Chapter 4

Simulating polarized growth factor delivery in fission yeast

4.1 Introduction

How different cell types can produce different shapes is a fundamental question in cell biology. As a relatively simple eukaryotic single-cell organism that is easy to control and manipulate, fission yeast (*Schizosaccharomyces pombe*) became one of the most intensely studied model systems of single-cell morphogenesis. Fission yeast cells have an elongated, rod-like shape with a highly reproducible diameter of ca. 3 µm and a cell-cycle-dependent length on the order of 8 – 14 µm [90]. The rod-like shape results from restriction of cell growth to two opposite regions on the cell cortex that are marked by accumulations of growth factor proteins. Knock-out experiments have identified many key components of fission yeast polarization. Intriguingly, they suggest that the localization of growth factors to the growing poles of the cell is established via a symmetry-breaking mechanism that combines active transport on the cytoskeleton, diffusion on the membrane and shuttling of proteins between cytoplasm, cytoskeleton and membrane.

To elucidate this interplay we developed a spatially-resolved model of growth factor polarization that features microtubules and a fully membrane-enclosed cell volume, and conducted stochastic simulations of the model with eGFRD. Our model is representative of the Tea1/Mod5-system, which is described in detail in section 4.2. It contains two species: a permanently membrane-bound species M (Mod5) and species T (Tea1), which can diffuse in the cytoplasm, bind microtubules on which it can drift towards the cell poles, and form TM-complexes with M on the membrane. The TM-complexes have a finite lifetime; after TM-complex dissociation T moves back into the cytoplasm. This sets up a cycle in which T is transported actively from the cytoplasm to the membrane at the poles and passively returns from the membrane to the cytoplasm via diffusion on the membrane.

In order to identify the components of the considered system that are critical to establish proper growth factor polarization we compared models with different ways
Simulating polarized growth factor delivery in fission yeast

of TM-complex formation at the membrane and systematically varied the membrane diffusion constant and the lifetime of the TM-complexes. We find that a “direct binding” model, in which growth factors T can bind to their membrane-bound partners M directly, exhibits better polarization properties than a “two-step binding” model, in which a T-particle first binds the membrane and then must find its reaction partner M via membrane diffusion. In the “direct binding” model, the simulations further reveal that cell polarization is enhanced with decreasing membrane diffusion constant of the TM-complexes. Moreover, there exists an optimal lifetime of TM-complexes on the membrane that maximizes both the efficiency of polarization, measured as the ratio between polar and central surface-density of TM-complexes, and the growth factor concentration at the poles.

In this chapter, we first give an overview of the current knowledge on fission yeast morphogenesis, discussing recent experimental findings. We then describe how we model the yeast cell and simulate polarization of growth factors with eGFRD. In the last part of this chapter we present and discuss our results.

4.2 Experimental facts

Elongated growth of fission yeast cells is controlled by polarity markers that agglomerate at the opposite cell poles in a mechanism that involves microtubules, actin and the cell membrane [43, 90, 91]. Once established, the agglomerations persist throughout interphase and promote continuous cell elongation from the poles, until growth stops during mitosis. Directly after cell division, cells only continue growing at the “old” pole opposite of the division site until the growth machinery is reconstituted at the “new” pole in G2 phase (“new end take off” / NETO) [92].

An essential polarity factor is the GTPase Cdc42, whose active form accumulates preferentially at the cell poles; cells with deleted or overexpressed cdc42 produce under- or oversized round cells, respectively. Cdc42 also plays a crucial role in growth of other yeast types and is thought to activate a range of proteins that promote actin polymerization. Indeed, the growth sites at the cell poles feature patches of actin which are necessary to localize enzymes involved in cell wall synthesis and remodelling [43, 90]. Recently, the active form of Cdc42 was shown to exhibit oscillations between the cell poles [93]. Here, we do not focus on the Cdc42 system, but rather on another polarization system.

