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Glazenburg, Marieke M.; Laan, Liedewij

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REVIEW

SUBJECT COLLECTION: POLARITY

Complexity and self-organization in the evolution of cell polarization

Marieke M. Glazenburg and Liedewij Laan*

ABSTRACT

Cellular life exhibits order and complexity, which typically increase over the course of evolution. Cell polarization is a well-studied example of an ordering process that breaks the internal symmetry of a cell by establishing a preferential axis. Like many cellular processes, polarization is driven by self-organization, meaning that the macroscopic pattern emerges as a consequence of microscopic molecular interactions at the biophysical level. However, the role of self-organization in the evolution of complex protein networks remains obscure. In this Review, we provide an overview of the evolution of polarization as a self-organizing process, focusing on the model species *Saccharomyces cerevisiae* and its fungal relatives. Moreover, we use this model system to discuss how self-organization might relate to evolutionary change, offering a shift in perspective on evolution at the microscopic scale.

KEY WORDS: Cdc42, Biochemical network, Budding yeast, Evolutionary theory, Fungi

Introduction

How cells work and how they develop has intrigued biologists ever since their discovery in the 17th century (Gest, 2004). Following Crick's seminal lecture in the late 1950s (Crick, 1958), our understanding of cell biology has been rooted in a principle known as the central dogma of molecular biology: DNA determines RNA determines protein, where the flow of information is limited to this order (Cobb, 2017). However, despite the apparent simplicity of this statement, it has become clear that having complete genomic knowledge of an organism does not equate to complete understanding of the inner workings of a cell. A protein's intracellular function is not uniquely determined by the amino acid sequence encoded in its gene, but rather by its interactions with other proteins and molecules in complex cellular networks – in other words, by the environment it exists in (Botstein and Fink, 2011; Brenner, 2010; Eisenberg et al., 2000; Gavin et al., 2002; Larance and Lamond, 2015; Sharan et al., 2007; Sleator and Walsh, 2010). This gives rise to the key questions cell biology faces today: how do the vast, interconnected networks of individual proteins together perform reliable functions in the cell, and, more fundamentally, how do these intricate structures evolve?

A distinguishing feature of living cells compared to lifeless matter is their ability to actively establish and maintain non-equilibrium conditions (Koshland, 2002). The maintenance of the 'ordered state' requires constant energy dissipation to overcome entropic forces driving the system into a disordered equilibrium, which is equivalent to cell death (Prigogine, 1978). An example of such an order-creating process is cell polarization. When no work is performed, molecular components within a cell are distributed homogeneously throughout the cytoplasm, meaning that the cell has no intrinsic orientation. However, some circumstances cause a cell to break its internal symmetry and establish a preferential axis, a process known as polarization. Polarization is required for a range of different cellular activities, such as cell division, migration or functional specialization. In certain microbial model species like yeasts, the protein networks underlying polarization have been characterized in great detail (Bi and Park, 2012; Chiou et al., 2017; Irazoqui and Lew, 2004; Klünder et al., 2013; Martin and Arkowitz, 2014; Park and Bi, 2007; Pringle et al., 1995; Pruyne and Bretscher, 2000). The availability of such elaborate mechanistic descriptions allows for precise conceptual and quantitative modeling and can help unveil general governing principles behind complex biomolecular networks and their evolution.

When describing cellular biology at the molecular level, biochemical and physical processes become increasingly relevant. A particularly interesting phenomenon at the intersection of physics, chemistry and biology is self-organization, referring to the spontaneous and maintained emergence of macroscopic order from microscopic interactions, both in animate and inanimate systems (Halatek et al., 2018; Karsenti, 2008; McCusker, 2020; Wedlich-Söldner and Betz, 2018). Although the importance of self-organization to the coordination of protein networks has been recognized, the effect of self-organizing properties on the evolution of complex pattern-forming processes is still poorly understood.

In this Review, we will describe our current knowledge about the evolution of cell polarization from a molecular perspective. We will continually refer to a particularly well-studied model species, namely the budding yeast *Saccharomyces cerevisiae*, and its relatives in the fungal kingdom. Based on this model system, we will explore the role of self-organization in evolutionary trajectories in terms of our current knowledge base and provide a perspective onto potential future directions of the field.

In the following sections, we will start by providing a basic overview of the mechanisms involved in polarization and their relation to self-organization. Then, we will describe what we can learn from the evolution of the polarity machinery, both over long timescales considering major evolutionary transitions and over short timescales in terms of single-step mutational trajectories. Finally, we will discuss the possible role of self-organization in the evolution of complex structures, in particular in relation to natural selection.

Polarity mechanisms

Although polarity is achieved differently and serves different purposes depending on the species and its environment, there are several universal principles to be recognized across these systems. Here, we will briefly introduce the context of the discussion by

Department of Bionanoscience, Kavli Institute of Nanoscience, Faculty of Applied Sciences, Delft University of Technology, 2629 HZ Delft, The Netherlands.

*Author for correspondence (l.laantudelft.nl)

M.M.G., 0000-0003-2586-641X; L.L., 0000-0002-7138-9004

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reviewing these general mechanisms and then discuss the role and meaning of self-organization in this regard.

