How to build a robust intracellular concentration gradient

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Abstract: Concentration gradients of morphogens are critical regulators of patterning in developmental biology. Increasingly, intracellular concentration gradients have also been found to orchestrate spatial organization, but inside single cells, where they regulate processes such as cell division, polarity and mitotic spindle dynamics. Here, we discuss recent progress on understanding how such intracellular gradients can be built robustly. We focus particularly on the Pom1p gradient in fission yeast, elucidating how a variety of buffering mechanisms operate to ensure precise gradient formation. In this case, a systems-level understanding of the entire mechanism of precise gradient construction is now within reach, with important implications for gradients in both intracellular and developmental contexts.

Keywords: intracellular, gradients, morphogens, fluctuations, robustness, precision

Gradients on multiple scales

The concept of a concentration gradient has been pivotal in explaining how development in biology is spatially regulated[1-6]. Typically, a spatially varying concentration of a morphogen protein drives spatially differentiated gene expression through a concentration thresholding mechanism, whereby morphogen concentrations above a particular threshold can, for example, activate the expression of a specific gene. In this way a continuously varying concentration in space can be converted into a discrete pattern of gene expression territories.

Classical morphogens often act over relatively long distances; the intensively studied Bicoid morphogen, important for anterior cell fate specification in the early *Drosophila* embryo, extends for hundreds of microns away from its anterior source[7]. At slightly shorter length scales, the morphogen Decapentaplegic (Dpp) extends tens of microns away from the anterior-posterior compartment border in the center of the *Drosophila* wing imaginal disc[8, 9]. In both cases, the gradients span many cells (or nuclei for Bicoid in the syncytial blastoderm) generating long-range patterning potential. The mechanism of formation of these gradients is thought to be due to localized protein production, followed by effective diffusion away from the source, and then eventual degradation (Box 1). The Bicoid system largely upholds this mechanism; although an underlying spatial *bicoid* mRNA gradient does contribute to the Bicoid protein gradient, protein movement is also required[10, 11]. For Dpp, although the system is governed by effective diffusive transport on longer length scales, the mechanism of morphogen movement on shorter scales is still controversial[12].

Over the past ten years or so, studies have shown that concentration gradients are not the exclusive preserve of developmental biology, but can also have a crucial role in spatial

organization inside single cells[13-17]. Brown and Kholodenko showed theoretically that concentration gradients could exist inside an individual cell with biophysically relevant parameters[13]. Hence, an individual cell was not so small that homogeneous concentrations were inevitable (Box 1). Since then, a host of different gradients have emerged, ranging from cell division regulators[18, 19] and cell fate determinants[20, 21] to mitotic spindle organizers[22-24]. Even some of the smallest of bacterial cells contain gradients with spatial extents of only around a micron[25, 26]. An important difference exists between intracellular gradients and their developmental cousins, however: the role of degradation. In developmental systems, the morphogen protein is typically degraded[27, 28] and this, together with diffusion, is believed to be responsible for the decrease in morphogen concentration as a function of distance away from the source. In intracellular systems, the lifetimes required for this mechanism to generate a meaningful gradient are too short to be realistic (Box 1). Instead, it is typically a modification of the protein (for example, its phosphorylation status) that is modulated as part of a gradient. In this way, a typical protein with a lifetime of hours or more, can potentially participate in (phospho-) gradient formation many times over before being degraded (Box 1).

Making development precise

The study of developmental morphogen gradients has recently been reinvigorated by a novel focus on noise and precision[15, 29-32]. All biological systems inevitably contain sources of noise, which can potentially corrupt the ability of the system to generate reliable outcomes. Noise can broadly be separated into two classes: extrinsic and intrinsic. Here, extrinsic noise refers to fluctuations from one gradient to another in two different cells or

embryos (Figure 1a). This might arise, for example, from different amounts of mRNA laid down from one embryo to another, leading to differing gradient profiles. Intrinsic noise refers to fluctuations inherent even within a single copy of the gradient (Figure 1b). In the latter case, such noise arises from the inevitable biochemical fluctuations inherent in processes such as diffusion that are needed to make a gradient (Box 2), and which will be particularly prevalent at low molecular copy numbers. Fluctuations, both extrinsic and intrinsic, will degrade the precision of the positional information provided by a gradient (Figure 1).

A variety of mechanisms are available to potentially buffer these sources of noise, thereby increasing the precision of the resulting positional information. Time-averaging will clearly reduce positional error introduced by intrinsic fluctuations[15, 31] (Box 2), as can spatially averaging the gradient read-out provided by neighboring cells or nuclei in a developmental biology context[31, 33]. Time-averaging is performed by a downstream signal-processing network, with time scales determined by the transcript and/or protein lifetimes of the target gene (e.g. for Bicoid, this would be the lifetimes of the products of its target gene *hunchback*). Extrinsic noise can be reduced by changing the degradation process involved in the removal of the morphogen protein, thereby altering the gradient shape (see also *[34]* and [29] for alternative methods). For example, it was demonstrated that morphogens with self-enhanced degradation exhibit, at large distances, profiles that decay as power laws (Box 1)[32]. Theoretical analysis has shown that such profiles can potentially better buffer extrinsic noise in morphogen production rates, as compared to profiles with standard (i.e. linear) morphogen degradation, which generates exponentially decaying profiles. More generally, if both extrinsic fluctuations in morphogen production rates as well as intrinsic

fluctuations need to be buffered then the gradient shape that maximizes precision depends on which source of noise is most important[35]. Power law profiles are potentially best for systems dominated by extrinsic noise in morphogen production (as expected from [32]), straight line profiles for intrinsic noise, and exponential profiles for systems where both types of noise were important[35, 36].

