 mutants, despite not enriching on the cortex. Transient and less pronounced following NEBD. MEX-5 also forms a gradient

**Beatty, 2013**

Higher myosin accumulation in par-2/par-6 double compared to par-6 alone (as also mentioned but not shown in Munro 2004). Best evidence for a direct effect of par-2 on myosin accumulation. However, lgl has no additional effect, indicating that its role in reducing posterior cortical myosin is dependent on aPARs. Suggest that PAR-2 and LGL influence myosin distribution via different mechanisms. Not sure what the mechanism is in the case of PAR-2, a bit confusing, but mrck-1 is implicated.

Posterior accumulation of myosin can be repressed (by mrck-1 mutation) in a par-2 mutant, but this doesn’t rescue the absence of par-2, indicating that the antagonism role of par-2 (via par-1) is crucial. Need to double check that I’m interpreting this correctly.

In the case of LGL, the effect on the cortex seems to be via its ability to remove aPARs. Its effect on par-6 amounts (i.e negatively regulating) may explain some of the phenotypes.

**Sailer, 2015** (possibly something here but need to check)

See posteriorly directed cortical flow at maintenance phase in par-2 and mutants (slightly enhanced when lgl mutant added), leading to redistribution of par-6 and par-3. Abolished by codepletion of mrck-1. Indicates that PAR-2 acts to restrict posterior-directed contraction at maintenance phase. In the absence of this flow, aPAR asymmetry can be maintained even without PAR-2 or LGL

**Bailey, 2015**

Bioinformatics study. PAR-2 classed as PRBH containing protein (phospho-regulated basic-and-hydrophobic domain)

**Arata, 2016**

By TIRF imaging, the authors were able to resolve distinct particles of varying size at the cortex. Based on fluorescence intensity, the largest particles were estimated to be at least tetrameric. However, the molecular mechanism of this association were not addressed, and oligomer size not verified by biochemical or biophysical methods.

Able to track particles. This is surprising, given the crowded appearance usually displayed by PAR-2. How much did Lars have to bleach by to be able to track? However, bleaches this high would be expected to remove multi-labelled particles

Tagged PAR-2 able to pull down untagged PAR-2 (GST-pull down and worm lysate immunoprecipitation). However, in both cases the amount pulled down is very small, indicating a weak interaction. Indicates that self-association is, at least partially, independent of membrane association.

Very weak particle size asymmetry (slightly more larger particles in the posterior)

Observe higher cortical residence time in the posterior of the embryo, and for larger particles regardless of location, indicating that off rate is dependent on both particle size and local PKC levels.

Off rate asymmetry insufficient to explain concentration asymmetry, implying an additional on rate asymmetry. Confirmed by higher appearance rates, but this isn’t a robust method

**Rodriguez, 2017**

PAR-2 domains able to resist invasion by aPARs after domains are established. Forced uniform recruitment of PKC-3 leads to a reduction in domain size, but a domain nevertheless persists. This resistance appears to be condition-dependent. Uniform PAR-2, which is lower in concentration, is unable to resist removal. Unclear whether this is due to PAR-2 concentration or lack of PAR-3.

Emb-27 embryos show a PAR-2 domain despite uniform PKC-3 (why is this?)

**Ramanujam, 2018**

Fig S1B shows predicted PRBH domains of PAR-2. Some of the predicted phosphorylation sites are within these regions.

Found two residues in the KA domain of PAR-1 essential for the interaction between PAR-2 and PAR-1 in vitro (R1118, K1119), which is independent of PAR-1 phosphorylation state. Mutant displays significantly lower localisation at the posterior cortex than wt.

PAR-1 localises to the cortex uniformly at a high level in aPAR mutants, independently of association with PAR-2.

PAR-2 shown to inhibit the phosphorylation of PAR-1 by PKC-3 in a strongly concentration-dependent manner, indicating that PAR-2 can protect other PKC-3 substrates via competitive inhibition. Didn’t look at phosphorylation of PAR-2 in all conditions. However, the ability to protect is partially dependent on a direct interaction between PAR-2 and PAR-1, indicating dual protection mechanisms. Could be that PAR-2 interaction physically blocks the phosphorylation sites on PAR-1, or induces a conformational change in PAR-1, or promotes membrane binding which blocks the site. Site of interaction in PAR-2 is unknown.