Some of the concepts referred to here run the risk of being interpreted differently depending on the disciplinary background of the reader. To minimize ambiguity, we make our assumptions explicit and relate to concrete examples when possible. An overview of the terminology used is given in Box 1.

Symmetry breaking in cell biology

From an abstract perspective, cell polarization is a form of symmetry breaking. Although the word 'symmetry' in everyday language is usually associated with mirror images, the physical meaning we refer to here is more akin to 'invariance': an object possesses a certain type of symmetry if its description is invariant to operations of that type (Brading et al., 2003). For instance, a spherical cell with a homogeneous interior is rotationally symmetric, since it can be freely rotated without changing its appearance. When such an invariance is removed, we speak of symmetry breaking. This can occur by means of external cues or forces, driving the system into an asymmetric configuration, but it can also be established spontaneously. In the latter case, the initially stable symmetric state destabilizes as a consequence of some parameter variation, while simultaneously several stable asymmetric states become available. Stochastic fluctuations will drive the system into either one of the newly available stable states, thereby breaking its symmetry. Cell polarization is thus a symmetry-breaking event, in which the initial rotational uniformity of the cell is destabilized; this leads to the establishment of a preferential axis determined by the anisotropic distribution of cellular components (Arkowitz and Bassilana, 2011; Li and Bowerman, 2010). The direction of this preferential axis can either be decided by spatial cues or determined stochastically if it emerged spontaneously. The full process is schematically illustrated in Fig. 1.

Symmetry breaking by polarization plays a role in many different biological systems, such as establishing apicobasal asymmetry in epithelial cells (Nelson, 2009), the formation of the anterior–posterior axis during embryonic development (Goehring et al., 2011) or ensuring unidirectional signal flow by axon specification in neurons (Tahirovic and Bradke, 2009). In our model species *S. cerevisiae*, cells need to polarize prior to cell division in order to select a budding site (Pruyne and Bretscher, 2000). Although the macroscopic pictures of these polarization events are quite different, the underlying mechanisms can be generalized (Fig. 1A). Successful polarization typically requires two elements: initial selection and marking of the polarity axis, and subsequent reinforcement to stabilize the asymmetry. Axis selection is often directed by spatial cues, such as extracellular contact in the epithelia (Manninen, 2015) or the location of the bud scar in *S. cerevisiae* (Kang et al., 2010). In some cases, polarization can still occur in the absence of spatial landmarks, in which case the polarity axis is randomly determined by local stochastic accumulation of signaling components (Altschuler et al., 2008; Drubin and Nelson, 1996). Once an initial polarization axis is chosen, it is reinforced and maintained by positive feedback loops, for instance by directed cytoskeletal transport or downstream effectors establishing effective self-recruitment of polarity proteins.

Self-organization and pattern formation

Spontaneous symmetry-breaking events in cell biology, such as polarity establishment, are often characterized as 'self-organized'. To consider the evolution of such processes, we should first clarify what is meant by self-organizing properties. The term self-organization generally refers to the emergence of global order emerging only from local interactions between individual components (Camazine et al., 2001; Halatek et al., 2018; Karsenti, 2008; Wedlich-Söldner and Betz, 2018). Self-organized ordered systems exist far from thermodynamic equilibrium and exchange energy with their environment, which is why they are sometimes referred to as dissipative structures (Prigogine, 1980). The term has its origins in the physics and chemistry of inanimate systems, but interest in the role of self-organization in living systems is growing, in particular at the cellular and molecular scale (Dhroso et al., 2014; Karsenti, 2008; Kitano, 2004; Wedlich-Söldner and Betz, 2018).

Perhaps more relevant than a fully exhaustive definition are the possible observable implications of what self-organization does and does not imply. Some important properties are summarized in Fig. 2. Firstly, self-organized order is a consequence of interactions between individual constituents at the (local) microlevel, in contrast to top-down arrangement, which utilizes an external (global) template that predetermines the pattern to be formed (Anderson, 2002; Halley and Winkler, 2008). In yeast, polarization can be established in a top-down manner when spatial cues are present inside or outside of the cell to prescribe the orientation of the

Box 1. Glossary and conceptual relations

In the discussion of cell polarity and self-organization, we refer to a multitude of concepts that often lack a universal definition or are used in ambiguous ways. Here, we provide our working definitions of frequently used terminology. The relations between the various concepts are visualized in the diagram below.

Complexity: a measure of (1) the number of components and (2) the interactivity of the components of a system.

Natural selection: the process through which better-adapted organisms produce more offspring than ill-adapted individuals, which increases the proportion of the fitter phenotypic variant in the population. This is traditionally considered the main driver of evolution.

Order: regularity or deviation from a fully homogeneous or isotropic state. Ordered systems have reduced symmetry compared to disordered systems.

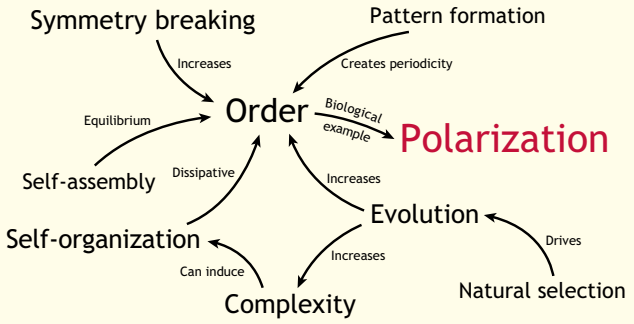
Pattern formation: a process by which (periodic) regularities or ordered structures are formed.

