**Discussion of robustness**

Given the importance of cell polarity, the presence of multiple, potentially semi-redundant feedback pathways to ensure robust polarity maintenance is not surprising. Whilst mutual antagonism alone might be sufficient to capture polarity in these systems, theoretical studies have shown that the addition of further mechanisms, such as positive feedback motifs, to polarity networks makes them more resistant to changes in factors such as protein dosage, diffusion coefficients and reaction rates \citep{Chau2012}. In a system that must be robust in the face of changing environments and biological noise, this presents a clear evolutionary benefit. Furthermore, having redundant interactions can help to ensure that systems can polarise (or at least partially polarise) even when components of the system are broken or missing.

Footnote: In this thesis I use the term ‘robustness’ to mean the ability of a system to maintain its functions despite internal and external perturbations (as discussed in x). In the case of the PAR network, robustness can be considered at two levels: the ability of the PAR network to maintain asymmetric patterns, and the ability of downstream polarity outputs to maintain normal functionality. I mostly focus on the former when discussing PAR network robustness in this thesis.

Systems level approaches, involving the use of mathematical models, have been instrumental in enhancing our understanding of the links between molecular interactions, feedback pathways and patterning behaviours in the PAR network. However, as most of the information that we have about the PAR network is qualitative rather than quantitative, our ability to fully understand systems level behaviours with models is currently limited. Additionally, many of the molecular mechanisms surrounding the proposed feedback pathways in the network are poorly understood.

Amongst many areas of uncertainty in the PAR network, PAR-2 is particularly enigmatic. Specific to C. elegans, PAR-2 is nonetheless essential for polarity maintenance, through its role as a scaffold for PAR-1 and via downstream regulation of the cortex. As discussed above, PAR-2 also defies predictions from simple mutual antagonism models in its apparent ability to polarise without aPAR asymmetries. A positive feedback pathway has been proposed, but we lack sufficient quantitative data to understand the contribution of this proposed pathway to polarity. Furthermore, the mechanistic basis of PAR-2 feedback is not known, and many uncertainties exist surrounding the roles of the RING domain and protein oligomerisation.

In this thesis, I present an interdisciplinary study of the molecular circuitry of feedback reactions in the PAR network, focussing on PAR-2. In light of a need for good quantitative data, the first aim of my project was to set up an image quantification pipeline that allows us to easily obtain quantitative information about the membrane-binding behaviour of the PAR proteins. I will describe this pipeline in chapter 2. In chapter 3 I use these tools to quantitatively analyse the membrane association behaviour of wild type PAR-2 and several mutant alleles, revealing evidence of a positive feedback circuit and a potential role for the RING domain. In chapter 4, I focus on understanding the mechanisms of RING domain action, exploring hypotheses relating to ubiquitination and dimerisation, and find a role for the RING domain as a concentration-dependent dimerisation domain. In chapter 5 I draw a theoretical link between dimerisation and positive feedback using thermodynamic models. In chapter 6, I incorporate the PAR-2 positive feedback pathway into patterning models, and explore its implications for bistability, symmetry breaking and pattern robustness. In chapter 7, I investigate the link between dimerisation strength and membrane association behaviour in vivo, revealing a key role for intermediate dimerisation strength, and providing novel and unexpected insights into the origins of PAR-2’s plasma membrane specificity.

In the introduction to chapter 3, I give a more detailed overview of the known roles and mechanisms of action of PAR-2 in polarity development and the key areas of uncertainty.

The minimal PAR model presented earlier, in which nonlinear feedback terms are required, is an example that highlights the importance of quantitative information about interactions in the PAR network.

Quantitatively identifying the essential feedback components underlying polarity remains a key outstanding gap in our understanding of the design principles of the PAR polarity network.