

# Cellular self-organization: generating order from the abyss

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**ABSTRACT** While the organization of inanimate systems such as gases or liquids is predominantly thermodynamically driven—a mixture of two gases will tend to mix until they reach equilibrium—biological systems frequently exhibit organization that is far from a well-mixed equilibrium. The anisotropies displayed by cells are evident in some of the dynamic processes that constitute life including cell development, movement, and division. These anisotropies operate at different length-scales, from the meso- to the nanoscale, and are proposed to reflect self-organization, a characteristic of living systems that is becoming accessible to reconstruction from purified components, and thus a more thorough understanding. Here, some examples of self-organization underlying cellular anisotropies at the cellular level are reviewed, with an emphasis on Rho-family GTPases operating at the plasma membrane. Given the technical challenges of studying these dynamic proteins, some of the successful approaches that are being employed to study their self-organization will also be considered.

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## INTRODUCTION

In a Perspective article on what important problems lay ahead in biological research in the 21st century, self-organization was proposed as a relatively underdeveloped, yet important area of biological research (Kirschner *et al.*, 2000). A more recent review by Karsenti also highlighted the importance of self-organization in cell biology (Karsenti, 2008). These reviews provide an excellent historical and conceptual background on key experiments in the physical and biological sciences that illustrate the importance of self-organization, distinguishing it from other types of physical organization such as self-assembly (see Table 1 for similarities, differences, and examples of each). However, preceding these thoughtful commentaries by some 50 years, Schrödinger had grappled with the problem of whether the laws of physics and chemistry could adequately explain the organization observed in living matter, especially the “organism’s astonishing gift of concentrating a ‘stream of order’ on itself, thus

escaping the decay into atomic chaos—of ‘drinking orderliness’ from a suitable environment” (Schrödinger, 1944, p. 77). Schrödinger’s elixir that bestows orderliness is energy, since it is the dissipation of energy that fuels the cell’s ability to organize its constituents, thus propagating life and, at least fleetingly, holding entropy and the second law of thermodynamics at bay (Prigogine and Stengers, 1984).

How does energy consumption generate order and contribute to self-organization? Energy consumption enables cells to actively organize their constituents away from thermodynamic equilibrium, underpinning remarkable phenomena associated with life: cell growth, movement, and multicellular development (Figure 1). Consider the properties that actin confers on these processes, for example. The polymerization of monomeric actin into filamentous actin (F-actin), and the assembly of these filaments into higher-order structures via actin-binding proteins, contribute to a cytoskeletal network capable of concentrating or dispersing cellular constituents on demand (Pollard and Cooper, 2009). At a molecular scale, some of these dynamic properties emerge from the differential rate of ATP-actin incorporation into opposite ends of the polymerizing filament (Drenckhahn and Pollard, 1986; Pollard, 1986) and from the ATP-dependent motility of filament-associated motors along the filament (Kuznetsov *et al.*, 1992). This type of system is capable of transporting vesicles in a directed manner, resulting in the concentration of lipids and protein at specific sites on the plasma membrane at higher concentrations than would be expected from random, diffusion-based movement (Schott *et al.*, 2002; Schuh, 2011). By

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Abbreviations used: Cdk1, cyclin-dependent kinase; F-actin, filamentous actin; GEF, guanine nucleotide exchange factor; GTP, guanine triphosphate; n-WASP, neuronal Wiskott-Aldrich syndrome protein; PI3K, phosphoinositide 3-kinases; PIP<sub>2</sub>, phosphatidylinositol 4,5-bisphosphate; PIP<sub>3</sub>, phosphatidylinositol (3,4,5)-trisphosphate; ROP, Rho-like GTPase; TM, transmembrane.

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	Involves multiple, interacting components?	Displays increase in order in space/time?	Requires energy input for maintenance?	Distance from thermodynamic equilibrium	Example of structure or system
Self-assembly	Yes	Yes	No	Near	Liposome, ribosome, proteasome, bacteriophage
Self-organization	Yes	Yes	Yes	Far	Mitotic spindle, actin cytoskeleton, cell cycle

Note how the structures ensuing from self-organized systems tend to display higher rates of flux, or turnover of components, than structures derived via self-assembly.