Polarization: asymmetric or non-uniform redistribution of cellular components, establishing a preferential axis.

Self-assembly: the spontaneous emergence of microscopic order from interactions at the microscopic level, stabilizing at equilibrium.

Self-organization: the spontaneous emergence of macroscopic order from interactions at the microscopic level, requiring dissipation of energy.

Symmetry: invariance with respect to a particular transformation, meaning that the description of the system remains identical after the transformation is applied.



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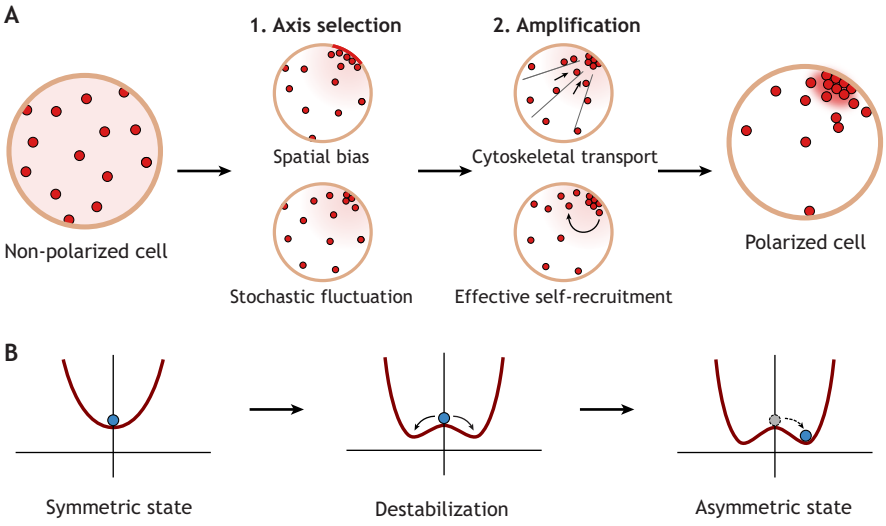

Fig. 1. Mechanisms of symmetry breaking in cell polarity. (A) Schematic overview of the mechanisms behind the two steps required to break cellular symmetry, going from a uniform non-polarized state without preferred direction to an inhomogeneous state with a clearly defined preferential axis. Step 1 (axis selection) can be driven by a spatial bias, such as an internal landmark complex or external gradients, or by stochastic fluctuations in the protein distribution leading to local accumulation. Step 2 (amplification) relies on positive feedback loops, for instance by means of cytoskeletal reorganization and directed transport or by effective self-recruitment through effectors and scaffolds. (B) Simplified energy landscape (vertical axis) of a symmetry-breaking process. In the symmetric state, the parameter describing the order of the system (horizontal axis) rests at zero. When asymmetric (non-zero) states become available, the symmetric state destabilizes, and the system will choose one of the newly available states. As soon as a particular asymmetric state has been selected, the others become energetically inaccessible.

polarity axis. When these cues are removed, however, the formation of the polarity spot is governed exclusively by local molecular interactions and its location is determined randomly, revealing the self-organizing capacity of the process (Altschuler et al., 2008). Secondly, self-organization requires energy dissipation and exists far from thermodynamic equilibrium, which sets it apart from passive self-assembled structures, which stabilize in equilibrium (McCusker, 2020). This property manifests in yeast polarization by its highly dynamic nature: rather than forming a static complex, the central regulator Cdc42 constantly cycles on and off the membrane through an energy dissipating GTPase cycle, which is required for normal polarization (Caviston et al., 2002; Woods and Lew, 2019). Thirdly, since self-organizing systems behave as a collective, their constituents are often interdependent. This means that applying a local perturbation is likely to also induce changes in the network architecture as a whole (Bandyopadhyay et al., 2010; Costanzo et al., 2021; Kim et al., 2012), for instance by a shift in importance of previously irrelevant (specific or non-specific) interactions between other components.

Evolutionary trends in cell polarity

Over the years, the molecular mechanisms of cell polarity and the role of self-organization in this process have been studied extensively. Moreover, polarization and defects therein are linked to easily observable morphological features. These properties make this system an attractive model to study the evolution of cellular processes.

Our current knowledge base enables us to explore cellular evolution on different levels. We can consider long timescales with the help of comparative genomics, using bioinformatics tools to identify protein homology and diversification in large genomic databases. Moreover, we can study short timescales using experimental evolution, tracking evolutionary adaptation occurring through individual mutations. In the following sections, we will discuss observations on both levels to build a picture of the molecular evolutionary mechanisms behind cell polarity.

Long timescales – throughout the fungal phylogenetic tree

Examining the clades of fungi from which *S. cerevisiae* originated, the most pronounced phenotypic differences related to polarization appear in growth morphology. Whereas *S. cerevisiae* and other budding yeasts primarily grow by bud formation, other fungal species proliferate by symmetric fission or filamentous extension (Naranjo-Ortiz and Gabaldón, 2019a). *Schizosaccharomyces pombe* is the typical fission yeast model species, which proliferates by dividing in the center of the cell to produce two daughter cells. Filamentous species, such as *Neurospora crassa* and *Ustilago maydis*, continually grow at their tips, forming elongated hyphae. Species do not necessarily commit to a single morphology; instead, growth patterns can change depending on factors such as the absence of nutrients or cell ploidy. *S. cerevisiae*, for example, can form pseudohyphae in nitrogen-poor conditions (Gimeno et al., 1992).

