**CELL POLARITY**

Almost all cells display some form of polarity.

The ability of cells to polarise is essential for development.

The ability to divide asymmetrically involves movement of fate determinants. Directed motion involves cytoskeletal rearrangements along a certain axis. This begs the question: what’s regulating these processes.

In most cases, cell polarisation is driven by a small network of molecules, known collectively as polarity proteins, that drive downstream processes by forming polarised patterns on the membrane of cells.

A whole field of research has emerged aiming to understand what these proteins are, how they regulate the downstream processes of cell polarity and, perhaps most importantly, how they become polarised in the first place.

Introduce self-organisation here?

A number of polarity proteins exist

A particularly important network of proteins that organises cell polarity in metazoa is the PAR network

**THE PAR NETWORK**

The PAR proteins (table \ref{tab:par\_proteins}) were first identified in \textit{C. elegans} in screens of mutants that disrupt asymmetric zygote divisions \citep{Kemphues1988, Morton2002, Watts1996}. In this particular context, it was later shown that a subset of these proteins becomes enriched asymmetrically at the cell cortex in distinct anterior and posterior domains (as seen in fig x). Once in this distribution, the PAR proteins then direct asymmetric cell divisions by controlling both the segregation of fate determinants and asymmetric positioning of the cleavage plane \citep{Guo1996}. When the zygote divides, this activity results in the formation of two cells that differ in size and fate.\\

Since their initial discovery in C elegans, work has gone on to show that these proteins act in numerous different cell types and organisms to set up polarity. EXAMPLES, why important…

Work has also gone on to find out more about what these proteins are and what they do, which has shed considerable light on the mechanisms of cell polarity. We now know that the PAR network consists of a mix of kinases, scaffolds and adaptor proteins, each of which has their own specialised role.

A fundamental process for polarity is a mutual antagonism between the two distinct PAR groups, in which each one acts to locally exclude proteins from the other group \citep{Boyd1996, Etemad-Moghadam1995, Guo1995}. Due to this interaction, two distinct domains are able to form on the membrane, with each one acting to locally exclude proteins from the other group. NEED MORE HERE AS IT SOUNDS LIKE ITS SOLVED! Variations are possible, such as unipolar polarity without an opposing domain in some cases, and junctional domains in other cases. Nevertheless, many of the core principles underlying polarity in different systems appears to be conserved.

In this project, I am interested in how the PAR proteins create these patterns, specifically using the C elegans embryo as a model system. I will begin this introduction by giving a broad overview of the system. I will then discuss mathematical modelling frameworks that have been instrumental in understanding how the PAR proteins form patterns. Finally, I will give a more detailed account of the known molecular interactions in the PAR network

**PAR POLARITY IN C ELEGANS EMBRYOS**

The system in which the PAR proteins were first discovered, the \textit{C. elegans} embryo, has continued to be an important model system for research into the PAR proteins. As large, transparent cells, \textit{C. elegans} zygotes are particularly suitable for quantitative imaging, and, there are a range of genetic tools available in \textit{C. elegans} that allow researchers to perturb protein function. Additionally, being confined to a single cell, the system is simpler than many others, which allows for easier identification of core principles of PAR polarity that would be more difficult to uncover in complex tissues.

As previously mentioned, this system comprises of two groups of proteins that define distinct anterior and posterior domains. We refer to these two groups as aPARs (anterior PARs) and pPARs (posterior PARs) respectively. The anterior domain is composed of the oligomeric scaffold protein PAR-3, the adaptor protein PAR-6, the kinase PKC-3 and the GTPase CDC-42. The posterior domain is composed of the kinase PAR-1, the RING-domain protein PAR-2, the tumour suppressor LGL-1 and the GTPase-activating protein CHIN-1. Two additional proteins, the kinase PAR-4 and 14-3-3 protein PAR-5, are not asymmetrically localised but play a role in setting up asymmetry of the other PARs.\\

