Adaptive molecular networks controlling chemotactic migration: dynamic inputs and selection of the network architecture

Hao Chang and Andre Levchenko

Department of Biomedical Engineering, Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD 21218, USA

Eukaryotic signalling networks underlying the cell’s ability to sense the gradient of chemotactic cues frequently have the dual property of perfect adaptation to spatially homogeneous inputs, and persistent activation by inputs that are spatially graded. This property is also shared by bacterial chemotaxis networks, raising the question of whether these two types of chemotactic processes also have similar organization of the underlying biomolecular processes. Interestingly, perfect adaptation can only be achieved robustly by a handful of mechanisms, and while eukaryotic chemotactic networks appear to rely on one of these—the incoherent feed-forward loop, bacterial chemotaxis depends on another—the negative feedback loop. In this review, we discuss how this conclusion can be reached even if the details of the molecular networks are incompletely understood. Furthermore, we argue that the use of distinct network architectures is not accidental and may be a consequence of the nature of the signalling inputs and the limitations of the sensory properties of different cell types.

1. Introduction

Cell migration mechanisms have evolved to enable cell navigation in chemically and mechanically complex microenvironments along with guidance by a range of signalling inputs reflecting spatially complex extra- and intracellular conditions. Responsiveness to diverse cues permits individual cells to integrate diverse pieces of information and convert this information into the directionality of cell polarity and migration. This decision-making process is frequently complex, presenting interesting challenges to the experimentalists and modelers alike. Indeed, it is frequently hard to unravel complex intracellular signalling networks distributed in space and differentially activated in time, and it can be even harder to understand how activation of these networks can control both qualitative and quantitative characteristics of something still poorly understood, i.e. how cells define where their front and rear parts should be localized. The experimental approaches require sophisticated and still largely immature tools allowing simultaneous imposition of multiple extracellular cues, precisely controlled both in space and in time. The modelling approaches need to rely on the appropriate level of description of biological complexity, so that the model and experiment can inform one another, a notion still not quite rooted in the modelling community. In this review, we will suggest how these important and pressing challenges can be addressed in the context of eukaryotic cell polarity and migration, highlighting insights that begin to emerge as a result of promising developments in this area.

2. Adaptation to spatially homogeneous stimuli: why and how?

Perfect adaptation to step-like increases in the chemoattractant concentration has been observed both in bacterial and eukaryotic chemotactic signalling networks [1–3]. It has been argued that the ability to perfectly adapt to persistent...
ligand stimulation can provide a signalling system with many important advantages, including but not limited to the ability to extend the range of chemoattractant concentrations over which the gradient sensing would be sufficiently precise [4–7]. This property can, in turn, enlarge the overall spatial extent of guided navigation, increasing the probability that a cell can successfully locate the source of the signal.

Perfect adaptation of a signalling response turns out to be quite restrictive in terms of the number of possible ways it can be achieved. A recent analysis suggested that the ‘architecture’ or topology, of the underlying signalling networks would be expected to fall into two classes: those containing a negative (integral) feedback (NFB) and those that contain two parallel initially diverging and ultimately converging pathways, affecting the output in opposite ways. The latter network type has been termed an ‘incoherent feed-forward’ loop (iFFL) [8]. It is of interest, and as suggested below, of possible importance, that the current biochemically informed models of bacterial chemotaxis postulate a type of negative feedback that carries information about a time integral of the input [6]. On the other hand, the prevailing model of an adaptive chemotactic signalling network, particularly in the archetypal examples of eukaryotic chemotactic motility of the amoebae cells of Dictyostelium discoideum and mammalian neutrophils, postulates an underlying iFFL [7,9–12]. Whereas the NFB model of bacterial chemotaxis has rarely been questioned, the iFFL model in eukaryotic cells has been under continuous scrutiny, in part because the molecular components constituting the feed-forward loop are still not established with certainty [13]. However, recent analysis has argued particularly convincingly that iFFL is indeed the main driving molecular network type underlying both the perfectly adaptive response to spatially homogeneous changes in chemotactic stimuli and persistent non-adaptive signalling in the presence of spatially graded distributions of the same stimuli [14,15]. The experiments used to provide this evidence relied on exposing cells to dynamic stimuli more complex than a simple step-like increase in the stimulant concentration, with the temporal complexity of the inputs used in particular not only to argue in favour of iFFL model, but also against NFB models.