Tracing back fungal lineages reveals how growth morphologies can shift and reappear throughout evolutionary development, suggesting a degree of flexibility and the presence of shared mechanisms in the underlying polarization strategies. The evolutionary pathways leading to *S. cerevisiae* exhibit several transitions in growth morphology occurring throughout the fungal phylogeny. Early ancestors of modern yeast species were most likely filamentous (Harris, 2011). Predecessors of these ancestors

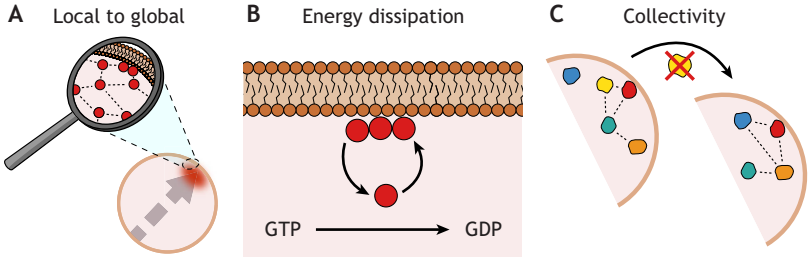

Fig. 2. Properties of self-organized structures. (A) The global ordered patterns of self-organized structures have their origin in local microscopic interactions, as opposed to an external template at the macroscopic level. (B) In contrast to self-assembly, self-organization requires energy dissipation to maintain the ordered structure, for instance through the GTPase cycle present in many polarity systems. (C) Components of self-organized structures behave as a collective: perturbing one element of the structure is likely to change the relations between other elements as well.

presumably inhabited marine biomes, where their mobility was driven by a flagellum. Subsequent terrestrialization of early fungal species then led to loss of the flagellum and development of hyphal growth (Naranjo-Ortiz and Gabaldón, 2019b). After terrestrialization, some fungal clades lost their filamentous growth mode and instead adopted the unicellular yeast-like lifestyle by either budding or fission (Dujon, 2010). Interestingly, the reverse transition also occurred: in some branches, hyphal growth reappeared from yeast species. A prime example is the hyphal species *Ashbya gossypii* (also known as *Eremothecium gossypii*), which is a close relative of *S. cerevisiae* and part of the originally yeast-like Saccharomycotina clade (Wendland and Walther, 2005).

In general, the evolution of yeast morphology is characterized not by gradual adaptation, but rather by relatively long periods of phenotypic stability interrupted by sudden change, presumably as a result of repeated bottleneck events drastically reducing population size (Dujon, 2010). The phenotype emerging from a particular polarization strategy is thus fairly constant throughout yeast evolution, demonstrating strong conservation of morphological properties. However, this constancy is not observed when inspecting the polarization machinery in fungal species on a molecular level. Comparative studies have shown considerable genomic divergence between species with similar growth morphologies (Diepeveen et al., 2017). This holds true even for species that are closely related on the phylogenetic tree.

Two observations have been made here. Firstly, morphologically similar fungal species can achieve the same function – successful polarization – with different sets of proteins. Although some core polarity proteins are strongly conserved, most notably the central GTPase Cdc42 and some of its associated regulators and effectors, there exists a vast diversity in the particular sets of proteins that underlie polarity establishment in different species. In a previous large-scale study across 298 fungal species, we identified a core set of 23 polarization proteins (Diepeveen et al., 2018). Over 95% of all studied species possess 14 or more of these core proteins; however, the majority of species contain a unique set of polarization proteins and none of the identified core proteins are 100% conserved among fungal clades. This suggests that in the combinatorial space spanned by these core polarization proteins, a large fraction of different subset compositions can lead to viable polarity establishment. Moreover, no individual protein could be identified as universally essential to polarization. Not even the highly conserved key player Cdc42 is essential in all fungal species: in *U. maydis*, for instance, the role of Cdc42 seems to be largely taken over by a different small Rho GTPase, Rac1 (Banuett et al., 2008).

A second important observation pertains to functional relations between the proteins that make up the polarity network. Even when protein sequences are conserved between species, functional conservation is not necessarily implied. For example, Bud3 is a protein that is shared by several fungal lineages, among which are the filamentous species *Aspergillus nidulans* and the budding yeast *S. cerevisiae*. Remarkably, Bud3 functions as a guanine-nucleotide-exchange factor (GEF) to the GTPase Rho4 in *A. nidulans*, whereas Rho4 is absent in budding yeast (Harris, 2011). Instead, Bud3 is thought to play a role in axial budding in *S. cerevisae*, localizing to the bud neck and marking the bud site in the next division cycle (Sanders and Herskowitz, 1996). Thus, a molecularly identical protein can fulfil different functions depending on the context in which it exists.