The polarisation process is generally described in terms of two distinct phases: establishment phase, in which PAR asymmetries are first set up, and maintenance phase in which PAR asymmetries are maintained until cell division. Prior to the establishment phase, aPARs are uniformly enriched on the cortex, and exclude pPARs through antagonistic interactions. Polarity is triggered by the sperm donated centrosome, which causes symmetry of the system to be broken via two redundant pathways. Firstly, signals from the centrosome cause there to be an inhibition of cortical actomyosin contractility in the posterior, triggering cortical flows that segregate aPARs to the anterior \citep{Munro2004, Goehring2011a}. Secondly, microtubules associated with the sperm centrosome can directly promote PAR-2 binding to the cortex in the posterior \citep{Motegi2011, Wallenfang2000, Zonies2010}. Together, these mechanisms break the symmetry of the system, establishing an aPAR rich anterior and a pPAR rich posterior. \\

After polarity is established, flow velocities are markedly reduced \citep{Gross2018}, and the sperm centrosome moves the centre of the cell, where it will help set up the mitotic spindle. Nevertheless, despite this loss of spatial cues from the system, aPAR and pPAR domains are able to remain stable and keep their axis of polarity until cytokinesis.\\

Rather than relying on spatial cues, maintenance phase is thought to reflect a dynamic steady-state, involving continuous exchange of proteins between the cortex and cytoplasm, diffusion, and protein-protein interactions \citep{Goehring2011}. Especially important are a set of antagonistic interactions between the aPAR and pPAR proteins, known as mutual antagonism, which prevents domains from mixing. In support of this view, pPAR proteins are known targets of PKC-3 and their phosphorylation is sufficient to displace them from the membrane \citep{Hao2006, Hoege2010}. Similarly, PAR-1 and CHIN-1 can inhibit the activity of PAR-3 and CDC-42, respectively \citep{Sailer2015}. Moreover, if either set of PAR proteins is disrupted, the other set of proteins localises uniformly on the cortex and polarity is lost, highlighting the essential competitive nature of the interactions between the two groups \citep{Etemad-Moghadam1995, Boyd1996, Cuenca2003, Guo1995}.\\

Signalling from asymmetric PAR proteins leads to asymmetric segregation of cell fate determinants \citep{Cuenca2003, Daniels2010, Griffin2011, Wu2018} and asymmetric placement of the cleavage plane (REFS). The net result is an asymmetric cell division, with daughter cells differing in both size and fate. This process repeats in subsequent cell divisions, playing an essential role in early patterning of the embryo. \\

To understand how interactions between the PAR proteins can lead to the stable maintenance of polarised patterns, researchers are increasingly making use of mathematical models to simulate the network. The system has hallmarks of a self-organising reaction-diffusion system, a phenomenon that applies not only to cell polarity, but has been hugely influential for understanding biological patterning more broadly.\\

**PATTERNING VIA SELF ORGANISATION**

The notion of self-organisation was first put forward by Alan Turing in his seminal 1952 paper ‘The Chemical Basis of Morphogenesis’ (REF). In this paper, Turing showed that diffusion of molecules, coupled to feedback reactions (in which the molecules themselves regulate their own local accumulation) is sufficient to spontaneously induce pattern formation from a uniform system with stochastic fluctuations. \\

Later work by Gierer and Meinhardt formalised this theory in a more intuitive framework, and emphasised a number of core principles that are required in reaction-diffusion systems to form patterns (REF). They proposed that a minimal patterning system requires at least two substances that diffuse at different rates, one of which (the slowly diffusing chemical) must act to locally enhance its own concentrations (local amplification). At the same time, a long-range signal, mediated by the faster diffusing species, must act to confine amplification of the first species to a local region (long-range inhibition).\\

Local amplification may be direct (autocatalysis), or via two negative reactions (mutual antagonism) (fig x). Long-range inhibition can be achieved in multiple ways, such as activator-inhibitor systems and activator-depletion systems (fig x). \\

The core ideas of Gierer and Meinhardt’s models are general, and can be applied to explain patterns across biology. One can interpret the chemical species as diffusing across tissues, controlling gene expression, and interacting to set up patterns of expression. In this way, these models can create 2D patterns that very much resemble the patterns one sees on the skin of animals (fig x), and a whole range of other patterns are possible. One could also think of diffusing chemicals as patterns of neural activity. In this way, these models can explain the development of patterned neural connections in the brain (MORE, REF). Finally, and as discussed in the next section, these principles are also readily applied to the context of an individual cell.