So far this type of analysis has been mostly applied to developmental morphogen systems. Here, we review how similar ideas concerning robustness and precision are being introduced into the field of intracellular concentration gradients.

The intracellular Pom1p gradient in fission yeast

One of the best studied intracellular gradients is that of the Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) Pom1p inside the fission yeast *Schizosaccharomyces pombe*[37]. Pom1p forms a cortical gradient, with highest concentrations at the cell tips reducing towards the cell center[18] (Figure 2a). Interestingly, detailed imagery has demonstrated that Pom1p profiles exhibit large dynamical fluctuations (Figure 2b). The Pom1p gradient is believed to have multiple important roles, regulating cell polarity, cell length control[38-40] and positioning of the cell division plane[18, 19, 37, 41]. In particular, Pom1p is believed to negatively regulate the mitotic activator Cdr2p, which is cortically located at the center of the cell. As the cell grows, the concentration of Pom1p at the cell center is proposed to drop, thereby activating Cdr2p and enabling the cell to couple cell size with the cell cycle[38, 39]. Furthermore, the midcell localization of the cytokinesis factor Mid1p, responsible for division site positioning, is also believed to be regulated by Pom1p[18, 19]. These biological functions have been intensively studied in recent years and are the subject of several recent reviews[42-44]. Below, we will therefore focus on a slightly

different question, namely the mechanisms of gradient construction themselves. In particular, we will examine how these mechanisms can buffer fluctuations, so that the relevant downstream biological processes are supplied with precise positional information[15, 45].

At heart, the cortical Pom1p gradient is generated by localized binding of Pom1p at cell tips, followed by spreading out on the membrane by diffusion, followed by membrane unbinding. A cycle of Pom1p phosphorylation underlies these events, consistent with the considerations of Box 1[46]. When Pom1p is targeted to the cell tips it is dephosphorylated, a process that enhances its affinity for the cell membrane. Once bound to the membrane, Pom1p is thought to undergo autophosphorylation. Such a process lowers the protein's membrane affinity, eventually leading to unbinding from the membrane. Pom1p can then diffuse in the cytoplasm before potentially being recycled into the cortical gradient by rebinding to cell tip regions, where it is again dephosphorylated. Consistent with this overall picture, a kinase-dead version of Pom1p localizes much more broadly over the cell periphery[46, 47], with a role for autophosphorylation favoured by the inability of wild-type Pom1p to restore the localization of inactive Pom1p[46].

We next examine how the Pom1p gradient can be made robust, focusing on three key elements: (i) precise polar binding followed by (ii) robust spreading away from these regions, followed by (iii) reliable gradient interpretation by downstream elements.

Precise polar binding through self-focusing

Polar targeting of Pom1p is dictated in part by the dynamic properties of microtubules, as well as by the overall rod-shaped geometry of the fission yeast cell. Incipient microtubules

initially tend to grow in a random direction before contacting the edges of the cell, sliding along them, and eventually orienting along the long axis of the cell[48]. The protein Tea1p is then delivered to cell tips through a microtubule-based delivery system; Tea1p rides on growing microtubule plus ends and is released when microtubule ends reach the cell tip[49, 50]. Tea4p localizes in a similar manner, but is dependent on Tea1p[51, 52]. Proper Pom1p membrane targeting is believed to depend on the cell-tip localization of both Tea1p and Tea4p. In *tea4Δ* cells, Pom1p fails to localize to the cortex and in *tea1Δ* cells, Tea4p does not localize to the cortex and Pom1p exhibits a weakly uniform cortical localization pattern[18]. Furthermore, Tea4p both directly interacts with Pom1p (in two-hybrid assays) and recruits the phosphatase Dis2p to cell tips[46, 53]. Dis2p can dephosphorylate Pom1p, thereby aiding in Pom1p membrane binding, as described above[46]. In this way, the microtubule/Tea1p/Tea4p/Dis2p program predominantly targets Pom1p to cell tips (see Figure 3a).