The evolution of polarity thus demonstrates that the polarization machinery harbors a remarkable level of complexity. The proteins that play a role in polarity establishment together form a network with a robust and conserved collective function, yet with a highly variable composition. Phenotypically similar species each possess a unique collection of dozens of different proteins, which together realize reliable polarization. Theoretically however, simple models consisting of only a few components (two-state Cdc42, GTPase-activating proteins and GEFs) can already be sufficient to account for spontaneous symmetry breaking (Goryachev and Leda, 2017; Goryachev and Pokhilko, 2008). This raises questions about the origin of the complexity observed *in vivo*.

We can think of complexity as related to the number of interacting proteins or genes within a functional network; the more components in a system and the more they interact, the higher the complexity. When viewing evolution as a process of gradually selecting fitness increases, the emergence of novel complex structures can seem unlikely. To resolve this, it has been proposed that the phenotypic variation required for innovative evolution is predominantly generated by regulatory changes to core processes, which are themselves highly conserved (Gerhart and Kirschner, 2007). This means that evolutionary change will occur in particular on the regulatory level, while the constituents of core processes remain mostly stable. These core conserved processes are said to have characteristics that make innovative phenotypic variation more accessible, such as robustness, modularity and ease of regulatory tuning, also known as weak regulatory linkage. Therefore, novel phenotypes could emerge relatively easily from redirecting and recombining existing core mechanisms.

The fungal polarization network, however, seems to match this picture only partially. On the one hand, several relevant conserved processes, as well as regulatory linkages, can been identified on a molecular level. For instance, the Cdc42 GTPase cycle, actin-mediated transport and exocytosis have been classified as the core processes underlying polarization (Wedlich-Söldner and Li, 2008). Many of the most highly conserved proteins we identified indeed have a role in either of these three core polarization processes (Diepeveen et al., 2018). On the other hand, however, we also observed many regulatory proteins to be part of our core set, implying that the distinction between conserved core and regulatory non-core cannot be easily made from these data. Moreover, the core was not stably conserved but instead varied strongly between species, as illustrated in Fig. 3. This suggests that the idea of regulatory evolution might not provide a complete explanation of the variety and complexity observed within the polarity network (Diepeveen et al., 2018).

Apart from functional benefit, high complexity might also be a contingent feature of evolving biological systems, in the sense that it emerges without providing selective advantage. In fact, there is evidence that many complex cellular structures have developed at least partially as a consequence of a non-selective bias driving evolving systems towards complexity, a phenomenon known as constructive neutral evolution (Schulz et al., 2022; Stoltzfus, 1999). It has been argued that increasing complexity is an inevitable consequence of optimization by self-organizing systems, due to the fact the addition of components is often more readily allowed than their removal (Saunders and Ho, 1976). This inherent tendency of evolving systems towards complexity has been demonstrated *in silico* (Soyer and Bonhoeffer, 2006; Yaeger et al., 2008) and can be observed *in vivo* in 'entrenched' multicomponent interactions in, for instance, receptors, enzymatic processes, RNA editing and membrane complexes (Finnigan et al., 2012; Gray et al., 2010; Lukeš et al., 2011; Schulz et al., 2022). The different mechanisms behind functional and neutral complexity increase are depicted in Fig. 4. Thus, although this has not been studied in detail for polarization and the influence of constructive neutral evolution is in itself not uncontested (Ho et al., 2017; Speijer, 2011), it is possible

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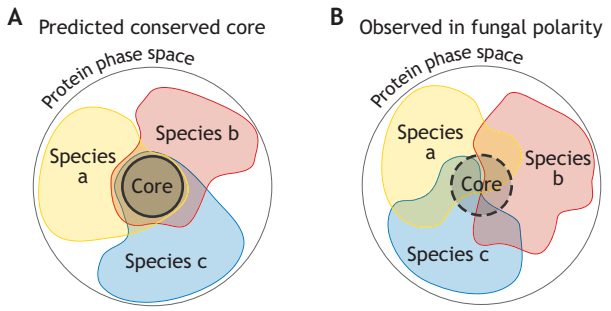

Fig. 3. The regulatory evolution hypothesis compared to observations from fungal polarity. Large circles represent a collection of proteins associated with a certain cellular function, with three species (a, b and c) each possessing a different subset of these proteins. (A) Regulatory evolution predicts a hard core of proteins that is strongly conserved (i.e. present in all three species). Phenotypic differences between species arise due to differences in the sets of regulatory proteins surrounding this core. (B) The picture arising from study of fungal polarity evolution (Diepeveen et al., 2018). The core is defined more loosely. All species possess a large but different fraction of the core, and none of the core proteins are found in all three species.