3. Deciding between two mechanisms of perfect adaptation

What signalling inputs can help distinguish between two possible distinct network architectures, both of which can account for adaptive behaviour, particularly if the molecular components of these networks are not precisely established? A popular test of network connectivity widely used in engineering and, more recently, in biology is to expose cells to oscillatory inputs [16–22]. Measurement of the signalling outputs when the frequency of the input is varied can reveal inherent filtering capabilities of the molecular circuit, so that the response is maximized at high (‘high-pass filter’), low (‘low-pass filter’) or intermediate (‘band-pass filter’) input frequency. In the case of yeast osmoregulation, the signalling pathway mediation cell adaptation to high osmolarity has been shown to have band-pass filtering properties, the property that was used to infer the existence underlying a negative feedback network structure. Unfortunately, although band-pass filtering requires considerable network complexity, both negative feedback and iFFL circuits can be powerful band-pass filtering devices. Indeed, if simple NFB and iFFL models are matched in such a way that the adaptation to a step input yields identical timing and amplitude of the transient response (‘matched iFFL and NFB models’), their responses to various dynamic stimuli [23] can be meaningfully contrasted (figures 1 and 2). If such matched iFFL and NFB models are subjected to oscillatory stimuli, both show essentially indistinguishable dynamic response characteristics and qualitatively similar band-pass responses (figure 1). Thus, even though a recent analysis has demonstrated that the network underlying adaptation in D. discoideum chemotaxis has band-pass filtering characteristics [14], this fact could not be used to determine whether the adaptive response relied on iFFL or on NFB signalling circuits.

What then might be the input that discriminates between the underlying iFFL and NFB architectures? It turns out that ‘ramp’ inputs, i.e. inputs that persistently increase with constant rates, can be such discriminating dynamical test inputs. Indeed, as can be easily seen from stimulation of the matched iFFL and NFB networks (figure 2), and shown on more theoretical grounds, the NFB does not adapt perfectly to ramp stimulation, instead coming to a steady-state activity proportional to the slope of the ramp [14]. In this sense, its output is proportional to the time derivative of the input, with the NFB serving as the ‘differentiator’. On the other hand, the iFFL can perfectly adapt to the ramp stimuli, with the transient response but not the steady-state behaviour affected by the slope of the ramp. This test, when applied to the D. discoideum chemotactic signalling network, strongly suggested that it indeed has the embedded iFFL but not NFB structure. However, when applied to the high osmolarity response pathway in yeast or in bacterial chemotaxis, the lack of adaptation to ramp stimulation strongly supported NFB architecture [24].

4. Can there be different selective pressure for evolution of different adaptation mechanisms?

Mounting evidence suggesting that adaptive molecular networks responsible for chemotactic responses can rely on both iFFL and NFB architectures begs the question of whether this evolutionary choice is biased by the demands of the chemotactic response itself for different organisms or whether it is more random. Where can the selective pressure stem from? As shown above, the key difference in dynamic responses between these network types can lie in their response to the dynamically complex stimuli, in particular, ramp inputs. Can this difference also suggest why these networks can present unique advantages in the real-world chemotactic behaviour? This is an important consideration, in part because laboratory analysis is frequently designed to present cells with simplified inputs versus those faced by the cells in more natural situations. Fortunately, for both bacterial chemotaxis and the chemotaxis of the amoebae D. discoideum, much is known about their natural environmental inputs, thus permitting the analysis of what the relevant input distribution is and what the cells might in fact be responding to.
The key difference between bacterial chemotaxis and chemotactic responses by eukaryotic cells, including *D. discoideum*, is that bacterial cells, given their small size, have to actively explore the chemotactic fields by actively moving within them, whereas the eukaryotic cells are large enough to reliably measure the difference between the concentrations of the chemoattractant across the cell length. Therefore, bacterial cells effectively measure the spatial gradients of chemoattractant by exploring them in time, sampling concentrations as they actively navigate through them during their own locomotion. Thus, the measurement that the cells rely on is the measurement of the rate of change of the chemotactic input, corresponding to the ramp stimulation discussed above. As the rate of the change increases, the cells would tend to maximize their response, recognizing that they move in the direction leading them more precisely towards the source of the chemoattractant. As suggested above, NFB circuits are perfectly suited for this type of computation, with the steady-state response proportional to the slope of the ramp stimulus (a complication here can arise from the properties of the receptors involved in bacterial chemotaxis, so that in fact exponential rather than linear ligand ramps need to be delivered to ensure linear ramp input into the NFB signalling network downstream of the receptor [25–27]).