**TABLE 1:** Characteristic properties and examples of structures generated by self-assembly versus self-organization.

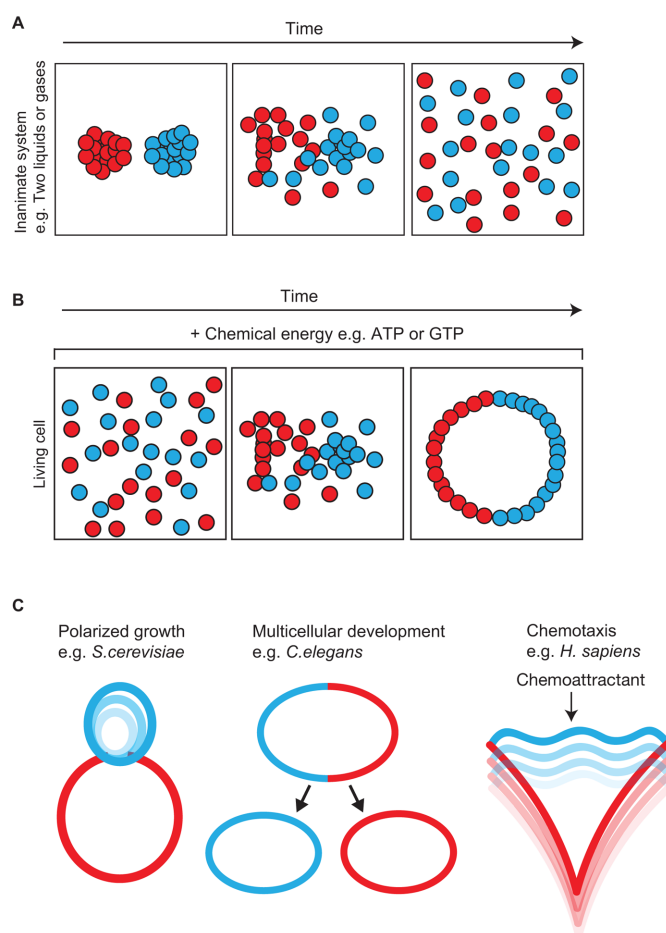
concentrating reactants locally on the plasma membrane, active transport increases the rates of reaction kinetics and concomitant signaling. This example illustrates how energy dissipation contributes to cellular organization, but in what sense does this reflect self-organization? To appreciate this, we might first consider how actin dynamics are integrated within signal transduction pathways that control the actin cytoskeleton temporally and spatially. We will then see how the actin cytoskeleton, in turn, influences signaling proteins via feedback mechanisms. The ordered structures that emerge constitute a self-organized system endowed with self-regulatory properties.

### THE MESOSCALE: RHO GTPase REGULATION, DYNAMICS AND RELATIONSHIP WITH ACTIN

Actin organization is intimately linked to Rho guanine triphosphate (GTP)ase function in eukaryotic cells. Rho-family GTPases use GTP as an energy currency to drive conformational changes in GTPase structure that toggle them between active and inactive states. This is a feature shared among the wider Ras superfamily of GTPases to which Rho GTPases belong (Bourne *et al.*, 1990, 1991). When bound to GTP and active, Rho GTPases interact with effector proteins, including actin nucleation-promoting factors such as formins and neuronal Wiskott–Aldrich syndrome protein (n-WASP) (Kohno *et al.*, 1996; Evangelista *et al.*, 1997; Rohatgi *et al.*, 1999) and membrane trafficking regulators such as the exocyst tethering complex (Guo *et al.*, 2001; Inoue *et al.*, 2003). At steady state, the localization of active Rho GTPases on membranes is frequently anisotropic, reflecting their function as critical regulators of cellular polarity. This anisotropic localization and activation is essential, enabling Rho GTPases to exert control over the actin cytoskeleton and cellular polarity more generally. Thus, Rho GTPases have been likened to the cell's compass, enabling the organization and definition of the cell's front and back (Bourne and Weiner, 2002; Yang *et al.*, 2016).