Among the most prominent polarity markers are further the proteins of the Tea family. While certain Tea protein complexes (Tea1/Tea4) play an essential role in establishing bipolar patterns of Cdc42 [94], the detailed interactions between Tea proteins and Cdc42 are yet poorly understood. Figure 4.1 summarizes the current picture of Tea-protein-based polarization in fission yeast. Proper localization of the Tea-family markers requires interaction with microtubules [45]. In fission yeast, microtubules form dynamic antiparallel bundles emerging from the cell center, with plus-ends growing towards and transiently remaining at the cell poles. The polarity marker Tea1 is delivered directly to the membrane at the poles by microtubule tips, to which it associates with the help of protein Tip1 [44, 95]. Tip1 is rapidly transported by the motor protein Tea2 towards the plus-end, where both proteins
Figure 4.1: Schematic drawing of the currently assumed model of fission yeast growth polarization. Fission yeast forms elongated cells by restricting growth to opposite cell poles. This involves microtubules (dark green), which in fission yeast form dynamic antiparallel bundles with outwards-growing plus ends. Growth is restricted to the cell poles by accumulation of polarity factors Tea1 (green) and Tea4 (blue). Tea1 and Tea4 are transported towards microtubule plus-ends via Tip1 (light red) and kinesin motor protein Tea2 (dark red). These complexes accumulate at the plus-end with the help of tip-tracking protein Mal3 (magenta). Membrane-bound protein Mod5 (yellow) is required for direct delivery of the markers to the membrane at the poles. On the membrane, Tea1 and Tea4 engage in complexes that promote actin assembly and subsequent recruitment of cell-wall-remodelling enzymes. Note that the drawing does not include other prominent polarity factors such as Cdc42, whose connection to the Tea-proteins is yet unclear.

form clusters [96]. To accumulate at the microtubule tip, they require the protein Mal3 [97], which belongs to the EB1 family of tip trackers. Cells with knocked-out tea1 or its transporter tea2 fail to establish two oppositely located growth sites; instead, a single growth site forms in a random spot on the cell wall, producing L- or T-shaped cells [98, 99, 100]. The same is observed in cells with shorter or completely depolymerized microtubules [45, 101]. While microtubules thus play a crucial role in Tea-protein polarization, there is also evidence that proper anchoring of Tea1 to the membrane requires the membrane protein Mod5 [102]. Consistently, Mod5 is localized to the cell poles in wild-type, whereas in a tea1-knockout it spreads out uniformly over the membrane. Since Mod5-turnover at the cell poles was found to be much faster than Tea1-turnover, it was proposed that Mod5 catalyzes the formation of membrane-bound Tea1-clusters [103]. An important role was also found for Tea4: like Tea1, it associates with growing microtubule plus-ends and together with Tea1 forms membrane-complexes that act as nucleators of actin assembly [94, 95, 104, 105].

Importantly, polarity factors also influence microtubule dynamics. At the microtubule plus-ends Tip1 acts as a stabilizing factor, reducing the microtubule catastrophe rate in the regions far from the cell poles [44]. Increase of the catastrophe rate at the poles was also found to depend on pushing forces and tip-accumulation of motors from the kinesin-8 family, which contribute to depolymerization of long microtubules at their plus-ends [81, 106]. Moreover, microtubules are hampered in directing their growth towards the cell poles in tea1-deletion mutants [82, 83].
Taken together, these observations suggest a polarization mechanism that involves 1D active transport on microtubules and diffusion in 2D and 3D: Microtubules recruit polarity markers such as Tea1 and Tea4 from the cytosol and direct them via active transport towards their plus-ends, where the markers accumulate aided by tip-trackers like Mal3. The plus-end clusters have a stabilizing effect on the microtubules, enhancing the probability to reach the cell poles. At the poles, the polarity markers are delivered to the cell membrane, where they bind and anchor with the help of highly mobile membrane-bound proteins such as Mod5. After forming membrane-bound complexes, the markers stimulate actin polymerization, leading to actin-mediated recruitment of wall-remodelling enzymes, and further cell growth.