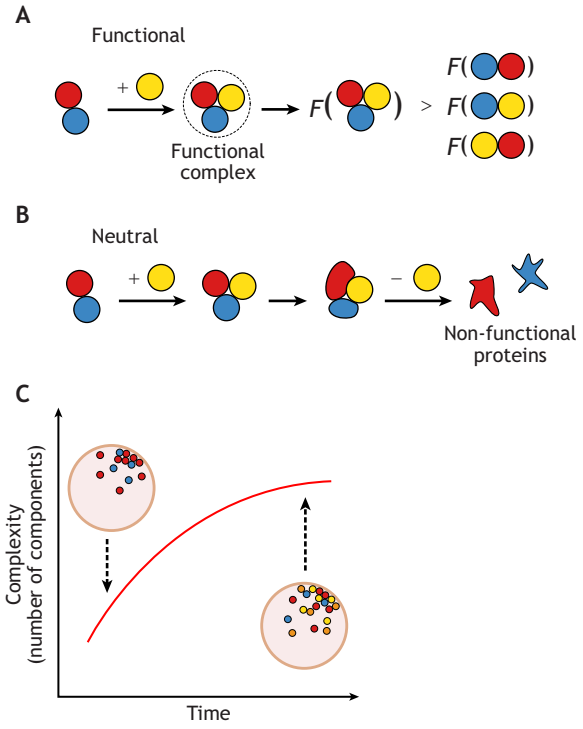

Fig. 4. Mechanisms behind increasing complexity. Evolving systems have an inherent tendency towards complexity, which is often irreversible. (A) Complexity increase can be functional when adding a component leads to the formation of a functional complex with an increased collective fitness *F*, causing selective pressure to maintain the multicomponent structure. (B) Complexity can also increase without fitness benefits. In this example, two components together fulfil some function. By chance, a third component is added to the complex, which does not cause a difference in fitness. This addition, however, relieves certain selective constraints on the first two components, allowing them to evolve such that removal of the new component becomes detrimental. (C) Generally speaking, evolving systems are biased towards component addition over component loss, leading to an ever-increasing level of complexity.

that part of the complexity of the polarity network is a consequence of neutral development.

It should be noted that the tree of life also provides many examples of reductive evolution, where complexity reduces as evolutionary time progresses. Most prokaryotic lineages in particular are characterized by highly streamlined and simplified genomes (Zhang, 2000). It has been suggested that the prokaryotic kingdom diverged from the more complex Eukaryota by a reductive trajectory (Kurland et al., 2007). Genomic simplicity is taken to its limits by endosymbiotic bacteria, which live symbiotically inside a host organism and often have a highly reduced genome (McCutcheon and Moran, 2012). This phenomenon is not exclusive to prokaryotes; certain eukaryotic parasites exhibit the same pattern (Galindo et al., 2021; Heinz et al., 2012). However, these instances of reductive evolution all occur in the context of highly specific selection pressures. In prokaryotes, energy shortage is a much more limiting factor than in eukaryotes, whose mitochondria significantly increase their energy budget (Lane, 2011). Moreover, endosymbiotic or parasitic organisms are adapted to the specific ecological niche of their host organism, adding or removing selective pressures that do not apply to free-living organisms occupying more diverse habitats. Therefore, the observation that evolution generally tends towards complexity appears to hold, at least in the absence of such explicit selective constraints.

Short timescales – the impact of individual mutations

We have seen how studying protein network evolution in terms of phylogenetic differences can elucidate the complex relation between genotypic divergence and phenotypic consistency. This has illustrated how a similar mechanistic function can be achieved by highly variable network compositions across long timescales. We will now turn our attention to the dynamics on shorter timescales by zooming in from interspecies variety to evolutionary trajectories within the same species, at the level of single mutational steps. The advent of accessible whole-genome sequencing techniques has facilitated new methods of studying evolution in unprecedented detail. In particular, it allows us to directly follow microbial evolution as it takes place in the controlled environment of a laboratory. This approach is referred to as experimental evolution.

Studies of experimental evolution have been used to illuminate, for instance, the principles that govern evolutionary rates of change. It is commonly observed that adaptation in evolving populations initially occurs rapidly but eventually decelerates until mutations only provide a minor fitness effect. This is known as diminishing-returns epistasis: beneficial mutations have a relatively smaller impact on fitness in fitter individuals (Helsen and Jelier, 2021). However, although adaptation slows down, it does not fully come to a halt; rather than the population gradually reaching a stationary optimum, population fitness appears to increase without bound (Wiser et al., 2013) and innovation keeps occurring (Lenski, 2017). This suggests that the molecular composition of a cell is subject to change even in the artificial static environment of experimental evolution studies, as genomes continually vary over evolutionary time.

Experimental evolution also lends itself well to studying the effects of genetic perturbations on cellular function. From the yeast deletion project, we know that less than 20% of yeast genes are essential for growth under laboratory conditions, meaning that knockout of those genes causes lethality or infertility (Giaever et al., 2002). However, recent studies show that gene essentiality is highly context dependent (Bosch-Guiteras and van Leeuwen, 2022; Rancati et al., 2018) and can often be overcome through additional mutations. In budding yeast, it has been found that 9% of genes initially deemed ‘essential’ can in fact be evolutionarily compensated for (Liu et al., 2015). Among the evolvable knockouts are several proteins known to play a role in cell polarity, such as

exocyst components, a septin recruited by Cdc42 and another small Rho GTPase (Diepeveen et al., 2018). It is generally assumed that essential genes are less suitable as targets for evolutionary change since they are subject to negative selection (Wilson et al., 1977), even though this has been challenging to prove experimentally (Hirsh and Fraser, 2001). However, the possibility of compensatory evolution might alleviate this constraint, opening up otherwise inaccessible evolutionary pathways.