While many Rho GTPases appear organized anisotropically at steady state—they localize to the front of migrating cells, the tips of growing yeast, and the equatorial plane of dividing cells—they nevertheless display striking dynamics. For example, during wound healing in *Xenopus laevis* (frog) oocytes, concentric rings of active Rho and Cdc42 constrict independently of myosin contractility (Burkel *et al.*, 2012). In plant pollen tubes, the Rho-like GTPase (ROP)1 displays pulsatile oscillations at the cell tip during polarized growth (Hwang *et al.*, 2005). Similarly, in the rod-shaped fission yeast *Schizosaccharomyces pombe*, which grows at the cell tips, the Rho GTPase Cdc42 and its activators oscillate in an anticorrelated manner at the growing cell ends (Das *et al.*, 2012). Oscillations involve rapid signal amplification and attenuation and can display ultrasensitive, nonlinear kinetics that are driven by positive and negative feedback (Ferrell and Ha, 2014a,b,c). Thus, the enrichment of Cdc42 at one cell tip in fission yeast is thought to require autocatalytic Cdc42-GTP production by an activating guanine nucleotide exchange fac-

tor (GEF) and an associated scaffold protein, while subsequent depletion at this tip, which precedes accumulation at the opposite tip, requires delayed negative feedback generated by a p21-activated kinase, which phosphorylates and inhibits the GEF (Das *et al.*, 2015a). Oscillations in the Cdc42 GTPase module have also been observed in the budding yeast *Saccharomyces cerevisiae* (Howell *et al.*, 2012). These oscillations may also be driven by scaffold-mediated GTPase module regulation (Kozubowski *et al.*, 2008; Smith *et al.*, 2013; Rapali *et al.*, 2017). Thus, a simple GTPase module that



**FIGURE 1:** The dissipation of energy is used to drive dynamic cellular processes via self-organization. (A) When added together, two different colored liquids mix until they reach thermodynamics equilibrium. (B) Living cells are energy-dissipating systems that harness chemical energy to drive their constituents far from thermodynamic equilibrium. (C) Examples of nonequilibrium processes in biology include polarized growth, asymmetric cell divisions associated with stem cells during development, and chemotaxis, a stimulus-driven form of cellular migration.

comprises a GTPase, an activator, and an inhibitor can generate a complex oscillatory switch that contributes to the pattern of cell growth and cell shape in an apparently self-organized manner.

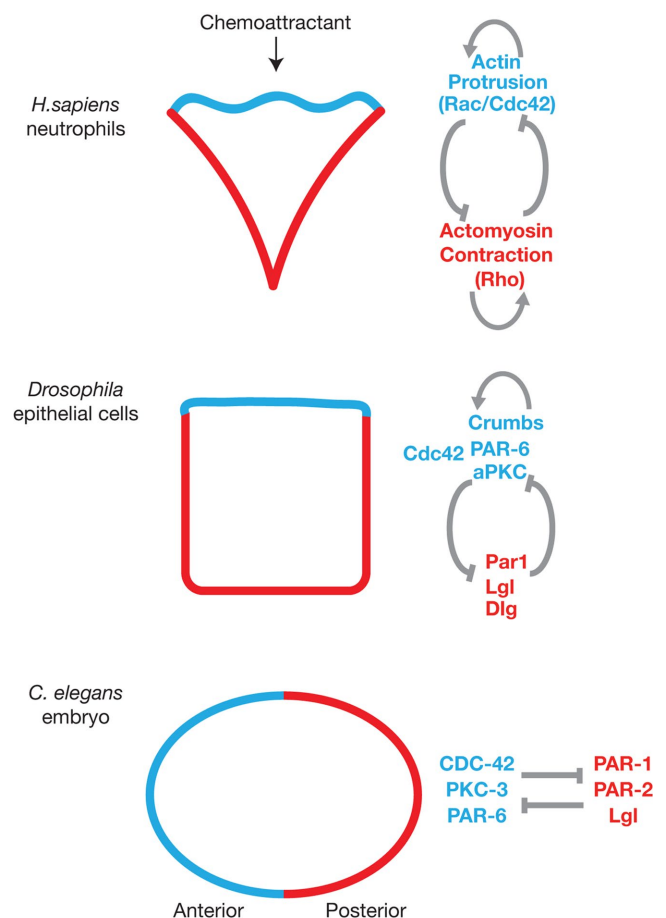
The use of positive feedback coupled to delayed negative feedback appears to be a systems-level property employed by other Rho GTPases. This has resulted in distinct, but equally striking, Rho dynamics in diverse species. For example, during furrow ingression in *Xenopus* and *Patiria miniata* (starfish) eggs and embryos, Rho-GTP and F-actin have been observed to move along the cortex as traveling waves where F-actin follows active Rho (Bement *et al.*, 2015). Similar to the oscillations in fission yeast, Rho-GTP is thought to be produced in an autocatalytic manner at the leading front of the wave by positive feedback. High spatial and temporal resolution imaging revealed that the product of Rho-GTP, F-actin, follows in the wake of the GTPase, inhibiting Rho-GTP production. While the mechanism by which F-actin inhibits Rho-GTP is not currently known in frogs, F-actin could inhibit Rho by recruiting Rho GTPase activating proteins, as has been demonstrated during pulsatile contractions of the *Caenorhabditis elegans* embryo (Michaux *et al.*, 2018). Collectively, these dynamics of the GTPase module fit well with the definition of an excitable system; that is, a system in which a threshold signal is exceeded, inducing an accumulation of the active signaling protein that subsequently produces its own inhibitor (Allard and Mogilner, 2013). The Rho-actin waves in frogs and starfish are coupled to the cell cycle machinery via cyclin-dependent kinase 1 (Cdk1) activity, facilitating the coordination of GTPase activation with cell cycle progression so that furrow ingression is initiated at the correct time during the cell cycle (Bement *et al.*, 2015). Interestingly, the activity of Cdk1 has itself been demonstrated to spread via a wave in *Xenopus* eggs (Chang and Ferrell, 2013). Traveling waves of Cdk1 are biologically important because frog eggs are very large cells (approximately 600  $\mu\text{m}$  in radius) in which the propagation of biochemical signals via waves is much faster than propagation via passive diffusion through the cytoplasm. Theoretically, an average-sized protein would take hours to diffuse across a frog egg, whereas a wave takes tens of seconds to traverse the same distance. As a consequence, the organization of a biochemical signal within a traveling wave enables large cells and embryos to rapidly and decisively respond to changes in Cdk1 activity. Consistently, Cdk1 activity is also organized as waves in large *Drosophila* embryos during syncytial nuclear divisions (Deneke *et al.*, 2016; Deneke and Di Talia, 2018). Examples of self-organization are widespread in multicellular development and morphogenesis (Schweisguth and Corson, 2019; Shahbazi *et al.*, 2019).