The overwhelming number of interacting proteins involved in fission yeast growth polarization, which in addition employ different transport modes and partly appear to follow distinct strategies to set up bipolar patterns, prompted us to ask: Which components of this system are indeed critical for efficient polarization? Following a bottom-up approach, we therefore aimed at reconstituting a minimal model of growth factor polarization in fission yeast, i.e. a model that includes as few of the experimentally identified system components as possible while producing a robust bipolar pattern under biologically realistic, meaning stochastic conditions.

4.3 Model

In order to reconstitute a minimal mechanism of fission yeast polarization we developed a model which features 1D active transport on microtubules, 2D diffusion on the cell membrane, cytosolic diffusion in 3D and particle interactions across the different dimensions. Using eGFRD, we performed particle-based stochastic simulations of the spatially-resolved model. We considered the system on the observed timescale of polarization, i.e. minutes. Therefore our model neglects elongation of the cell, focusing on the spatio-temporal dynamics of polarity factors. The cell is thus modelled as a static rectangular box consisting of six interlinked finite planes representing the cell membrane, with an aspect ratio typical of interphase fission yeast; exemplary 3D views of the model geometry are shown in Figure 4.3. At the mid-plane of the long axis of the box, we symmetrically place four pairs of antiparallel capped rods whose orientation vectors point outwards, representing microtubules with plus-ends directed towards the cell poles. Here we considered only static rods, i.e. microtubules with a fixed length; the influence of microtubule dynamics will be studied in forthcoming work. In our model, the microtubule bundles are almost as long as the box, with tips located in proximity to the poles.

As shown in Figure 4.2, the model features two principal chemical species: the cytosolic species T, which represents a polarity factor protein, e.g. Tea1, and the membrane-bound species M, representative of the Mod5 protein. T and M can form membrane-bound complexes TM with diffusion-limited on-rate; the lifetime $\tau$ of TM-complexes is a model parameter. The complex diffuses slower than the individual proteins. This is tuned via a "slowdown factor" $\chi$ that divides the standard 2D diffusion constant $D_2 = 0.1 \mu m^2/s$ of a single membrane-bound protein. Most importantly, in our model Mod5 (M) acts only as a recruiting agent for Tea1 (T), i.e. the model
Figure 4.2: Schematic of the simulated fission yeast polarization model. Our model includes two protein species: a bulk species $T$ (green), representative of (e.g.) Tea1, and a membrane-bound species $M$ (yellow), representing Mod5. Upon binding of $T$ to microtubules it is converted to $T^*$ and transported towards the microtubule plus-end (tip) with drift velocity $v$. $T^*$-particles can agglomerate at the tip, forming clusters $T^*_n$ (magenta), which "spawn" $T$-particles back into the cytosol close to the membrane. $T$ and $M$ can form membrane-bound complexes $TM$ (red) which have a reduced mobility, tuned by model parameter $s$. We compare a "direct binding" scenario, in which $T$ may bind $M$ directly (red arrows), to a "two-stage binding" scenario, in which $T$ first binds to the membrane to create $T_M$ before forming $TM$ (blue arrows).

does not assume a catalytic role for Mod5 in Tea1 polymerization on the membrane, as proposed previously [103]. We assume that species $M$ is tightly bound to the membrane, i.e. never dissociates on the considered timescale. Moreover, we assume that on the given timescale protein production and degradation is negligible. Particle numbers thus only change due to formation and dissociation of $TM$-complexes. As a further important model feature, $T$-particles may also bind to the microtubules and convert to species $T^*$, which is transported outwards with significant drift until it reaches the cylinder cap. $T^*$ represents the whole complex consisting of Tea1 or Tea4, Tip1 and the motor protein Tea2 as a single species. The cylinder cap (microtubule tip) can accumulate $T^*$-particles via a cascade of reactions of the type $T^*_n + T^* \rightarrow T^*_{n+1}$; for simplicity we represent a cluster of $n$ $T^*$-particles by a single, immobile particle with increased radius (species $T^*_n$). A cap-bound cluster particle can "spawn" $T$ particles at a rate $k^\text{tip}$ back into the cytosol. In order to limit the combinatorial explosion of modelled reactions here we only considered a model in which $T$-particles unbind from the cap-bound cluster one by one.

