Multi-objective identification from fluorescence recovery after photobleaching experiments: Understanding morphogenetic regulation of epithelial polarity

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Abstract: Many of the processes related to morphogenesis depend critically on the maintenance of the spatial distribution of certain polarity proteins on the plasma membrane of the cell. Maintenance of this epithelial polarity depends on the localization and concentration of the polarity proteins. The transmembrane protein Crumbs (Crb) is vital for the dimensions and integrity of the apical membrane. However, there is little knowledge on the molecular mechanisms controlling Crumbs dynamics during morphogenesis. Fluorescence recovery after photobleaching (FRAP) is a live cell imaging technique allowing for the functional measurement of protein mobility in living cells. FRAP experiments were performed in different parts of the Drosophila melanogaster embryonic epithelium and in different developmental stages show significant differences in their characteristics. We analyze the relationship of the changes in the FRAP experiments with the different morphogenetic states of the embryo. To this end, we develop a reaction-diffusion spatiotemporal model involving processes in the membrane and in the cytosol. Moreover, we model the fluorescent tags of proteins and the bleaching process to get in silico FRAP experiments. These in silico FRAPs are then used together with the experimental data to perform parameter estimation based on a multi-objective optimization design procedure. The multi-objective optimization is suitable to understand how morphogenetic events result in different sets of model parameters. Our parameter identification can reveal the mechanisms involved in the regulation of the Crb protein in the different stages of embryonic development and how morphogenesis affects these mechanisms.

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Keywords: Biological model, epithelial cell polarity, complex systems simulations

1. EPITHELIAL CELL POLARITY

Epithelial cells are the defining cell type of all metazoans. Their principal property is to form sheets that can be molded into different shapes, and then act as selective and dynamic barriers between body compartments and between the environment and the inner space of the body. The ability of epithelial cells to self-organize into sheets is based on the polarization of individual cells along the apical-basal axis. Cells adhere to each other with aligned polarity axes so apical membranes face the outside or luminal spaces of internal organs. An apical junctional complex segregates the apical membrane from the basolateral membrane in most epithelia. This belt contains adhesive elements that bind cells into sheets and a junction that seals the intercellular space to control diffusion of ions and molecules across the epithelial sheet. Bidirectional active transport together with the barrier function allows epithelia to regulate compartment composition and organ function (Tepass, 2012).

Formation and maintenance of apical-basal polarity requires the dynamic integration of several cellular mechanisms. Great progress has been made in the identification of components of different modules within the polarization network and in understanding the function of individual cellular mechanisms. However, how these mechanisms cooperate in epithelial polarization is only now emerging and much remains to be learned (Mogi1ner et al., 2012). In particular, the evolutionarily conserved transmembrane protein Crumbs (Crb) is crucial for the size and identity of the apical membrane (Tepass et al., 1990), yet little is known about the molecular mechanisms controlling Crumbs surface expression.

2. FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING

In order to quantitatively investigate the molecular mechanisms involved in epithelial polarity, we use fluorescence recovery after photobleaching (FRAP) experiments (see Fig. 1A). FRAP
is a functional live cell imaging technique allowing for the measurement of protein dynamics in living cells. Conventional FRAP analysis is based on curve-fitting approaches and focuses on parameters of the curve like recovery-time (see Fig. 1B and Fig. 2A) and value of the plateau (Rapsomanikis et al., 2012). To estimate the kinetic parameters of the underlying molecular processes, including association and dissociation constants, relative size of mobile and immobile pools and protein diffusion rates, model-based quantitative FRAP analysis is necessary (Phair et al., 2003). We perform FRAP experiments in different parts of the Drosophila melanogaster embryonic epithelium in different developmental stages (see Fig. 1C). This experiments show significant differences in their characteristics as it is possible to see in Fig. 2B. These differences, like different immobile fraction or different half times suggest that model-based quantitative FRAP analysis approaches like the one presented in (Rapsomanikis et al., 2015) are not enough to get the information we want. These approaches will give us either a mean model (if all the data is used together) or different models for different scenarios. However, we want to analyze the relationship between the changes in FRAP experiments with respect to the different morphogenetic states of the embryo.