In terms of fate determinants, preventing association between PAR-2 and PAR-1 seems to have a stronger phenotype than PAR-2 removal, which doesn’t make sense. Therefore it may be that their mutants are less active?

**Gross, 2018** (possibly something here about effects on cytoskeleton)

Nmy-2 dissociation rate in par-6 par-2 double RNAi is not much different to rate in posterior domain of unperturbed embryo, indicating that aPARs are dominant regulator off nmy-2 dissociation rate. Does this conflict with earlier studies? They suggest that flows are via feedback look from aPARs (need to double check)

They claim that the par-2 microtubule cue is persistant and operates over a large area, However, I’m not sure what their justification for this is. Seems like something that’s just been imposed.

**Klinkert, 2019**

Suggests that PAR-2 displays curvature sensitivity, based on microfabrication studies in which cells are compressed in triangular shapes. Suggested to explain spontaneous symmetry breaking (?) and restriction of PAR-2 domains to poles in air-1 embryos (centrosome independent polarisation), and may contribute to proper domain maintenance. Need to double check paper as I’m unsure what the air-1 condition represents and how to tie this together. Mechanistic basis for this proposed curvature sensitivity is unclear. Should look up literature around curvature sensitivity.

**Folkmann, 2019**

Implicates interaction between PAR-1 and nmy-2 as mechanism of PAR-1 activity gradient regulation

**Rodrigues**

Direct effect of PAR-2 on spindle pulling forces, which plays a role in setting the spindle position

Motegi review is good to guide this

Goldstein review, 2007 old but potentially useful

Lang 2017 review

**Discovery**

Identified through random mutagenesis screens as a 628 amino acid protein containing a RING-finger domain and an ATP binding domain (Kemphues, Levitan)

**Signalling to cytoskeleton**

PAR-2 has a negative effect on nmy-2 accumulation independently of aPARs (Munro, Beatty)

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, significant posterior-directed cortical flows are observed at maintenance phase which redistribute aPARs back towards the posterior (Beatty, Sailer).

**Membrane binding**

PAR-2 can bind to an array of phospholipids in vitro (Motegi)

Cortical localisation of PAR-2 in vivo depends on a central region of the protein rich in basic amino acids (Hao). This dependence on charge, as well as the protein’s promiscuity of binding to many phospholipids, raises the likely possibility that the interaction is electrostatics based, rather than a specific phospholipid interaction.

This mode of cortical localisation is common to many polarity proteins (Bailey).

**Microtubule binding**

In the absence of cortical flows, symmetry breaking still occurs, albeit later, indicating the existence of multiple symmetry breaking mechanisms. A second triggering mechanism involves an interaction between PAR-2 and microtubules emanating from the sperm-donated centrosome. In no flow regimes, PAR-2 symmetry breaking occurs late, and correlates spatially and temporally with the site of MTOC-cortex contact (Motegi). Treatments that disrupt microtubules, or mutants at the binding interface on PAR-2 prevent symmetry breaking in no-flow conditions.

Mechanistically, binding of PAR-2 to microtubules is thought to shield the phosphorylation sites on PAR-2, and has been shown to reduce phosphorylation by PKC-3 in vitro. This creates a zone of local protection in the posterior of the cell, allowing PAR-2, and thus PAR-1 to load, which in turn antagonises the aPARs

**Role of 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 role of the RING domain in promoting stable membrane association is poorly understood, and perhaps surprising given that this part off the protein isn’t thought to directly interact with the membrane, 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 due to disruption by the unfolded domain, as truncation mutants lacking the domain show a qualitatively similar phenotype, although a quantitative comparison hasn’t been performed.