**SELF ORGANISATION FOR CELL POLARITY**

Using these core principles, a number of models have been proposed to describe cell polarity. For the most part, models do not attempt to address the complex biochemistry of cell polarity networks, but compose systems as a small number of species and core reactions. Species in models generally do not refer to a single protein but to a set of proteins that interact together. Feedback reactions, which are generally complex, multi-step reactions in vivo are built as single (or a small number) of terms in partial differential equations. Because polarisation often occurs over a single axis, systems are usually modelled in one-dimension.\\

Some models are considered 'Turing-type', characterised by instabilities that cause symmetry to react to a small spatially varying stimulus and spontaneously break symmetry (e.g. REFS). These are attractive in the sense that they can account for spontaneous polarisation and can achieve high degrees of spatial asymmetry. However, they generally fail to account for resting nonpolar states which are often observed in vivo, in which systems wait for a sufficiently large trigger before polarity is kicked off.\\

A second class of models relies on bistable reaction kinetics driven by nonlinear feedback reactions to produce 'wave-pinning' behaviour \citep{Jilkine2011, Mori2008, Mori2011}. Unlike classic Turing instability models, in which small perturbations are always amplified, the model permits parameter regimes in which unpolarised rest states are stable to small stimuli and stochastic fluctuations. Systems in these regimes won't break symmetry spontaneously, and require a sufficiently large trigger to kick off polarity.\\

In this section I will describe two models that are particularly relevant to this work. I will first describe the original wave-pinning model, a simple model that shares many features with models of the PAR system (although the precise mechanisms are quite different), before moving onto a simple model of PAR polarity.\\

**WAVE PINNING MODEL**

The wave-pinning model is a minimal cell polarity that was initially built to describe patterning of RhoGTPases (Cdc42, Rac, Rho), a conserved family of polarity proteins that regulate cell polarity in a range of contexts and cell types. An example is budding yeast, in which asymmetric cell division ate controlled by the polarity protein Cdc42, which forms a polar cap on the cell membrane (fig xA). Cdc42 exists in a continuous cycle between an active, GTP-bound membrane state and an inactive GDP-bound cytoplasmic state. Switching between these two states is catalysed by two classes of proteins. The GEF Cdc24 switches Cdc42 to its active state, and a group of GAPs switch Cdc42 to its inactive state. Another key player is the scaffold protein Bem1, which is recruited to the membrane by the active form of Cdc42 and further recruits the GEF Cdc24. This reaction leads to a positive feedback loop, in which active Cdc42 accumulation leads to further accumulation. Meanwhile, growth of the domain causes the cytoplasmic form to be depleted, which eventually stalls growth of the domain (fig xB).\\

MATHS

A key feature of this model is that the reaction kinetics display bistability. This can seen by solving the reaction terms at equilibrium, which shows the permitted steady state combinations of $m$ and $c$ (fig xA). We can see from this that the resulting curve folds over itself, meaning that, within a range of cytoplasmic concentrations, two stable membrane concentrations (an upper and a lower state) are permitted by the reaction terms (a third solution between them is unstable). This bistability comes as a result of nonlinearities in the reaction terms, specifically in this case the exponents of 2 in the Hill function, which imply an ultrasensitivity relationship of the positive feedback reaction on membrane concentrations.\\

Starting from a uniform state, a transient stimulus can be locally amplified by the positive feedback terms, bringing part of the system into the upper state. Due to diffusion, this region then spreads, until depletion of the cytoplasmic pool causes this spreading to stall (a phenomenon known as 'wave-pinning'). An example of a stable pattern is shown in fig x, which shows stable upper and lower regions separated by an interface. As pinning is caused by cytoplasmic depletion, the position of the interface is strongly influenced by the total amount of protein in the system.\\