To assess the role of the membrane-protein Mod5 in the polarization of the polarity markers, we compare a system with "direct binding", in which $T$ directly associates with $M$, to a system with "two-stage binding", in which $T$ first binds to the membrane to form $T_M$ and then finds its reaction partner $M$ via 2D diffusion.
Figure 4.3: Typical 3D views of the polarization model simulated with eGFRD. We show here a typical initial (A) and final, polarized state (B) of the particle-based fission yeast model as simulated with eGFRD. The yeast cell is represented as a box consisting of interlinked finite planes, with lengths and aspect ratio corresponding to wild-type cells in interphase. Four microtubule bundles are modelled by eight pairwise antiparallel static cylinders oriented with their plus-ends outwards, starting from the mid-section of the box. Each bundle spans 95% of the box length, with cylinder caps close to the two opposite “cell poles”. Our model features two principal particle species, cytoplasmic species T (green) and membrane-bound species M (yellow), which can form membrane complexes TM (red; also see Fig. 4.2). T-particles can bind the microtubules to drift outwards, and return to the cytoplasm at the cylinder caps. The shown snapshots are for the system with direct binding, for complex diffusion constant $D_{TM} = 0.01 \, \mu m^2/s$ (slowdown factor $\chi = 10$) and complex lifetime $\tau = 10 \, s$.

4.3.1 Parameters

Here we briefly describe the choice of parameters in our model. Table 4.1 gives an overview of their standard values. Note that modelled species are not thought to represent single molecules but rather larger protein complexes, in particular in the microtubule-bound state.

The dimensions of the simulation box roughly correspond to the dimensions of a fission yeast cell in interphase. With the chosen aspect ratio between box length and width/height the surface-to-volume ratio of the box corresponds well to the surface-to-volume ratio of a spherocylinder of the same size. In our model, microtubules are static and their length is chosen such that their tips are close to the two box ends; the bundles therefore span 95% of the box length. Particle and rod diameters have typical values for proteins and microtubules.

Since diffusion constants for the involved protein species are unknown we resorted to typical values. Therefore we set $D_3 = 1 \, \mu m^2/s$ for the cytoplasmic diffusion constant and $D_2 = 0.1 \, \mu m^2/s$ for the standard diffusion constant on the membrane. The diffusion constant of the membrane-bound TM-complex is equal to $D_2/\chi$, where the slowdown factor $\chi = 1 - 100$ is varied.
<table>
<thead>
<tr>
<th>Quantity</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulation box length</td>
<td>$l_X$</td>
<td>9.5</td>
<td>µm</td>
</tr>
<tr>
<td>Simulation box width</td>
<td>$l_{YZ}$</td>
<td>4.0</td>
<td>µm</td>
</tr>
<tr>
<td>Microtubule (MT) length</td>
<td>$l_{MT}$</td>
<td>4.5</td>
<td>µm</td>
</tr>
<tr>
<td>Distance of MT axis from box sides</td>
<td>$\delta_{MT}$</td>
<td>1.0</td>
<td>µm</td>
</tr>
<tr>
<td>Total membrane surface area</td>
<td>$A_{mem}$</td>
<td>240</td>
<td>µm$^2$</td>
</tr>
<tr>
<td>No. of cytoplasmic (T) particles</td>
<td>$N_T$</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>No. of membrane-bound (M) particles</td>
<td>$N_M$</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Particle radius (TM-complexes)</td>
<td>$R_{TM}$</td>
<td>45</td>
<td>nm</td>
</tr>
<tr>
<td>Particle radius (all other species)</td>
<td>$R_0$</td>
<td>30</td>
<td>nm</td>
</tr>
<tr>
<td>Microtubule radius</td>
<td>$r_0$</td>
<td>25</td>
<td>nm</td>
</tr>
<tr>
<td>Cytoplasmic diffusion constant</td>
<td>$D_3$</td>
<td>1.0</td>
<td>µm$^2$/s</td>
</tr>
<tr>
<td>Standard membrane diffusion constant</td>
<td>$D_2$</td>
<td>0.1</td>
<td>µm$^2$/s</td>
</tr>
<tr>
<td>Standard 1D diffusion constant</td>
<td>$D_1$</td>
<td>0.1</td>
<td>µm$^2$/s</td>
</tr>
<tr>
<td>Membrane complex slowdown factor</td>
<td>$\chi$</td>
<td>10–10$^3$</td>
<td></td>
</tr>
<tr>
<td>Outwards drift velocity on MTs</td>
<td>$v$</td>
<td>0.5</td>
<td>µm/s</td>
</tr>
</tbody>
</table>