Occasionally, however, microtubules (particularly shorter ones) will still contact the membrane in non-polar regions (Figure 3a). This could trigger inappropriate localization of Tea1p/Tea4p/Dis2p and misplaced Pom1p binding. Therefore, an additional targeting mechanism exists to guard against this possibility, involving both Tea1p and another tip regulator, called Mod5p[54-56]. Anchoring of Tea1p at cell tips is dependent on Mod5p, and vice-versa. Hence, one possible hypothesis is that Tea1p/Mod5p function in a positive feedback loop to focus the precise localization of Tea1p at cell ends. Further support for this idea has recently been provided *[54]*. A simple potential model for this loop is that Mod5p acts as a molecular ‘glue’ to retain Tea1p at cell tips, with the Tea1p–Mod5p complex being relatively immobile. In this model, Tea1p and Mod5p should have identical diffusion

constants as part of such a complex. However, Fluorescent Recovery After Photobleach (FRAP) measurements made by bleaching half a cell tip indicated that Tea1p and Mod5p have distinctly different dynamics, with Tea1p barely recovering after 5 min in contrast to approximately 50% recovery for Mod5p. This result makes a simple ligand-receptor tethering mechanism unlikely. Instead, it was suggested that Tea1p forms a polymeric network at cell tips, with insertion of extra Tea1p mediated by Mod5p and autocatalytically promoted by existing polymeric Tea1p (Figure 3b)[54]. Because Mod5p is assumed to associate and disassociate from the Tea1p polymeric network on a fast timescale, this model can explain the rapid half-tip FRAP dynamics of Mod5p as compared to Tea1p, with Tea1p diffusing only very slowly when polymerized.

Although many of the microscopic properties of the above model could not be directly tested, several indirect predictions were examined[54]. In order to form a network over a two-dimensional area, Tea1p must have a local connectivity of three or higher. Consistent with this reasoning, mutating a region of Tea1p that is predicted to form a trimeric coiled-coil caused mutant Tea1p to fail to accumulate at cell tips, despite being delivered accurately by microtubules and interacting with Mod5p.

Also important is the model's self-focusing property; the model predicts that the steady-state distribution of Tea1p at cell tips should be much narrower than the spatial distribution of Tea1p microtubule deposition sites, due to autocatalytic amplification. This indeed turned out to be the case. The amplification was predicted to build up the Tea1p concentration close to the extreme cell tips, until such peaks were balanced by an outward diffusive flux of polymeric Tea1p along the membrane and eventual Tea1p unbinding. This mechanism provides a simple way to correct for any potential non-polar Tea1p deposition.

In such cases the local Tea1p concentration will be too low to trigger autocatalytic growth; instead, Tea1p will be rapidly recycled back to the cytoplasm where it can be re-targeted to cell tips. Hence, the self-focusing mechanism allows Tea1p membrane binding to be much more tightly localized to cell tips. Because Tea4p precisely colocalizes with Tea1p[51], this guarantees a narrow spatial window for Pom1p membrane binding, and thus a much more precise downstream Pom1p gradient (Figure 3b). Tea1p is, however, often distributed at cell tips as multiple discrete dots rather than as a single polymeric structure. It is currently unclear how this observation might affect the self-focusing model.

How spreading can buffer noise

Once present on the membrane, a simple mechanism for gradient formation would involve diffusive motion of cortical Pom1p, with autophosphorylation of Pom1p eventually leading to unbinding[46]. FRAP experiments on half a cell tip provide evidence for transport from one half-tip to the other at the same end of a cell[46, 57]. Moreover, gradient formation appears to be substantially unaffected by cytoskeletal or endocytic disruption[57]. These results are entirely consistent with diffusion as the dominant transport mechanism of cortical Pom1p, which spreads out on the membrane beyond the region occupied by its upstream recruiters Tea1p/Tea4p[46, 57]. These and other FRAP results indicate that the mean Pom1p diffusion constant is around $0.1\ \mu m^2 s^{-1}$ with a membrane lifetime of around *30* s[57]. Using the considerations in Box 1, these parameters can clearly generate a functional intracellular gradient inside single fission yeast cells (which are typically around *9-14* μm long).

However, confocal imaging of a cortical slice through fission yeast cells revealed an unexpected level of complexity for the Pom1p membrane dynamics[57]. Intriguingly, in

time-lapse imagery Pom1p was found in clusters that appeared to stochastically grow and shrink on timescales of a few seconds, much more rapidly than the overall membrane dwell time of around *30 s*. Moreover, tracking of the clusters (as well as Fluorescent Correlation Spectroscopy measurements) yielded a cluster diffusion constant much smaller than that suggested by half-tip FRAP. These results motivated a two-state model for Pom1p dynamics, with Pom1p either existing as a more rapidly diffusing, potentially monomeric state, or as slow-diffusing massive clusters (see Figure 4a)[57]. The slow-diffusing clusters can absorb fast-diffusing Pom1p through collisions, but are at the same time unstable and liable to disintegration. Mathematical modelling of these processes (with nonlinear cluster aggregation) then made an unexpected prediction; namely, that the peak cortical Pom1p concentration and the decay length of the gradient should be anti-correlated. This conclusion followed from higher levels of Pom1p leading to more clusters and, therefore, overall slower diffusion, thereby decreasing the decay length, whereas lower levels of Pom1p led to fewer clusters and overall faster diffusion, increasing the decay length. The anti-correlation persisted even though the clustered Pom1p cannot unbind from the membrane, thereby effectively decreasing the Pom1p membrane unbinding rate. Assuming extrinsic variation in all model parameters, such an anti-correlation does not typically arise in simple linear one-state models, in contrast to the two state model. Measurements of *in vivo* concentrations and decay lengths uncovered a clear anti-correlation, conforming well to the two-state model prediction.