3. MODELING

To this end, we develop a spatiotemporal reaction-diffusion model involving 2D and 3D processes for the membrane and the cytosol. Moreover, we model the fluorescent tags of proteins and the bleaching process to obtain in silico FRAP experiments. Then these in silico FRAPs are then used together with the experimental data in a multi-objective optimization based parameter estimation. Previous models of polarity include reaction-diffusion models in 3D only for Yeast polarity which is based in cytosolic membrane-bound proteins (Goryachev and Pokhilko, 2008). In this model, the polarity determinant protein Cdc42 diffuses in the cytoplasm and then binds to the membrane. This mechanism is intrinsically different to the transmembrane proteins involved in polarity of insects and mammals (Tepass, 2012). A more complete mechanistic model, but still related to Yeast polarity, is presented in (Savage et al., 2012) and combines reaction-diffusion with vesicular trafficking. This model relies on reinforcement of spatial asymmetries by the directed transport of molecules along the cytoskeleton to specific locations in the plasma membrane, but in a very simplistic approach disregarding the spatial components in this transport. A model of Crumbs protein (Crb) has been developed by (Fletcher et al., 2012). This model is a first approach, but it is only in 1D and it fails to capture the mechanisms involved in the regulation of the Crb protein as it is not modeled as a transmembrane protein but as the previous membrane bounding proteins.

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Fig. 1. Fluorescence recovery after fotobleaching experiment pipeline. A. Example of a D. melanogaster embryonic epithelium being bleached. B. Typical recovery curve with its main features. C. Areas and stages of the D. melanogaster embryo used in the experiments.

Fig. 2. FRAP curve-fitting. A. Example of experimental curve and fitted curve. B. Curve parameters obtained for different scenarios.

Fig. 3. Schematic model of Crumbs protein interactions.
Here, we propose a combined model of the individual mechanisms known for Crb protein regulation in order to unravel the underlying molecular mechanisms responsible for its localization and regulation. Combining mechanisms (Fig. 3) we present a reaction-diffusion model in a 2D and 3D environments for the membrane and the cytosol respectively. Moreover we include the dynamics of the fluorescent and bleached states for the corresponding proteins and complexes.

The known molecular mechanisms present in the regulation and localization of Crumbs protein have been studied separately in the past and they include:

- Stabilization in the plasma membrane by the cytosolic protein Stardust (Sdt) (Bachmann et al., 2001).
- Incorporation into the endocytic pathway by Adaptor protein 2 (AP-2) (Lin et al., 2015).
- Recycling to the plasma membrane by Vacuolar protein sorting-associated protein 35 (VPS35) (Pocha et al., 2011).

We combine them into a single model. As a first approximation we do not model the synthesis of these proteins and the total number of proteins is initialized in the initial conditions of each species. This approach allows to easily study different scenarios depending on the initial conditions of the molecules. The protein Crb is a transmembrane protein and it can only leave the membrane by the endocytic pathway. The Adaptor Protein 2 which has been shown to be responsible for the initiation of the process of endocytosis. Interestingly, it has been shown that Sdt has a role preventing AP-2 from binding Crb, and hence stabilizing it in the membrane. Finally, the fate of the protein Crb, once it is in the endocytic pathway is modeled through a virtual sorting element from where the possible routes are Crb recycling though the retromer via VPS35 to the plasma membrane and its proteolytic degradation through the lysosome.

The implementation of this model has been done the simplest geometry (cube) with a regular grid using a C++ version (OpenFPM) of the parallel particle mesh methods from (Sbalzarini et al., 2006). With this it is possible to make a 3D simulation of the system together with an in silico FRAP experiment (See Fig. 4A) and obtain different FRAP curves by changing the parameters of the model (Fig. 4B).

4. MULTI-OBJECTIVE OPTIMIZATION

We propose to use the methodology based on using multi-objective optimization design (MOOD) to perform parameter identification (Boada et al., 2016) of the previous model in order to understand how morphogenesis changes the regulation of epithelial cell polarity. The methodology uses a global multi-objective evolutionary algorithm (MOEA) and a multi-criteria decision making (MCDM) strategy to select the most suitable solutions (Reynoso-Meza et al., 2014). Although the identification problem itself can be naturally expressed as a multi-objective problem (MOP), this approach has never been used in the context of FRAP experiments in developmental biology (Rapsomaniki et al., 2015). Our approach finds an approximation to the Pareto set of model parameters that correspond to each experimental scenario (i.e. different parts of the embryo, different developmental stages, even different mutants). The Pareto set together with the Pareto front can be then analyzed with a MCDM generating the best model parameters that explains each scenario.