The situation in the natural *D. discoideum* responses is more complex. During the developmental response, individual cells assemble into a multicellular organism by virtue of both secreting and responding to a chemoattractant, presenting in the form of self-sustained waves propagating through the cell community. Therefore, individual cells are exposed to a complex input presenting them with crests and troughs of chemoattractant concentration, and gradients that can change their sign (direction) as the crest (trough) of the wave passes over a cell. Although a cell can measure the gradient without moving or while moving relatively slowly, the conflicting cues have the potential to force its movement both towards and against the direction towards the source of the chemoattractant. Therefore, it can be hard to make progress, yet individual cells appear to solve this problem well. This fact strongly suggests that the cells can be sensitive to the spatial gradient on only one side (trench to crest) but not the other (crest to trench) of each incoming wave. Furthermore, the cells can maximize the response if the fact that the average concentration changes during passage of each wave does not interfere with their spatial gradient measurement. Hence, adaptation to temporally changing input can be a key in this response, necessitating the iFFL but not the NFB architecture.

These considerations, although still largely speculative, strongly suggest that the complexity of the environmental inputs faced by individual chemotactic organisms as well as the internal limitations inherent in these organisms (e.g. the size of a cell undergoing chemotaxis) can impose important constraints in selection of one of the available adaptive network architectures. This observation links to a more general question often ignored in the analysis of signalling networks: which aspect of the input do cells really ‘care about’? More precisely put, which aspect of the input...
contains the most information relevant to the cell decision-making? [28,29] The information about spatial gradients can be contained both in space and in time, and individual networks may evolve to respond to one of this information sources while ignoring the other.

Another important aspect of functioning of different molecular networks is their noisy nature. Indeed, chemical reactions can be subject to considerable fluctuation in concentration and thus rates of various reactions. Again it may be instructive to determine whether the ability of iFFL and NFB circuits to withstand such fluctuations may be similar. Simulation results for the matched models (figure 3) suggest that the NFB can robustly ensure constant duration of the output to the step increase in the chemoattractant input (the duration of the transient peak) but not the amplitude of the output (the height of the peak). On the other hand, the response of the iFFL model is much more variable in terms of the peak duration, since, in this response, the amplitude is linked more tightly to the duration of the peak. This result may suggest lower robustness of the iFFL circuit to the ‘noise’ or small variations in activity of the signalling pathways. However, the tight coordination between the amplitude of the peak and its duration also implies that correcting for variability in either amplitude or duration can also decrease the overall variability of the response (figure 3), making iFFL more robust to noise than NFB. This property of the iFFL circuit can be connected to the so-called ‘fold-response’ capacity, i.e. the ability of the sensory system to robustly measure fold-changes in response, while being less sensitive to either average value of the input or the less substantial input changes. This property may in turn relate to inherent ability of iFFL to adapt to linear ramp inputs, as discussed earlier, suggesting that nonlinear inputs characterizing fold responses can be more effective in eliciting sustained responses. The consequences of this property for gradient sensing capabilities of D. discoideum and other chemotactic cells and organisms remain to be explored.

5. Analysis of other signalling networks with incompletely known chemical composition and organization

Understanding the structure of signalling networks is paramount for investigation into their dynamical behaviour and function. However, frequently, the molecular components of these networks and their inter-molecular interactions may not be completely known or easily assayed using classical tools of genetics and molecular biology. As illustrated earlier, the ability to expose live cells to tightly controlled dynamically complex stimuli can help distinguish between alternative models that can account for observed signalling responses. This analysis involves a combination of mathematical analysis and matched quantitative experimentation, which together can help unravel the functional characteristics of the signalling apparatus. Importantly, this analysis can also be suggestive of the nature of the environmental inputs processed by the networks. The fact that the natural, in vivo signalling fields are known for at least some chemotactic
responses, along with the knowledge of limitations on the sensory capabilities of chemotactic cells, has been used above to justify the use of alternative sensing strategies. However, for many other signalling networks, the dynamics and spatial distribution of signalling inputs are frequently unknown. Controlled presentation of large sets of distinct, dynamically complex stimuli can allow one to determine which aspects of the input can be sensed best and which stimuli can trigger qualitatively distinct outputs. This analysis should inform both the hypotheses about the wiring and composition of signalling networks, and the analysis of how evolutionary selection of a particular network type might have taken place. Signalling networks underlying cell polarization and chemotactic migration can thus serve as important and influential archetypes for the new era of quantitative analysis of noisy and dynamical cellular information processing.

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References


Figure 3. Comparison of iFFL and NFB model outputs under noisy parameter sets. Variation of parameter values by 10% introduces variability of responses to both step (a,c) and ramp (b,d) inputs for the matched iFFL (red; a,c) and NFB (blue; b,d) models. Normalization to the value of the amplitude of the peak responses suggests the possibility for a substantial reduction of variability in all responses, but in particular in the response of the iFFL model to the step input.

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