As schematically illustrated in Figure 4b, the above mechanism has noise-buffering properties in mitigating against the effects of variation in Pom1p tip concentration levels. Indeed, measurements showed that the anti-correlation could reduce extrinsic fluctuations

by up to *40%*[57]. How the intricate cortical Pom1p dynamics fit with its phosphorylation state is currently unclear, however, as cluster formation appeared to be independent of Pom1p kinase activity[57]. Moreover, the assumption of a two-state model is rather crude. Although it does contain the essential idea and provides verifiable predictions, the reality is likely to be more complex, with Pom1p clusters of many different sizes (rather than just two) and varying biophysical properties.

Quantifying and reducing intrinsic noise

Intracellular concentration gradients will clearly be subject to intrinsic noise arising from the inherent stochasticity of the biochemical processes that make the gradient. In particular, in order to measure the gradient concentration at a detector, gradient molecules must first arrive by diffusion. This arrival process is highly stochastic [58, 59] and therefore sets physical limits on the precision of positional information[15, 31] (Box 2). However, these fluctuations can be reduced by time-averaging the signal measured at such a detector. In this way, intrinsic (though not extrinsic) fluctuations can be reduced (Box 2). In the case of Bicoid, the effects of intrinsic noise have been carefully analyzed, and, theoretically, time (and spatial) averaging could reduce relative intrinsic noise in the gradient to the *10%* level within minutes (considerably less than the time period of a single nuclear cycle)[31]. For the Bicoid system, precision is limited by the rare, stochastic arrival of Bicoid molecules at its detector: the promoter of its downstream target, the *hunchback* gene.

For the case of Pom1p, the precision with which the gradient can be read is again determined by the noisy arrival of Pom1p molecules at downsteam 'detectors'. These detectors could include, for instance, molecules of the protein kinase Cdr2p, an activator of mitotic entry that is negatively regulated by Pom1p. Although the impact of these

fluctuations on intracellular gradient precision had been considered theoretically[15, 45], until recently there was no direct experimental measurement of the size of intrinsic noise, and the extent to which time-averaging could reduce it in an intracellular gradient system. Such measurements were carried out recently for Pom1p (which is present in copy numbers of around 5000 per cell) and showed that intrinsic noise could be significant, particularly away from the tips[57]. However, time-averaging over *30 s* reduced intrinsic noise in the Pom1p gradient to significantly lower levels (lower in all locations than the cell-to-cell variation). Interestingly, the potential downstream targets of Pom1p, particularly Cdr2p, have relatively long membrane dwell times (of about *90 s* or more), and could therefore integrate Pom1p levels over that time, thereby significantly reducing intrinsic noise[57].

In general, intrinsic fluctuations set physical limits to the precision of any signal transduction process[58], a consideration that is highly relevant to the precision of gradient-based systems. A quantitative analysis is thus vital to determine whether the signaling system in question can produce reliable outcomes within experimentally relevant timescales[15, 31, 45].

Lessons from a robust intracellular gradient

As we have seen, multiple mechanisms are employed to ensure that the Pom1p gradient can provide reliable positional information. A multidisciplinary approach involving both cell biology and mathematical modeling has enabled these mechanisms to be dissected in considerable detail, with a fairly complete systems-level understanding of the entire gradient-forming mechanism now available[18, 46, 48, 54, 57]. A number of lessons can be learned from such an approach. Perhaps most important is that the dynamics of biological gradients can be considerably more complex than often envisioned. For example, Pom1p

gradient dynamics involve not only localized binding, diffusion and then disassociation, but also intricate and novel self-focusing and clustering dynamics. Furthermore, these novel mechanisms could be widely relevant to developmental systems. For example, there is nothing in the dynamic clustering mechanism that is necessarily specific to the Pom1p system. Indeed, the output of the clustering mechanism -- an anti-correlation between the peak concentration of the gradient and its decay length -- has also been observed in the Bicoid system, although its origin there is currently unexplained[36, 60]. It will be interesting to see whether other intracellular gradients also employ noise-reduction mechanisms, or whether Pom1p is an outlier in this regard. Currently, it is probably fair to say that other intracellular gradients have not been investigated in sufficient depth to be able to answer this question. A particularly interesting example in which to study this issue might be the intracellular gradient of the cell fate determinant MEX-5 in the one-cell *C. elegans* embryo[20, 21]. This gradient also manifests complex behaviour, in that overall gradient formation depends on phosphorylation of MEX-5 altering its effective diffusion constant[14, 21]. Finally, the gradient-forming and noise-buffering mechanisms we have discussed in this review would be very interesting to reconstitute *in vitro*, as has already been achieved for the dynamics of the bacterial Min proteins that regulate cell division positioning in *E. coli[61-63]*. With *in vitro* reconstitution as a first step, control mechanisms used in the Pom1p system could eventually form a useful toolkit for precise positioning in synthetic biology contexts.