Table 4.1: The standard parameters of the simulated fission yeast system.

We assume all binding rates to be diffusion-limited; the binding rates at contact are therefore set to high numerical values. For simplicity we set the unbinding rate from the microtubules to zero. Unbinding thus only happens from the microtubule tips at a rate $k_{tip} = 1.0$/s. The dissociation rate of TM-complexes is a model parameter that we vary.

### 4.3.2 Simulations and analysis

We conducted stochastic, particle-based simulations of the simplified yeast model defined in section 4.3 using eGFRD, with the new features defined in chapters 2 and 3. In all simulations, initially we randomly placed $N_T$ T-particles in the cytoplasm and $N_M$ M-particles on the membrane (the state shown in Fig. 4.3A). The system then was propagated for a fixed number of steps, usually resulting in several hundred seconds of simulated time. Particle data (positions and species) was acquired at approximately regular time points with a measurement interval of $\Delta t = 0.5$ s.

Initial simulations showed that the polarity-complex formation model indeed is capable of establishing polarity along the long axis of the simulation box on a timescale
of minutes. To quantify and compare the extent of polarization we introduced the following protocol: For all recorded time points, raw particle position data was binned into a coarse (six-bin) histogram along the long axis of the system. This was used to compute the average (area) density $\langle \rho \rangle$ of TM-complexes on the membrane for each bin by dividing the total particle number by the total membrane surface area in the bin. We then define the “polarization score” or “polarity” as the ratio between the average density in the two outermost bins at the poles $\langle \rho_p \rangle$ and the average density $\langle \rho_c \rangle$ in the two central bins:

$$\Pi \equiv \frac{\langle \rho_p \rangle}{\langle \rho_c \rangle} \quad (4.1)$$

The polarity $\Pi$ quantifies the anisotropy of the TM-complex density in a straightforward way. However, high anisotropy does not necessarily imply a sufficiently high density of the complexes at the poles. Indeed, $\Pi$ could be high, while the overall density at the poles is low. To capture the combined effect of polarization and density enhancement at the poles, we therefore also compute a “combined score” which increases with both TM-complex density and polarity, defined as:

$$\Gamma \equiv \langle \rho_p \rangle \Pi = \frac{\langle \rho_p \rangle^2}{\langle \rho_c \rangle} \quad (4.2)$$

### 4.4 Results

We reasoned that two parameters of our model are crucial for the magnitude of TM-complex polarity: the slowdown of membrane diffusion upon complex formation, characterized by the slowdown factor $\chi$, and $\tau$, the mean lifetime of the complexes on the membrane. Since in our model particle binding to microtubules occurs (quasi) instantly after contact and microtubule-bound particles drift outwards very efficiently without interrupting unbindings, $\tau$ is the main determinant of the timescale of polarity marker recycling via cytoplasm and microtubules. To assess the dependence of polarity on $\chi$ and $\tau$ we performed a parameter sweep over these two quantities and compared the values of polarity $\Pi$ and combined score $\Gamma$. We find that while increasing slowdown is beneficial for proper polarization, there exits an optimal lifetime $\tau_{\text{opt}} = 10 \text{ s}$ of the complexes on the membrane that maximizes $\Gamma$.