The fact that a large number of genes in yeast are to some extent dispensable illustrates the remarkable robustness of biological systems. For polarization in yeast specifically, experimental evolution has demonstrated the complexity of evolutionary robustness in this system at the level of single proteins. Deletion of the near-essential scaffold protein Bem1 has been found to induce a reproducible evolutionary trajectory of three subsequent rescuing deletions, involving two Cdc42 regulators and one unknown putative RNA binding protein. At the end of the evolutionary trajectory, the mutated cells recover fitness levels comparable to the fitness of wild-type cells (Laan et al., 2015). Recent work suggests distributed redundancy of multiple self-organizing polarization modules, which rely on different concentrations of Cdc42 regulators (Brauns et al., 2020 preprint). Inactivation of one module can cause other modules to take over by appropriate regulatory adjustments, which could explain some of the mutations in the trajectory. However, much is still unclear about the exact mechanisms behind the evolutionary recovery. For instance, one of the rescuing mutations occurs in a gene that had not previously been related to polarity and whose function is unclear (Laan et al., 2015). Interestingly, although there is still a bias towards functionally related genes (Szamecz et al., 2014), compensatory evolution outside of the immediate functional network has been observed before (Harcombe et al., 2009). This might be related to the collectivity that is characteristic to complex self-organizing systems, as the response to perturbations is not limited to the direct environment in which the perturbation took place.

The above example illustrates that even for such a relatively well-studied system as polarization in budding yeast, our understanding of evolutionary dynamics in terms of intracellular molecular mechanisms is still limited. To date, the cell biological mechanisms behind evolutionary robustness have been relatively underexposed in experimental evolution research. Efforts have been made in, for example, the yeast cytokinesis machinery, where deletion of the myosin motor protein Myo1 causes rapid evolutionary recovery, mostly by means of changes in cell ploidy (Rancati et al., 2008). This is consistent with the recovery trajectories following deletion of many other highly essential genes, as discussed previously, which is often accompanied by aneuploidy (Liu et al., 2015). However, specific knowledge about the molecular principles behind evolutionary recovery of complex biological systems remains scarce.

Self-organization and evolution

The evolution of polarization highlights the variability and flexibility of the network, as well as its adaptive capacities. Given the presumed role of self-organization in the establishment of cell polarization, this raises the question how self-organizational properties relate to these evolutionary dynamics. More specifically, the relation between self-organization and natural selection has been the subject of vivid debate (Batten et al., 2008; Swenson, 2010; Weber and Depew, 1996). How do complex ordered structures change over evolutionary time, and how does selection act upon them?

In the classical neo-Darwinian sense, evolutionary development is a consequence of natural selection acting on existing variation within a population. Better-adapted variants will produce more offspring and thus pass along their inheritable beneficial traits more efficiently, eventually leading to a change in the average phenotype of the population. In this view of evolution, function is primary: development on a species level occurs because of functional differences between variants, which can be selected for. In other words, selection of contingent order (i.e. formed 'by chance') is the main driver of evolutionary change (Szathmáry and Smith, 1995). We can refer to this as a 'function-first' mode of evolution (schematically drawn in Fig. 5A, top). Importantly, this implies that in order for complex structures to arise, the population as a whole needs to randomly explore an extensive range of genotypes until a favored

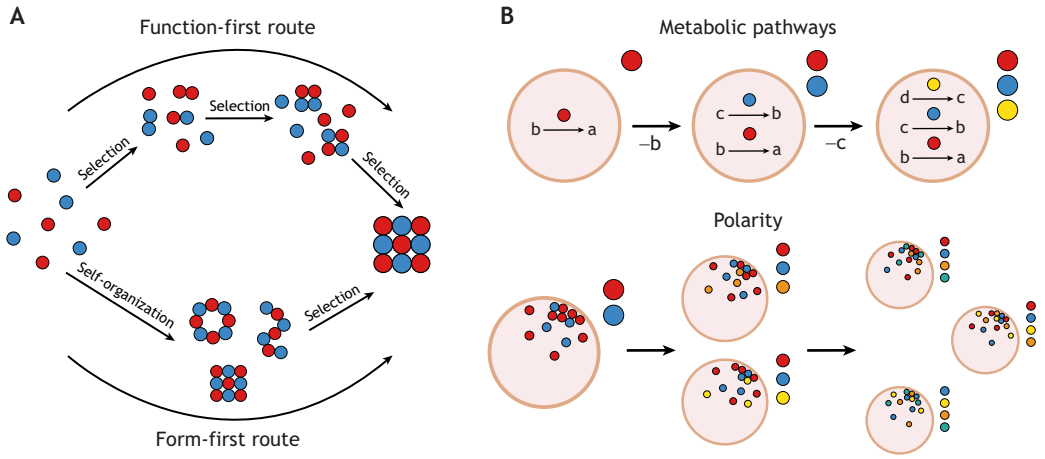

Fig. 5. Self-organization in evolutionary development. (A) Two possible routes towards ordered structures through evolution. The 'function-first' route (top) consists of stepwise addition of components, where each addition is the consequence of direct selective advantage. In the 'form-first' route (bottom), self-organization first determines the available collective structures, after which selection acts on these collectives to fix the best-adapted structure. (B) The evolution of metabolic pathways (top) can be viewed as an example of primarily function-first evolution. Here, the addition of each new protein (colored spheres) is driven by a specific selection pressure – the absence of an essential metabolic substrate (a, b, c and d). Component count increases linearly, and proteins cannot easily be lost. In contrast, a self-organizing process like polarization (bottom) might exemplify form-first evolution. After an initial functional configuration is contingently discovered, new components can be freely added and removed, as long as the macroscopic pattern remains conserved. This facilitates more non-linear trajectories and even the loss of previously important proteins.

phenotype is found. Moreover, phenotypic change is expected to be incremental, as each step has to be individually selected for. The (early) evolution of metabolic pathways is an example of mostly selection-driven development: here, every added enzymatic step is primarily a consequence of selective pressures specific to that step, such as the absence of an essential nutrient (Fani and Fondi, 2009) (illustrated in Fig. 5B, top).