While self-organizing oscillations and traveling waves have only been discussed here in the context of polarized growth and furrow ingression during cytokinesis, similar dynamic phenomena have also been documented in diverse biological contexts (Inagaki and Katsuno, 2017), including neuronal outgrowth (Ruthel and Banker, 1998; Winans *et al.*, 2016), neutrophil chemotaxis (Weiner *et al.*, 2007), *Dictyostelium* locomotion, and keratinocyte migration (Vicker, 2000; Barnhart *et al.*, 2011). Many of these cells migrate. A common theme in cell migration, and cellular polarity more generally, is the generation of a cell "front" and "back." In the example of migration, actin polymerization is harnessed at the front to power protrusion and myosin contractility is concentrated at the cell back. These two activities are under the control of Rac and Rho GTPases, respectively, and importantly, while each GTPase is thought to be enriched to the front or back via positive feedback, the two GTPases also display mutual antagonism (Xu *et al.*, 2003). Positive feedback within GTPase modules and antagonism between modules at the front and back of cells appears to be a mechanism that establishes

distinct functional domains. For example, the Par6-aPKC module and the Par1 module display mutual antagonism in worm embryos, fly oocytes, and mammalian epithelia that underpins cell development and tissue organization (Figure 2) (St Johnston and Ahringer, 2010). Mechanistically, positive feedback combined with mutual antagonism are proposed to make polarity pathways more robust, and when incorporated into synthetic regulatory signaling networks, these systems-level properties have been observed to support the self-organization of stable lipid domains in the plasma membrane of cells (Chau *et al.*, 2012).

## THE NANOSCALE: MEMBRANES, LIPIDS, AND GTPases

The function of Rho GTPases and the wider Ras superfamily requires their association with membrane (Buss *et al.*, 1989; Hancock *et al.*, 1990). In the case of Rho GTPases, they frequently interact with the plasma membrane via isoprenylation and carboxyl-methylation of a C-terminal cysteine residue (Ghomashchi *et al.*, 1995). This, together with juxtaposed polybasic residues, facilitates the high affinity interaction of these GTPase with membrane lipids



**FIGURE 2:** Examples of signaling network configurations associated with self-organizing systems. The signaling networks shown on the right are required for the formation of the discrete domains illustrated in red and blue on the left. Feedback is thought to play a role in these examples of self-organization, although additional mechanisms are also likely to be important. Positive and negative feedback are illustrated as gray arrows and lines, respectively. References for the examples shown: neutrophil chemotaxis (Wong *et al.*, 2006); *Drosophila* epithelial cells (Fletcher *et al.*, 2012; Tian and Deng, 2008); *C. elegans* embryo (Motegi *et al.*, 2011; Ramanujam *et al.*, 2018).