In Figure 4.4 we plot the time evolution and a time average of the TM-complex surface density along the long ($x$-) axis of the system; we compare the system in which the cytoplasmic species $T$ binds membrane-bound species $M$ directly (4.4A) to the system in which $T$ first has to associate with the membrane itself before it can form the complex with $M$ (4.4B), for $\tau = 10 \text{ s}$ and $\chi = 10$. In both cases the density of the membrane-bound polarity marker complexes is enhanced at the poles. The systems are capable of establishing polarization on a timescale of $t \simeq 60 \text{ s}$. However, while in the system with two-stage binding the overall complex density is slightly higher, polarization along the $x$-axis is significantly more pronounced in the system with direct binding ($\Pi = 4.32 / \Gamma = 3.17$ with direct binding vs. $\Pi = 1.64 / \Gamma = 1.64$
Figure 4.4: Establishment of TM-complex density polarization. Here we plot the time evolution of the average TM-complex surface density along the long ($x$-) axis of the simulation box for the systems with direct binding (A) and with two-stage binding (B), for complex lifetime $\tau = 10$ s and slowdown factor $\chi = 10$. The black dashed line shows the time average of the density over the last 30 s. Note that initially the complex density is zero because no membrane-complexes have formed yet. The expected system-wide average density when all T-particles have formed membrane complexes is $0.83/\mu m^2$.

As a next step we quantified the dependence of polarity on the two principal parameters of our system, the TM-complex lifetime $\tau$ and the complex slowdown factor $\chi$. In Figure 4.5 we plot, for the system with direct binding, the polarity $\Pi$ and the combined score $\Gamma$, which is the product of $\Pi$ and the pole density $\langle \rho_p \rangle$, as a function of $\tau$ for different slowdown factors $\chi$. The figure reveals the requirements for optimizing polarization in the considered system: First, for slowdown factors $\chi \geq 10$, i.e. for membrane-complex diffusion constants $D_{TM} \leq 0.01 \mu m^2/s$, the system reaches significantly higher scores than for $\chi = 1$ ($D_{TM} = 0.1 \mu m^2/s$). Moreover, figure 4.5A suggests that faster recycling of the complexes, corresponding to short complex lifetimes, leads to more efficient polarization. However, this neglects the fact that with decreasing complex lifetime $\tau$ also the complex density on the membrane decreases. Therefore there is a trade-off between polarity and membrane occupancy when reducing $\tau$. This is captured by the combined score $\Gamma = \langle \rho_p \rangle \Pi$ (4.5B), which displays a maximum at $\tau_{opt} = 10$ s.
4.5 Discussion

In order to reconstitute a minimal model of yeast polarization we conducted stochastic, particle-based eGFRD-simulations of a model that features a cytoplasmic polarity marker species T (representing Tea1), which can bind microtubules which transport it towards the cell poles, and a membrane-bound species M (representing Mod5), with which T can form slow-diffusing heterodimeric polarity complexes TM on the membrane. The results presented in this chapter demonstrate that this simple system, with static microtubules that reach the proximity of the cell poles, is capable of establishing considerable polarity ($\Pi \gtrsim 4$) along the long axis of the cell if two conditions are met: First, for efficient polarization the polarity markers should diffuse slowly ($D \lesssim 0.01 \mu m^2/s$) once bound to the membrane. Second, there is an optimal lifetime of polarity marker complexes on the membrane $\tau_{opt} = 10$ s that maximizes both polarity and the surface density of the markers at the poles. The optimum arises from a trade-off between two opposing effects: On the one hand, fast complex recycling aids polarization by rapidly returning polarity markers that were “misanchored” at the membrane far from the poles back to the microtubules, and by preventing correctly localized complexes from diffusing too far away from the poles; on the other
hand, it also reduces the overall number of complexes at the membrane. Importantly, in contrast to earlier studies [103, 107], our polarization model does not explicitly assume polymerization, i.e. formation of larger clusters of Tea1 on the membrane.