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Boxes

Box 1: Making a simple concentration gradient. Morphogen gradients in a developmental biology context often rely on local protein production, followed by diffusion and eventual degradation. The symmetry breaking necessary for gradient formation therefore relies on a localized source, which can be provided by previous localization of morphogen mRNA. Provided each morphogen protein is degraded independently at a constant rate, a simple mathematical analysis reveals a concentration profile that decays exponentially with distance from the (planar) source. The decay length of the gradient (the distance over which it decays to *1/e* of its highest value) has also been precisely measured in several cases (and is *≈100 μm* for Bicoid and ≈ 20 *μm* for Dpp). Modulating the decay process can produce qualitatively different gradient shapes: for example, if a dimerization reaction is required for decay (an example of self-enhanced degradation) then a power law decay results at large distances. In this case, at large distances from a planar source, the concentration decays with distance as $1/(\text{distance})^{\alpha}$, where α is equal to two for the dimerization reaction.

In an intracellular context, the question arises as to whether a cell is big enough to support an intracellular gradient, or whether internal concentrations are necessarily homogeneous. If we consider a molecule with a diffusion constant D and a lifetime τ, then it will typically move a distance on the order of the decay length $d \propto \sqrt{(D\tau)}$ before being degraded. For a diffusion constant on the order of $D \sim 1\ \mu m^2 s^{-1}$, and a protein lifetime of hours, the characteristic decay length d will be far larger than typical cellular dimensions. However, the distinguishing feature of a gradient protein could be a modification of the protein, for example, phosophorylation. In that case the effective lifetime of the protein with the appropriate modification could be far shorter. For example, the protein could be

dephosphorylated at the same time as being localized at a specific site within the cell (as is the case for Pom1p in fission yeast). The protein could then diffuse away while being continuously subject to attempted phosphorylation. If the latter process occurred on a timescale of approximately a second, then with $D \sim 1\ \mu m^2 s^{-1}$, a gradient with a typical decay length of around $d \sim 1\ \mu m$ would emerge, significantly smaller than typical eukaryotic cell sizes, and comparable with the size of bacteria. Hence, intracellular gradients are not only possible, but can be expected to be fairly ubiquitous. Once phosphorylated, the gradient protein itself could then be recycled for reuse in the gradient, a much more energy-efficient mechanism for gradient maintenance than rapid degradation combined with protein resynthesis.

Box 2: Intrinsic fluctuations in gradient formation. Intrinsic fluctuations inevitably reduce the precision of positional information provided by a gradient. When the gradient concentration is measured in a detector volume, the number of gradient molecules present will fluctuate. Indeed, at a particular instant there could be no gradient molecules at all within the target volume. The detector will then have to wait for the noisy arrival of gradient molecules by diffusion before reliable measurement becomes possible. This effect is largest at low concentrations, but can be large even for surprisingly high concentrations of gradient molecules if the measuring volume is small. Small effective measuring volumes are likely to be common for morphogens that act as transcription factors (even if the morphogen transcription factor attempts to increase the effective target size by diffusing in one dimension along the DNA[64]). Morphogens that bind to receptors covering an entire cell surface, on the other hand, will have much larger measuring volumes, meaning that intrinsic noise is likely to be small. In order to determine the quantitative importance of

intrinsic noise for precision, a theoretical analysis is essential. If the dominant source of fluctuations is diffusion, then the variance in the number of molecules in the detector volume will simply be equal to the mean. However, time averaging will reduce these fluctuations by an amount that depends on the length of the time-averaging period. Essentially, after time averaging for a period, τ, the noise will be reduced by an amount proportional to $\sqrt{(\tau/\tau_0)}$, with a characteristic timescale, τ_0, set by the magnitude of the gradient molecule diffusion constant and the physical size of the detector. Reduction by the square root of the time-averaging period follows for the same statistical reasoning that the error of the mean in any series of measurements decreases essentially according to the square root of the number of independent measurements.

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Figure legends

Figure 1: Extrinsic and intrinsic noise both affect precision of positional information provided by concentration gradients. (a) Extrinsic noise in morphogen production rate leads to varying profile from one gradient to another. Through variation in the position where the gradient concentration drops through a critical level (ρ_T), this leads to imprecision in specification of position x_T. **(b)** Similarly, intrinsic noise within a single gradient also leads to imprecise positional information.

Figure 2: Pom1p forms a noisy cortical gradient. (a) Confocal image of wild type fission yeast cells expressing pom1-tomato in medial focal plane (scale bar, *2 μm*). **(b)** Four separately normalized pom1p cortical intensity profiles from *0.5 s* exposures taken *15 s* apart in same cell. *d* is distance measured along the membrane from a cell tip. Image and data reproduced from [57].