**MINIMAL PAR MODEL**

TO DO. Make sure to talk about cortical flow trigger too

Such non-linearity could, in theory, be achieved in a number of ways \citep{Ferrell2014, Ferrell2014a, Ferrell2014b}, and several mechanistic details of the PAR network give justification to this. One example is multisite phosphorylation \citep{Serber2007}, which is common to many targets of PKC-3, including LGL-1 \citep{Graybill2014} and PAR-2 \citep{Hao2006}. The non-linearity of such reactions can be enhanced through the presence of cooperativity between phosphorylation sites \citep{Gunawardena2005}, or the presence of redundant sites \citep{Wang2010}, giving a switch-like behaviour. Alternatively, oligomerisation of proteins, coupled to antagonism, has been shown to provide sufficient non-linearity to support bistability \citep{Sailer2015, Dawes2011, Lang2022}. Such reactions have been proposed for PAR-2 \citep{Arata2016}, PAR-3 \citep{Li2010a} and CHIN-1 \citep{Sailer2015}. I will discuss mechanistic details relating to these ideas further in the next section.

**THE MOLECULAR CIRCUITRY OF THE PAR NETWORK**

The model in the previous section describes a system in which proteins are in exchange between the cytoplasm and membrane, and feedback reactions between the two groups of proteins drive patterning. AND CUES. Here, I will give an overview of the molecular details thought to underlie these processes. I will begin by discussing mechanisms of cortical association. I will then discuss how this association is biased to set up polarised domains, firstly by cues which kick off polarity establishment, and secondly by antagonistic feedback. Finally, I will discuss the downstream roles of these proteins that drive an asymmetric cell division. It is worth noting that the mechanisms vary somewhat between different organisms and cell types, and I will focus on mechanisms of relevance for the \textit{C. elegans} embryo, although some of the studies cited were performed using other model systems.\\

**MECHANISMS OF CORTICAL ASSOCIATION**

The PAR proteins associate with the cortex in a number of ways. Some of the PARs display direct and intrinsic cortical localisation activity (PAR-3, PAR-2, CDC-42, CHIN-1, LGL-1). Other PARs rely instead on other PARs to act as scaffolds (PAR-6, PKC-3), or fall somewhere in between with a mix of direct and scaffold-mediated membrane binding (PAR-1).\\

**Association with lipids**

A number of the PAR proteins localise to the cortex by interacting with the inner leaflet of the plasma membrane. For some PAR proteins, this is mediated by interactions with specific phospholipids that are enriched at the plasma membrane. Other PARs, rely on electrostatic interactions with the membrane via charged domains.\\

PAR-3, an aPAR scaffold protein, associates with the membrane via its PDZ2 domain \citep{Li2010a}. PAR-3 has three PDZ domains, of which only PDZ2 is required for cortical association. PDZ domains most commonly recognise C-terminal sequences on proteins \citep{Tonikian2008} or other PDZ domains (ref), however the PDZ2 domain of PAR-3 is thought to interact directly with plasma membrane phosphoinositides \citep{Wu2007}.\\

The membrane localisation of the RHO GTPase CDC-42 is largely due to a c-terminal geranylgeranyl moiety \citep{Ziman1993}, which promotes hydrophobic attachment to cell membranes. 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 PIP$\_2$ containing membranes \citep{Johnson2012}, which is likely responsible for the protein's plasma membrane specificity.\\

Cortical localisation of PAR-2 in vivo depends on a central unstructured region of the protein rich in basic amino acids \citep{Hao2006}. 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 \citep{Motegi2011}. Given this promiscuous nature, its apparent specificity for the plasma membrane in vivo is poorly understood, but may be a consequence of the increased charge associated with the plasma membrane compared to other membranes \citep{Yeung2008}.\\