Interestingly, we find that the two-stage binding scenario, in which T must first associate with the membrane to form TM-complexes with M, performs significantly worse in terms of polarization efficiency than the system in which direct binding of T to M is allowed. Since this is observed for equal slowdown factors, the reduced polarization efficiency in the system with two-stage binding must be the product of reduced recycling efficiency for complexes forming at the central parts of the membrane. In the system with two-stage binding the whole membrane constitutes a reactive surface for cytoplasmic T-particles and thus the probability of a cytoplasmic T-particle to bind the microtubule and be directed to the cell poles is lower than in the direct binding scenario. Moreover, in the two-stage binding scenario T remains bound to the membrane for a certain time after complex dissociation; this enhances the probability to reform the complex with a closeby M-particle.

We believe that in addition the following positive feedback mechanism is at work: The formation of complexes at the poles is accompanied by depletion of freely diffusing M. For high slowdown factors, there is a significant difference between the mobility of M and TM-complexes. This leads to an effective influx of M into the pole regions upon formation of slow complexes; simultaneously, the concentration of M in more remote regions of the membrane is decreased. This way, formation of polarity marker complexes at the poles reduces the probability of complex formation at the central parts of the membrane. The above effect also manifests itself in the density profiles of the two species: If the diffusion constants of species M and TM were to be equal, \( D_M = D_{TM} \), the sum of their average densities \( \langle \rho_M \rangle + \langle \rho_{TM} \rangle \) would be constant, because the total number \( N_M + N_{TM} \) is conserved on the membrane. A rise of \( \langle \rho_{TM} \rangle \) at the poles then would be accompanied by a complementary drop of \( \langle \rho_M \rangle \), implying a peak of \( \langle \rho_M \rangle \) in the central parts of the cell. In contrast, if \( D_M \gg D_{TM} \), the total density \( \langle \rho_M \rangle + \langle \rho_{TM} \rangle \) can be high at the poles while \( \langle \rho_M \rangle \) does not exhibit the central peak because it is annihilated by fast diffusion of M. Indeed, in our simulations with direct binding we observe that while in the polarized state the density of M at the poles is somewhat reduced when compared to its density in the center of the system, this effect is far less pronounced than enhancement of TM-complex density at the poles (data not shown); in fact, to a good approximation, the M-particles equilibrate over the membrane, supporting the existence of the mechanism described above. This gives a rationale for the presence of the membrane-bound reaction partner (M / Mod5) in the system.

A combined theoretical-experimental study on the Pom1 protein [107], another important polarity factor in fission yeast that associates with the membrane to form concentration gradients decreasing from the cell poles, recently uncovered dynamics with striking similarities to our model: It was found that membrane-bound Pom1 molecules permanently change from a clustered to a non-clustered state, defining two separate Pom1 populations with markedly different diffusion speeds (unclustered form: \( D_u \simeq 0.026 \mu m^2/s \); clusters: \( D_c \simeq 0.006 \mu m^2/s \); cytoplasmic diffusion: \( D_1 \simeq 1.5 \mu m^2/s \)). While the average cluster lifetime was measured as \( \tau_u \simeq 3 \) s, the overall membrane-bound time of Pom1 was \( \tau_m \simeq 30 \) s. These findings not only are in very
good agreement with the values at which we find optimal polarization efficiency in our model, they also suggest that slowdown of polarity factors upon binding to the poles is a common ingredient in mechanisms that establish bipolar concentrations along the yeast membrane. While in [107] the authors demonstrate that two different diffusion speeds on the membrane aid in producing more robust Pom1 gradient signals, i.e. making positional information along the membrane more reliable, our results, in particular the uncovered trade-off between polarity and pole occupancy, give a slightly different rationale for the observed slowdown, also offering an explanation for the timescale of membrane association (∼ 10 s). To clarify whether true (i.e. multimeric) membrane clustering as proposed in [107] performs better or worse in terms of polarization reliability than the simpler (hetero-) dimerization considered in our model a direct comparison of these two “slowdown mechanisms” will have to be performed.

Continued simulations which account for the observed microtubule dynamics, and with more realistic copy numbers of the involved reaction partners, will help to further elucidate the mechanisms of fission yeast polarization in forthcoming work.

4.6 Acknowledgements

The author thanks P. Recouvreux, A. Mugler and N. Taberner for inspiring discussions on the topic presented in this chapter.

The author thanks A. Hoffmann for critical reading of this chapter.