**Evidence for ubiquitination**

PAR-2 shows a moderate interaction with several E2 enzymes (ubc-2, ubc-18 and uev-2) in yeast two hybrid assays (Gudgen)

Interestingly, RING mutant transgenes show elevated protein levels in whole embryo extracts compared to wild type transgenes, despite similar mRNA levels, indicating a potential regulatory mechanism at the protein level (Hao). An intriguing possibility is that autoubiquitination may play a role in regulating protein levels, as has been observed for other RING domain proteins (ref)

**Evidence for oligomerisation**

Tagged PAR-2 is able to pull down untagged PAR-2 in vitro, however the strength of this interaction appears weak (Motegi, Arata).

Cortical PAR-2 has a speckled appearance, with structures of variable intensity visible (Boyd, Petrasek, others). This size distribution of particles was characterised by Arata, who predict a size distribution ranging from monomer to approximately tetramer. A slight oligomer size asymmetry was observed, with larger particles found in the posterior, which may be compatible with a general concentration-dependence of oligomerisation reactions. Nevertheless, the molecular basis of PAR-2 oligomerisation, is unclear.

**Evidence for positive feedback**

However, the mechanistic basis of this is unclear. Hao show that RING domain mutants are unable to be protected by endogenous PAR-2, whereas Motegi show that RING mutants are able to maintain a domain under similar conditions.

**Phosphorylation by PKC-3**

Phosphorylation by PKC-3 has been shown to disrupt binding to phospholipids in vitro, which is thought to represent reduced electrostatic attraction via the addition of negatively charged phosphate groups (Motegi)

**Curvature sensing**

Additional mechanisms may underlie triggering in some circumstances. <Evidence of still breaking symmetry when other cues are lost>. Microfabrication studies show that PAR-2 has a preference for curved membranes. This may lead to preferential binding of PAR-2 at the poles of cells, which has been suggested to act as a symmetry breaking cue in cases where normal symmetry breaking is misregulated, (Klinkert). The mechanistic basis for this proposed curvature sensitivity is unclear.

**Protection of PKC substrates**

Once established, PAR-2 domains appear resistant to PKC-3, and are able to withstand forced recruitment of PKC-3 to the posterior cortex (Rodrigues). The reasons behind this are unclear, but may represent a positive feedback reaction able to keep the domain in place, or an ability to resist PKC-3 when concentrated.

**ATP-binding domain**

C-terminal mutants lacking the entire domain show largely normal cortical localisation patterns and rescue as efficiently as wild type (Hao)

**Spindle pulling forces**

PAR-2 also has a direct effect on spindle pulling forces through an unknown mechanism, which may contribute to placement of the division plane (Rodrigues).

**Recruitment of PAR-1**

PAR-1 cortical localisation occurs slightly later than PAR-2, and is absent in PAR-2 mutants (Boyd).

Recruitment of PAR-1 by PAR-2 is believed to be through two mechanisms. A first mechanism involves a direct interaction between PAR-2 an unknown site on PAR-2 and the KA domain of PAR-1 (Motegi, Ramanujam). <Evidence>. The molecular details are unclear. 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.

This can’t explain everything <details>. Additional protection is believed to occur through competitive inhibition <evidence>

In the absence of aPARs, PAR-2 loss reduces cortical localisation of PAR-1, but not to the same extent as in the full system (Boyd).

May be useful for proper segregation of PAR-1 in the P-lineage. Compatible with this, whilst PAR-2 is inessential for proper polarity of fate determinants in P0, polarity of fate determinants is impaired at the four stage cell and beyond (Kemphues)

**Fate determinants**

Despite lack of cortical binding, cytoplasmic gradients of PAR-1 appear intact (Griffin)

Despite reduced PAR-1 cortical localisation, asymmetry of fate determinants proceeds largely as normal in PAR-2 mutants at the P0 stage (Kemphues, Boyd)

**Domain resistance**

Although PAR-2 requires an initial aPAR asymmetry to establish domains, 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 (Rodriguez et al., 2017). Interestingly, the same study showed that this resistance of PAR-2 to aPARs is acquired only when PAR-2 is in a domain (i.e. PAR-2 is not resistant to removal by aPARs when uniform).