Fig. 4. A. In silico. FRAP experiment. After reaching a steady state, the selected section is FRAPed by instantaneously changing the state of the bleached species. Then the dynamic of all the states in analyzed to obtain the fluorescence recovery. B. Different in silico FRAP curves obtained by changing the model parameters.

Fig. 5. Multi-objective optimization design.

Now to successfully implement this approach, at least three fundamental steps are required (Miettinen et al., 2008): the multi-objective problem (MOP) definition, the optimization process, and the multi-criteria decision making (MCDM) stage. This overall multi-objective optimization design (MOOD) procedure enables to analyze current trade-offs between the objectives to accordingly select a preferable solution (Reynoso-Meza et al., 2014).

Multi-objective problem definition At this point the error measures between the experimental data for each scenario and the model predictions, are formulated as objectives to be optimized. The objectives to be optimized can be for example the mean square error (MSE) between the prediction and the experimental FRAP curve for each scenario. Then, it is possible to select the parameters of the model to be included in the optimization and which ones will be kept constant. Finally, the multi-objective problem is to find the set of values of the decision variables $\theta$ that minimize all objectives $J(\theta)$. These different objectives for the different scenarios are in conflict if one tries to identify a single ensemble of parameters. So a trade-off must be reached.
Multi-objective optimization process  The multi-objective optimization process finds the best parameters \( \theta_p \) which produce the best Pareto front approximation \( J_p^* \). For problems with a large number of decision variables, as our case, it is more efficient to use an appropriate multi-objective optimization algorithm to approximate this solution. In this work propose to use a multi-objective evolutionary algorithm based on differential evolution which uses a spherical pruning to approximate the Pareto front. The implementation used in this work is the sp-MODE\(^2\) algorithm, which \( i \) improves convergence by using an external file to store solutions and include them in the evolutionary process, \( ii \) improves spreading by using the spherical pruning mechanism (Reynoso-Meza et al., 2010), and \( iii \) improves pertinence of solutions by means of a basic bound approach that is suitable to understand how morphogenetic events result in different set of model parameters of the proposed reaction-diffusion model that includes the FRAP experiment. We propose to use a multi-objective optimization design approach that is suitable to understand how morphogenetic events result in different set of model parameters of the proposed reaction-diffusion model that includes the FRAP experiment. The multi-objective evolutionary algorithm used in this work is the spherical pruning algorithm, which \( i \) improves convergence by using an external file to store solutions and include them in the evolutionary process, \( ii \) improves spreading by using the spherical pruning mechanism (Reynoso-Meza et al., 2010), and \( iii \) improves pertinence of solutions by means of a basic bound approach that is suitable to understand how morphogenetic events result in different set of model parameters of the proposed reaction-diffusion model that includes the FRAP experiment. In this work propose to use a multi-objective optimization design approach that is suitable to understand how morphogenetic events result in different set of model parameters of the proposed reaction-diffusion model that includes the FRAP experiment.

Multi-criteria decision making stage  The selection of the preferable solution according to designer’s criteria takes place in an a-posteriori multi-criteria analysis of the Pareto front approximation. It is desirable that these tools simplify the visualization and the analysis of the trade-off among competing objectives. We use the visualization tool Level Diagrams (Blasco et al., 2008), which has a freely available implementation for designers LD-Tool\(^3\). LD-Tool allows to correlate design objectives with decision variables. The first graph displayed contains each objective, where its Y-axis is the \( p \)-norm \( \|J(\theta)\|_p \) of the objectives vector, and the X-axis corresponds to each objective value \( J_i(\theta) \). The second graph shows \( \|J(\theta)\|_p \) with respect to each decision variable. Thus, a given solution will have the same \( y \)-value in all graphs.

5. CONCLUSION

We propose to use a multi-objective optimization design approach that is suitable to understand how morphogenetic events result in different set of model parameters of the proposed reaction-diffusion model that includes the FRAP experiment. Our set parameter identification could reveal which mechanisms are involved in the regulation of Crb protein in the different stages of the embryo and how morphogenesis affects this mechanisms.

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