However, apart from natural selection alone, we have seen that the generation of order in biology can also be a consequence of self-organizing physics (Kauffman, 1993; Reid, 2007; Thompson, 1917). In cellular biology in particular, the formation of complex structures, such as protein folds or membrane configurations, is governed by biochemical interactions. This kind of order is not a result of gradual selection of contingently formed complexes, but rather of the thermodynamic laws of molecular interaction and energy minimization that predetermine the allowed collective states. From this perspective, selection for function still plays a role in evolutionary development, but this role is secondary to the universal self-organized primary forms that make up cellular life (Denton et al., 2003); this 'form-first' mode of evolution is illustrated in Fig. 5A (bottom). Here, selection does not act upon individual proteins in a stepwise fashion, but rather on the emergent collective. This could theoretically alleviate environmental constraints on the individual components of a system (Glancy et al., 2016), allowing for greater variety in composition, such as that observed in fungal polarity networks (Fig. 5B, bottom).

In reality, evolutionary trajectories are expected to be shaped by a combination of natural selection and self-organization, in a relation that can vary for different systems or stages of life (Batten et al., 2008). For instance, self-organization can act as a constraint to natural selection, while the latter is still the creative force behind evolution. In this case it is argued that, although the physics of self-organizing systems restricts biological systems and automatically generates much of the observed order, selection still plays the pivotal role in manipulating and controlling the parameters required for self-organized order to be functional within the cell (Johnson and Lam, 2010). This means that complex structures can emerge ('be discovered') abruptly by self-organizing processes, after which their stability and regulation is gradually fine-tuned by natural selection. The observed patterns of sudden instances of change in fungal morphologies interspersed by extended phenotypic stability, for instance in yeast–hyphal transitions, would be consistent with this mechanism.

The challenge in the near future will be to convert these hypotheses into feasible experiments. For instance, known evolutionary trajectories could be used to study the behavior of proteins in a collective in different species or genetic backgrounds. Shifts in behavior of larger fractions of a network as opposed to individual constituents would suggest a role of self-organization. Theoretically, it would also be informative to compare more ancient forms of polarization to the networks of modern species; if selection has acted to more firmly establish the spontaneously organized polarity pattern, ancient systems should be more sensitive to environmental changes (Johnson and Lam, 2010). In practice, however, such an experiment would likely require more molecular knowledge about ancestral polarity networks than currently available.

The influence of self-organization within the evolution of polarization remains an open question. From the available observations, it seems that selection does not act on individual proteins, but instead considers their collective self-organized behavior, which is dynamic and adaptive. Moreover, the robustness of the polarity network with respect to single mutations, as exemplified by evolutionary recovery trajectories, can be interpreted as a sign of self-organizing capacity: organization into stable forms by biomolecular interactions could facilitate the discovery and establishment of novel viable configurations (Denton et al., 2003). However, we are currently lacking appropriate theoretical frameworks and models to properly empirically demonstrate self-organization in evolution. Progress might require more holistic models that acknowledge the role of biophysics in cellular complexity, in order to arrive at concrete predictions and testable observables.

Conclusions and perspectives

Together, insights from long-term interspecies development and short-term evolutionary adaptation paint an interesting picture of the functionality of cellular protein networks like the one underlying polarity establishment. Networks are highly variable and do not converge into a single optimal composition, while even proteins with conserved sequences can have different functions depending on the genetic background they operate in. Deleterious knockouts can be compensated for by duplication events or loss-of-function mutations, and there is evidence hinting at an apparent degree of redundancy that is maintained within the network. Proteins can be gained and lost in diverse and seemingly unpredictable ways, even when the intra-individual mechanisms involved are considered well understood.

These observations advocate a view on protein network functionality that moves away from the common modular perspective (Hartwell et al., 1999), in which each protein has an assigned role within a (subset of a) network that together makes up a mechanistic function, in favor of a more fluid interpretation. In this view, the behavior of a protein is dependent on its environment and functionality is at least partially an emergent property of the full network, rather than something that can be reduced to individual protein functions or to a specific submodule. The process of evolution sheds light on the dynamic, shifting nature of protein collectives and questions their description in novel ways, revealing unknown mechanisms involved in well-known processes.

To be able to properly assess the influence of self-organization in evolutionary processes, a shift in perspective might be required. This could be towards a view in which the biochemical features of proteins take up a more prominent role in the description of cellular function. Elemental to the progression of this field is the ability of formal models to produce observables that can be empirically challenged, in order to expel some of the ambiguity that is currently still associated with the definition and interpretation of self-organization in biology. The toolkit of modern microbiology grants access to an unprecedented level of detail when it comes to studying cellular processes and their evolution. Armed with these possibilities, the study of the molecular workings of a cell could yield exciting new insights into the effect of biophysics on how modern-day life has come to be.

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Competing interests

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