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 \citep{Moravcevic2010}. This domain has been shown to be both necessary and sufficient for cortical localisation in vivo \citep{Motegi2011}.\\

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 \citep{Visco2016}. Independent of overall membrane charge, affinity is strongest for membranes enriched in diphosphoinositides \citep{Visco2016}, 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**

PAR-6 and PKC-3 are stable binding partners, interacting via PB1 domains at the N-terminus of each protein \citep{Hirano2005}, and in normal circumstances are dependent on each other for stable cortical association \citep{Tabuse1998, Hung1999}. 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 \citep{Aceto2006}. It can also interact with the PDZ1 domain of PAR-3 via its own PDZ domain \citep{Li2010}. However, this interaction does not appear to play an essential role in vivo in \textit{C. elegans} embryos, as mutations to this domain which disrupt the interaction in vitro have no effect on the in vivo distribution of PAR-6 \citep{Li2010}. PKC-3 engages with PAR-3 via its kinase domain. Two sites flanking the phosphosite direct binding to the CR3 domain of PAR-3 \citep{Soriano2016}). 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 disrupts an N-lobe required for catalytic activity, keeping PKC-3 in an inactive state.\\

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 \citep{Aceto2006, Beers2006}. The punctate pool represents PAR-6/PKC-3 directly associated with PAR-3 \citep{Dickinson2017}, whereas the diffuse pool represents PAR-6/PKC-3 bound to CDC-42. PAR-3 is usually essential for any PAR-6/PKC-3 cortical 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 \citep{Rodriguez2017}. This implies a model where the complex is first recruited into a PAR-3 associated complex, PKC-3 is then inactivated by PAR-3, 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, its cortical localisation is largely mediated by interactions with PAR-2. Again, this interaction is via the KA domain of PAR-1, which interacts with an unknown region of PAR-2 \citep{Motegi2011}. This reaction leads to local recruitment of PAR-1 by PAR-2, and cortical localisation in regions of PAR-2 enrichment. It has also been proposed that the interaction between PAR-1 and PAR-2 can protect PAR-1 from phosphorylation by PKC-3, perhaps by occluding the phosphorylation site on PAR-1 \citep{Ramanujam2018}.\\

**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. In oligomers, independent membrane binding domains can cooperate with one another to promote a high affinity membrane binding interaction \citep{Lemmon2008}.\\

PAR-3 contains a CR1 domain at the N-terminus, an oligomerisation domain which assembles into helical filaments in vitro \citep{Feng2007, Zhang2013a}. Whilst CR1 mutants can localise to the membrane transiently, stable membrane association requires an intact CR1 \citep{Dickinson2017, Li2010a, Rodriguez2017}. Clustering via the CR1 is negatively regulated by PLK-1 phosphorylation, which conveys cell cycle dependence on PAR-3 cortical association \citep{Dickinson2017}.\\

Whilst little is known about the mechanisms of CHIN-1 cortical association, it has also been observed to localise in discrete puncta \citep{Kumfer2010}. These punca only appears during late maintenance phase. It is therefore plausible that, similar to PAR-3, self-association might be under cell-cycle control.\\

PAR-2??? PAR-2 has also been suggested to self-associate and form clusters on the cortex \citep{Arata2016}.

**ESTABLISHMENT OF POLARITY**

As previously mentioned, PAR polarity does not occur spontaneously, requiring a trigger so that polarity happens at the correct time and with the correct orientation. Prior to symmetry breaking, the system starts an aPAR uniform state in which pPARs are held off the cortex by antagonistic interactions from the aPARs (see next section). Polarity is then triggered by signals from the MTOC which forms near the site of sperm entry, via two redundant pathways:\\

**Anterior-directed cortical flows**

Signals from the sperm-donated centrosome induce cortical flows towards the anterior of the embryo \citep{Cowan2004, Munro2004, Goehring2011a}. The cue is thought to lead to a local downregulation of RHO-1 activity, which downregulates actomyosin contractility in the posterior. This sets up a spatial gradient of contractility, which leads to anterior-directed cortical flows.\\