Figure 3: Tea1p/Mod5p/microtubule module ensuring precise binding of Pom1p at cell tips. (a) Rod-shaped cell ensures most microtubule tips grow until close to cell ends, where Tea1p is deposited. Occasional contact between short microtubules and non-polar membranes will, however, inevitably occur which could, without additional measures, lead to inappropriate Tea1p localization. **(b)** Schematic, illustrating the autocatalytic self-focusing mechanism for Tea1p, which ensures a more precise Tea1p polar localization than specified

by its membrane input distribution. Solid blue line represents autocatalytic amplification of Tea1p mediated by Mod5p; dotted green line represents spreading of Tea1p by diffusion followed by membrane unbinding.

Figure 4: Pom1p exhibits dynamical clustering. (a) Schematic illustration of Pom1p clustering dynamics, showing average Pom1p cortical gradient close to a cell tip, as well as detailed Pom1p cluster aggregation/disintegration dynamics. Note that the fast-diffusing species need not necessarily be monomeric. All arrows represent fast diffusive motion, but only the green/red arrows correspond additionally to cluster aggregation/disintegration dynamics. **(b)** Diagram showing how an anti-correlation between peak Pom1p cortical concentration and decay length can reduce extrinsic fluctuations in Pom1p concentration levels.

Figure 1

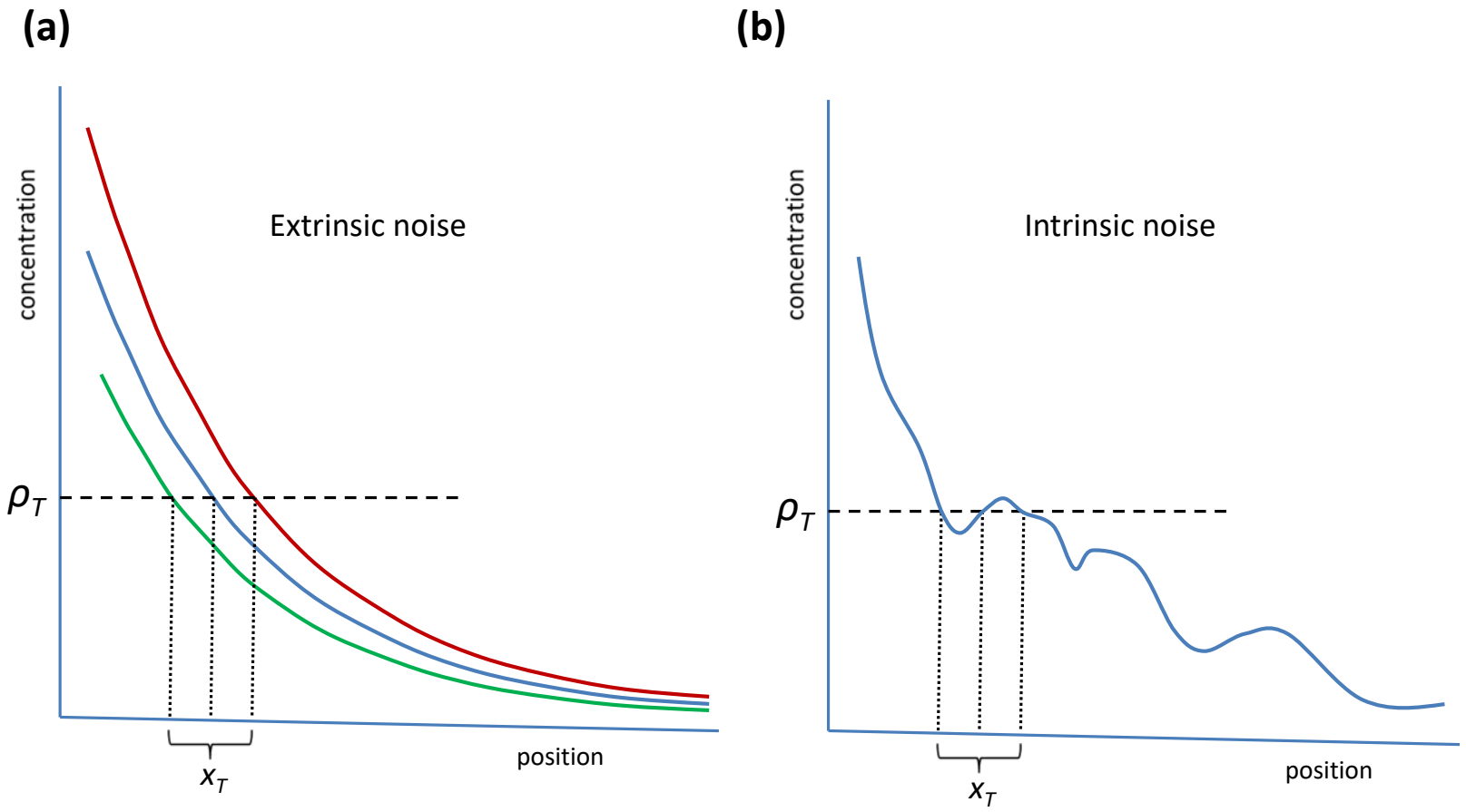
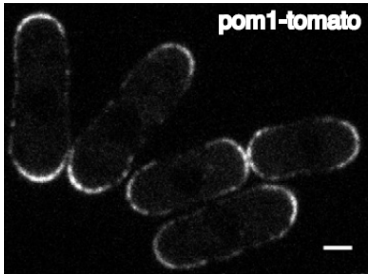