(Finegold *et al.*, 1991). Lipids provide an essential physicochemical environment in which GTPases of the Rho and the wider Ras superfamily reside. Lipids regulate GTPase activity, mobility in the plasma membrane, and the recruitment of downstream effectors. The diffusion of peripheral membrane proteins such as GTPases is reduced by around two orders of magnitude in membrane compared with their diffusion in the cytosol, potentially increasing the probability of encounters with protein partners via local concentration effects, although such effects must be investigated case by case (Groves and Kuriyan, 2010). Therefore, the mechanisms that confine GTPase diffusion in membranes, for example by promoting their clustering, contribute to signaling. Before focusing on the contribution of specific lipids to GTPase self-organization via these mechanisms, it is worth considering some aspects of membrane organization in cells that may be indicative of self-organization more generally.

Along the secretory pathway, the anionic nature of lipid polar head groups tends to increase, as does acyl chain saturation, sterol content, and membrane thickness, being most pronounced at the plasma membrane (Bigay and Antonny, 2012). Transmembrane (TM) proteins tend to self-organize along the secretory pathway to minimize energy cost, such that TM length matches membrane thickness. Consequently, the TM domain of plasma membrane proteins tends to be longer (around 20 amino acids) than Golgi-resident TM proteins (around 15 amino acids) (Bretscher and Munro, 1993; Munro, 1995; Sharpe *et al.*, 2010). This physical means of self-organization makes the plasma membrane thicker and thus a more protective barrier to the outside world.

The chemistry of the plasma membrane also contributes to self-organization (McLaughlin and Murray, 2005). The negative charge of anionic lipids serves as a beacon for the recruitment of peripheral membrane proteins containing clusters of basic residues (Honigsmann *et al.*, 2013). Electrostatic interactions between anionic lipids and basic residues in low-complexity regions of proteins can locally concentrate anionic lipids such as phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) (Denisov *et al.*, 1998), forming clusters in the plasma membrane to which additional proteins containing basic clusters are recruited (Picas *et al.*, 2014, 2016). Electrostatic interactions between specific protein domains such as bim, amphiphysin, and rvs (BAR) domains and PIP<sub>2</sub> can also initiate stable domain formation (Zhao *et al.*, 2013). The result of this lipid-protein feedback may be the demixing of anionic lipids from the membrane milieu and the emergence of organized membrane domains containing clustered proteins (van den Bogaart *et al.*, 2011; Recouvreur and Lenne, 2016).

The dynamic organization of signaling proteins is now amenable to investigation at single molecule resolution in living cells, facilitating insight into self-organization at the nanoscale (Garcia-Parajo *et al.*, 2014; Goyette and Gaus, 2017). For example, canonical Ras superfamily members H-, K-, and N-Ras are not organized randomly, but are concentrated in discrete nanoscale ensembles termed nanoclusters in the plasma membrane (Murakoshi *et al.*, 2004; Plowman *et al.*, 2005). Nanoclusters are signaling hotspots from which Ras signals emanate. Different Ras isoforms contain distinct polybasic sequences in their C-termini. These sequences, in combination with different Ras prenylation signatures, are thought to target the GTPases to specific nanoclusters (Zhou *et al.*, 2017). Recent work indicates that the propensity of Ras GTPases to undergo nanoclustering extends to the Rho family, since Rac has been observed in nanoclusters in the plasma membrane of human cells and Rho-related proteins are observed in nanoclusters in plants (Das *et al.*, 2015b; Remorino *et al.*, 2017; Platre *et al.*, 2019). Moreover, in budding yeast, the Rho GTPase Cdc42 has also been reported to be