These flows leads to transport of PAR-3 clusters towards the anterior \citep{Dickinson2017, Rodriguez2017, Wang2017}, which moves the kinase PKC-3. The resulting depletion of PKC-3 concentration in the posterior relieves antagonism of pPARs, allowing them to bind in the posterior and form a nascent domain. Further work has shown that the PAR proteins themselves are able to feed back onto the actomyosin cortex, amplifying contractility asymmetries as polarity progresses \citep{Gross2018}.\\

**The microtubule pathway**

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 cells lecking cortical flows, PAR-2 symmetry breaking occurs late, and correlates spatially and temporally with the site of MTOC-cortex contact \citep{Motegi2011}. Treatments that disrupt microtubules prevent symmetry breaking in these conditions.\\

Mechanistically, this is thought to be carried out by a direct interaction between PAR-2 and microtubules, which is thought to shield the phosphorylation sites on PAR-2 (discussed further in chapter 3), and has been shown to reduce phosphorylation by PKC-3 in vitro \citep{Motegi2011}. This creates a zone of local protection in the posterior of the cell, allowing PAR-2, and thus PAR-1 to load, kicking of self-organisation. Mutations at the microtubule binding interface on PAR-2 have a similar phenotype to treatments that disrupt microtubules.\\

**Additional pathways**

Additional mechanisms may underlie triggering in some circumstances. 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 \citep{Klinkert2019}. The mechanistic basis for this proposed curvature sensitivity is unclear.\\

**MAINTENANCE OF POLARITY BY MUTUAL ANTAGONISM**

TRANSITION. Involves antagonistic reactions from the aPARs to the pPARs and vice-versa. Several redundant pathways operate.

**PKC-3 pathway**

Antagonism from aPARs to pPARs is thought to be driven exclusively by PKC-3. The pPAR proteins PAR-1, PAR-2 and LGL-1 contain FxR motifs, which act as high affinity PKC-3 recognition motifs \citep{Soriano2016}. Phosphorylation of these substrates by PKC-3 occurs within the regions that regulate membrane binding, adding negative charge which electrostatically repels the proteins them from the membrane \citep{Bailey2015}. In the case of PAR-1, phosphorylation is at a single site within the membrane association domain, which is necessary and sufficient to exclude PAR-1 from the membrane \citep{Motegi2011}. LGL-1 phosphorylation occurs at three conserved sites, and phosphorylation at all three sites is required for full membrane displacement \citep{Graybill2014, Hoege2010}. PAR-2 has seven predicted phosphorylation sites \citep{Hao2006}, although these have yet to be verified biochemically. Phosphorylation by PKC-3 has been shown to disrupt binding of PAR-2 to phospholipids in vitro \citep{Motegi2011}. Mutation of all seven sites prevents phosphorylation in vivo, causing PAR-2 to localise uniformly to the cortex \citep{Hao2006}. I will discuss details relating to PAR-2 phosphorylation further in the appendix.\\

CHIN-1 does not have an FxR site, but is also excluded from the anterior by PKC-3 \citep{Sailer2015}. 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.\\

Continuous dephosphorylation is required to counteract phosphorylation by PKC-3 and maintain an active pool of substrate. For the most part, the mechanisms of PKC-3 substrate dephosphorylation are poorly understood, although phosphatases GSP-1 and GSP-1 have recently been shown to underlie dephosphorylation of PAR-2, which involves a PP1 binding motif in PAR-2 \citep{Calvi2022}.