(a)
Extrinsic noise
concentration
ρ_T
x_T
position
(b)
Intrinsic noise
concentration
ρ_T
x_T
position

Figure 2

(a)


pom1-tomato

(b)

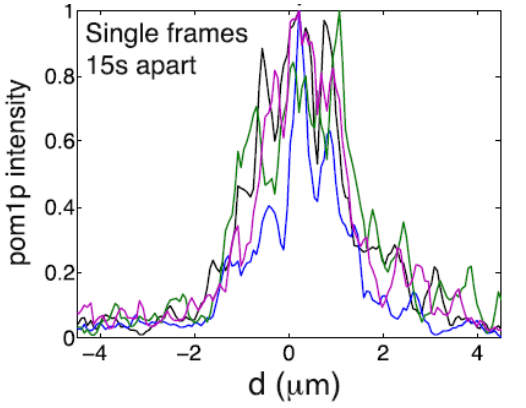

Single frames
15s apart
pom1p intensity
d (μm)
0
0.2
0.4
0.6
0.8
1
−4
−2
0
2
4

Figure 3

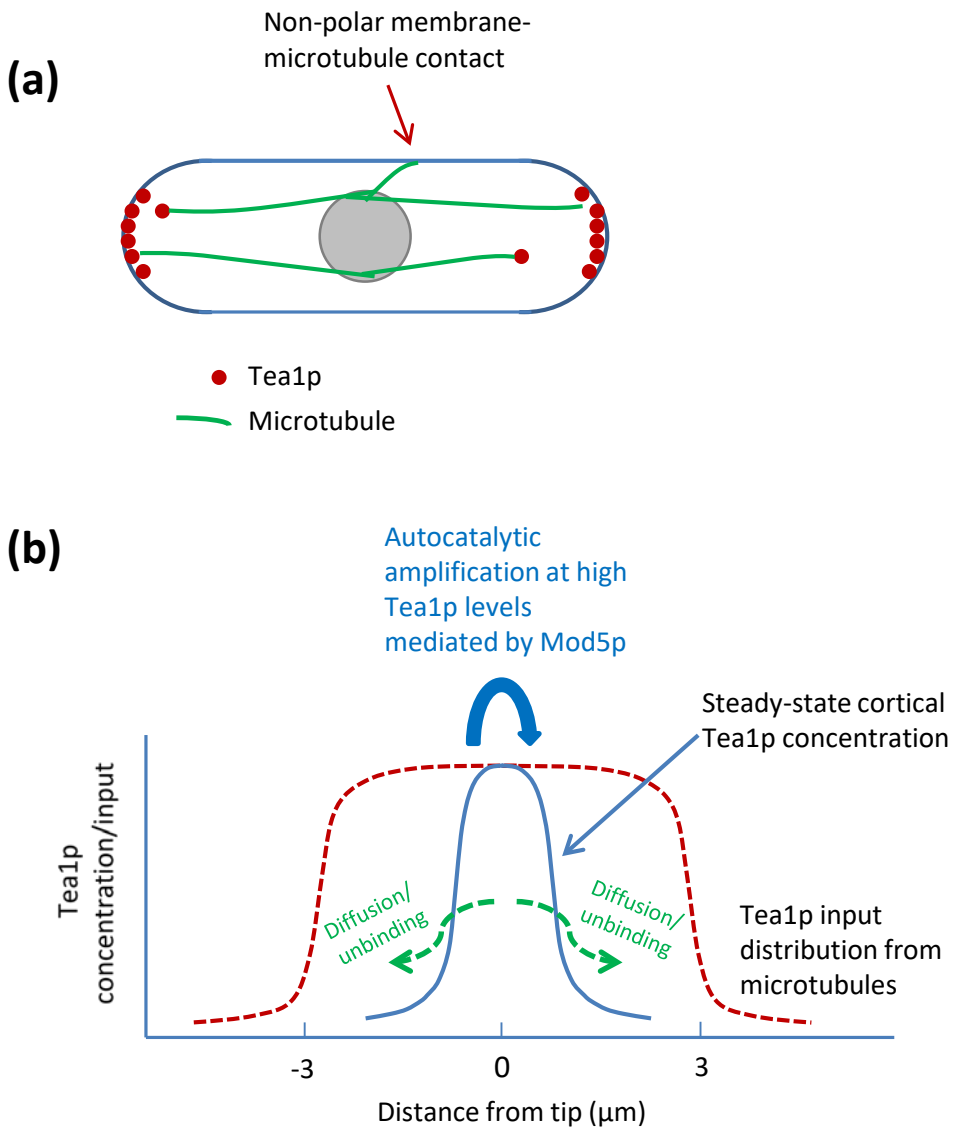
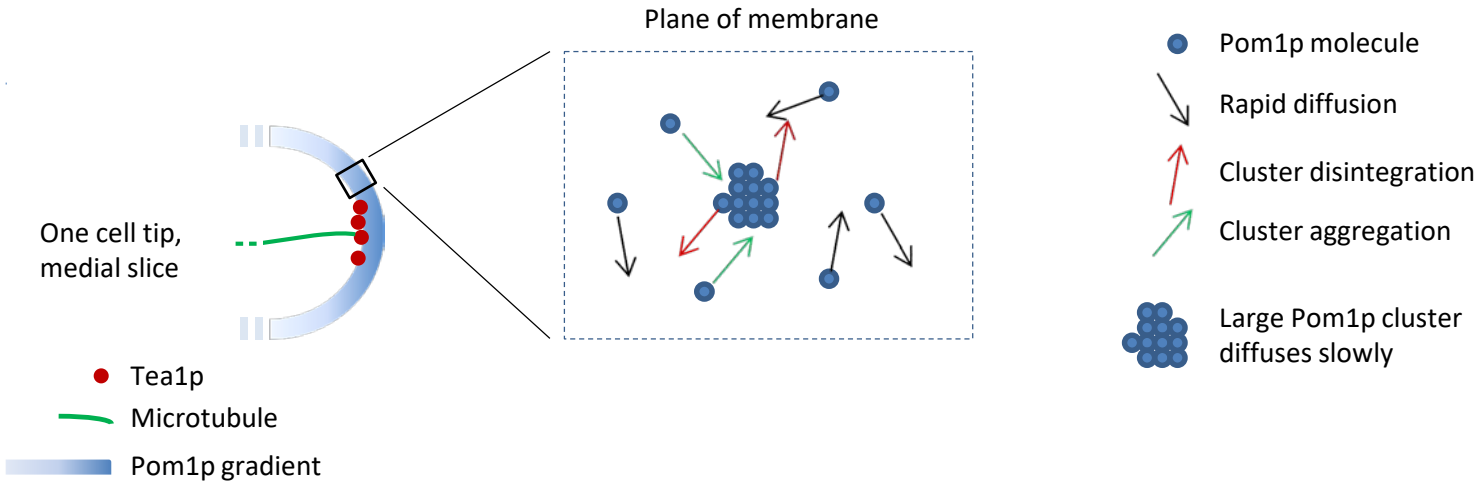
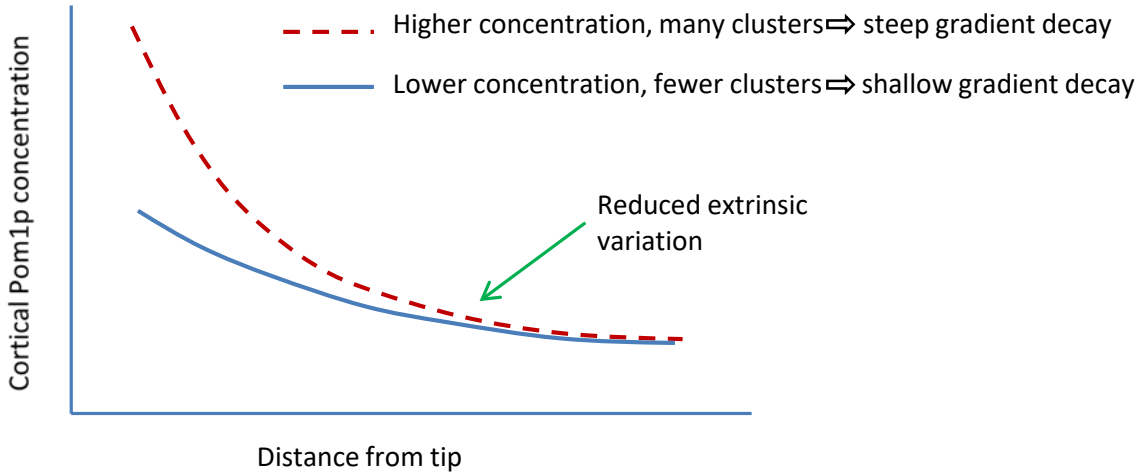

(a)
Non-polar membrane-microtubule contact
Tea1p
Microtubule
(b)
Autocatalytic amplification at high Tea1p levels mediated by Mod5p
Steady-state cortical Tea1p concentration
Tea1p concentration/input
Diffusion/ unbinding
Diffusion/ unbinding
Tea1p input distribution from microtubules
-3
0
3
Distance from tip (µm)

Figure 4

(a)


Plane of membrane
One cell tip,
medial slice
Tea1p
Microtubule
Pom1p gradient
Pom1p molecule
Rapid diffusion
Cluster disintegration
Cluster aggregation
Large Pom1p cluster
diffuses slowly

(b)


Higher concentration, many clusters⇨ steep gradient decay
Lower concentration, fewer clusters⇨ shallow gradient decay
Reduced extrinsic
variation
Cortical Pom1p concentration
Distance from tip