organized in nanoclusters, whose size correlates with GTPase activation (Sartorel *et al.*, 2018; Meca *et al.*, 2019). Both K-Ras and Cdc42 nanoclustering are controlled by specific anionic lipids including phosphatidylserine, and by regulatory components of their respective GTPase modules (Cho *et al.*, 2012; Blazevids *et al.*, 2016; Zhou *et al.*, 2017). These factors influence nanoclustering by controlling the lateral diffusion of GTPases in the plasma membrane and via mechanisms that await identification. Thus, nanoclustering is emerging as a critical property shared among Ras superfamily members in evolutionary distant species. The conservation of these features implies their importance. However, it is presently unclear how mechanisms operating on these nanoscale ensembles are propagated at the mesoscale to generate the complex, ensemble behavior exhibited by signaling systems *in vivo*, including the oscillations, traveling waves, and decisive, switch-like properties discussed previously (Bement *et al.*, 2015; Deneke and Di Talia, 2018; Fukushima *et al.*, 2019). Nevertheless, theoretical work has highlighted that positive feedback within signaling networks has the potential to promote protein clustering on membranes and the rapid dissemination of active species across membranes (Das *et al.*, 2009).

The reconstitution of dynamic protein assemblies in model membranes is proving to be a promising avenue to explore how self-organization may arise in the plasma membrane. For example, in addition to the effect of BAR domains on model membranes mentioned previously, actin polymerization has also been found to trigger the demixing of homogeneous lipid mixtures into domains enriched in PIP<sub>2</sub> (Liu and Fletcher, 2006). The addition of myosin to actin networks tethered to supported lipid bilayers has also enabled the reconstitution of a self-organizing actomyosin-membrane network (Koster *et al.*, 2016). Importantly, the system depended on a source of chemical energy, displayed actin organized in bundles or asters depending on the ratio of components added, and was capable of clustering an actin-associated protein. Remarkably, when an energy regenerating system was incorporated into this reconstituted network, the system displayed constant remodeling and confinement due to interactions between the system's components (Koster *et al.*, 2016). The formation of actin asters observed in the reconstituted system is also associated with the self-organization of the actin cortex observed in living cells (Gowrishankar *et al.*, 2012; Fritzsche *et al.*, 2017). Moreover, in the plasma membrane of human cells, a sizeable fraction of phosphatidylserine appears to be correlated in an actin-dependent manner, suggesting that the reconstituted systems recapitulate salient properties of plasma membrane self-organization (Kay *et al.*, 2012).

A final example of an anionic lipid involved in cellular self-organization is the product of phosphoinositide 3-kinase (PI3K), phosphatidylinositol (3,4,5)-trisphosphate (PIP<sub>3</sub>). PIP<sub>3</sub> has been identified as an important constituent of Ras and Rho-family GTPase self-organization in *Dictyostelium* migration (Sasaki *et al.*, 2004, 2007), mammalian neuronal polarity (Fivaz *et al.*, 2008), and neutrophil chemotaxis (Xu *et al.*, 2003; Wong *et al.*, 2006). In these systems, the GTPases activate PI3K at the leading edge of the cell, generating PIP<sub>3</sub> that is proposed to activate additional GTPase via positive feedback. Mechanistically, it seems likely that PIP<sub>3</sub> recruits cognate GEFs of the GTPases; however, this is not presently well understood. Additionally, the enrichment of PIP<sub>3</sub> levels at the cell front in *Dictyostelium* antagonizes the membrane recruitment of its own inhibitor, the PIP<sub>3</sub> phosphatase PTEN, providing another example of inhibition being used as a means of self-organization (Matsuoka and Ueda, 2018). In these cells, PIP<sub>3</sub> also forms waves on the plasma membrane that colocalize with active Ras, again underscoring the close relationship between GTPase activation and lipid dynamics

during self-organization (Gerisch *et al.*, 2011; Shibata *et al.*, 2012; Fukushima *et al.*, 2019).

## CONCLUSION

Self-organization appears to be an intrinsic property of at least some, and perhaps all, GTPase-driven pathways. Energy dissipation in these systems enables the emergence of a highly dynamic steady state in which multiple system components become organized in space and time. These properties appear to endow cells with timely responsiveness when perturbations are experienced. For example, migrating cells will rapidly move around encountered barriers before continuing on their path. Similarly, plasma membrane wounds are rapidly sensed and healed. Whether general principles of self-organization emerge from the study of GTPase-dependent processes is impossible to predict, but the use of diverse experimental models and approaches is providing insight into Schrödinger's wonder at the "organism's astonishing gift of concentrating a 'stream of order' on itself" (Schrödinger, 1944, p. 77).

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