**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 \citep{Motegi2011}. This is thought to disrupt membrane association by disrupting clustering. 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 still relatively weak \citep{Sailer2015}. It is unclear why some degree of asymmetry is maintained in these conditions, although this may be a result 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 loss prevents aPARs from polarising at all \citep{Motegi2011}.\\

**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 \citep{Hoege2010}. 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 \citep{Beatty2010}. Furthermore, LGL-1 overexpression is able to compensate for absence of PAR-2 \citep{Hoege2010}, 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 \citep{Kumfer2010, Beatty2013, Sailer2015}. CHIN-1 loss results in uniform CDC-42 activity, but this does not lead to uniform PAR-6/PKC-3 localisation \citep{Sailer2015}, indicating that active CDC-42 is not 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 now 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 to enforce a degree of asymmetry.\\

**ORCHESTRATING AN ASYMMETRIC CELL DIVISION**

TRANSITION

**Placement of the division plane**

Signalling from the PARs regulates the position of the mitotic spindle, which leads to a cell size asymmetry following cytokinesis. The PARs set up spindle displacement by setting up asymmetric pulling forces \citep{Grill2001}. This is thought to be carried out at least partially PKC-3-dependent phosphorylation of LIN-5 \citep{Galli2011}, which is part of a complex containing dynein and the G-protein regulators GPR-1/2 which attaches astral microtubules to the cortex. This results in decreased microtubule pulling forces in the anterior, leading to a shift in the position of the mitotic spindle towards the posterior. PAR-2 also appears to have a direct effect on spindle pulling forces through an unknown mechanism, independently of aPARs, although the role of this in division plane placement is unclear (Rodrigues et al., in prep).\\

**Segregation of fate determinants**

As well as differing in size, the two daughter cells differ in a number of cytoplasmic components which define cell fate during development, which is also set up by signalling from the PARs. Immediately downstream of the PARs is MEX-5, which is organised into a cytoplasmic gradient in response to asymmetry of PAR-1 \citep{Daniels2010}. PAR-1 phosphorylates MEX-5 \citep{Griffin2011}, which increases its mobility. Working against the action of a uniform phosphatase, PP2A \citep{Schlaitz2007}, this leads to an asymmetry in MEX mobility, which leads to accumulation at the anterior where mobility is lowest. Mathematical models have shown that the combination of states with different diffusion coefficients and spatially segregated kinase with a uniform phosphatase can create cytoplasmic gradients \citep{Lipkow2008}.\\

This MEX gradient then sets up a P granule asymmetry by regulating growth and dissolution of phase-separated P-granule droplets \citep{Brangwynne2009}. These granules dominate in the posterior, so are inherited by the P1 cell after cell division. The granules contain fate determinants which are responsible for specifying germ-line fate in the P lineage.\\

Interestingly, MEX gradients can still form in mutant embryos where PAR-1 is unable to bind to the cortex, indicating that this mechanism may be driven primarily by cytoplasmic PAR-1 \citep{Folkmann2019}. However, cytoplasmic gradients of PAR-1 alone are insufficient to explain MEX gradients in mathematical models \citep{Griffin2011}, and so a cytoplasmic activity gradient has been postulated.\\

**BEYOND MUTUAL ANTAGONISM**

So far, I have introduced the mutual antagonism model, and described the molecular details underlying this. However, wonk on the system is continuously revealing complexities beyond mutual antagonism that likely have an impact on polarity maintenance. The system is now understood to be comprised of many feedback reactions that synergise to create polarity, in which mutual antagonism is only a part (Motegi, Lang). In this section, I highlight some of these complexities.

**REGULATION OF THE ACTOMYOSIN CORTEX**

Whilst primarily important during establishment phase, continued regulation of the actomyosin cortex by the PAR proteins also plays a role in preventing the breakdown of polarity during maintenance phase. Maintenance phase contractility is controlled by CDC-42, which acts through MRCK-1 to activate myosin II. As CDC-42 activity is restricted to the anterior by the activity of CHIN-1, this leads to a gradient of myosin contractility towards the anterior \citep{Kumfer2010, Sailer2015}. CDC-42 RNAi embryos display reduced levels of myosin at the anterior cortex during maintenance phase \citep{Beatty2013}, which is similarly observed in embryos lacking MRCK-1 \citep{Sailer2015}.\\

The pPARs also play a role in cortex regulation. In \textit{par-2} mutants, whilst anterior-directed cortical flow proceeds as normal during polarity establishment \citep{Gross2018}, these are followed by aberrant posterior-directed cortical flows at maintenance phase, leading to significant spread of aPARs back towards the posterior \citep{Munro2004, Beatty2010}. The precise mechanistic reasons for this misregulation in \textit{par-2} mutants is unclear, but it suggests that signalling from PAR-2 plays some role in preventing rearwards cortical flows at maintenance phase. PAR-2 loss leads to higher cortical myosin accumulation independently of PAR-6/PKC-3 presence \citep{Munro2004, Beatty2013}, suggesting that this is not an indirect effect via PKC-3 signalling. Signalling appears to be through the myosin light chain kinase MRCK-1, as backwards flows can be prevented by MRCK-1 mutation \citep{Beatty2013, Sailer2015}.\\

LGL-1 can also regulate maintenance phase flows in a similar way. Dual loss of PAR-2 and LGL-1 leads to stronger rearwards flow \citep{Beatty2010}, and overexpression of LGL-1 is able to rescue rearwards flow in PAR-2 mutants \citep{Hoege2010}. However, unlike PAR-2, LGL-1 has no effect on myosin accumulation in the absence of aPARs \citep{Beatty2013}, suggesting that this LGL-1 dependent effect may be indirect via aPARs.\\

Coupling of PAR proteins to anterior-directed flows, counterbalanced by diffusive spread, has been shown to help stabilise the PAR boundary position in mathematical models \citep{Sailer2015}.\\

**DIVISION OF LABOUR**

TRANSITION**.**

As discussed previously, PKC-3 cycles between two states: an inactive PAR-3 state to an active CDC-42 state. PAR-3 clusters recruit PAR-6/PKC-3 to the cortex, and the complex is driven to the anterior cortex by flows. PKC-3/PAR-6 then switch to the active CDC-42 associated state. In this form, they are allowed to diffuse more freely and target pPARs for displacement. Cycling of PKC-3/PAR-6 between these states allows for functional segregation between cue sensing and effector complexes, which may allow the system to independently modulate the response to cues and the strength of the antagonism signal \citep{Rodriguez2017}. The full implications of this division-of-labour for pattering are unclear, and likely will require modelling studies to fully appreciate.

**SUBSTRATE COMPETITION**

Most current models group the pPARs together, and model PKC-3 antagonism as a single reaction to this grouped species. However, evidence suggests that PKC-3 does not act on all pPARs equally. Rather, there appears to be a hierarchy of interactions between PKC-3 and its substrates. LGL-1, for example, displays considerable localisation to the cortex prior to symmetry breaking, whereas PAR-2 is held mostly cytoplasmic, indicating that these two substrates have differing sensitivities to PKC-3 \citep{Beatty2010}. In kinase systems with multiple substrates, competition between substrates can contribute to nonlinear phosphorylation behaviour, whereby high affinity substrates act as competitor substrates to shape the response of lower affinity substrates \citep{Ferrell2014b}. However, whether the relationships between PKC-3 and its substrates are tuned to give ultrasensitive responses has not been explored. I will explore some ideas relating to substrate competition in the context of PAR-2 multiphosphorylation in the appendix.\\

**A pPAR POSITIVE FEEDBACK CIRCUIT**

**OUTLOOK**

Given the importance of cell polarity, the presence of multiple, potentially semi-redundant feedback pathways to regulate polarity maintenance is not surprising.

However, most of the information that we have about the system is qualitative, so our ability to fully understand these behaviours with models is limited.

The minimal PAR model presented earlier, in which nonlinear feedback terms are requires, highlights the importance of quantitative information about interactions in the PAR network.

The first aim of my project was to set up an image quantification pipeline that allows us to obtain quantitative information about the PAR proteins…

My project then led me to focus on the pPAR subnetwork, and to try to better understand how feedback within this network contributes to polarity maintenance.

Notably, whilst we have highlighted potential sources of non-linearity in the network, there have, to date, been no direct quantitative demonstrations of this key requirement for the PAR polarity model. Thus, the ability of the PAR network to support bistability in vivo is unknown.