MinE conformational switching confers robustness on self-organized Min protein patterns

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Protein patterning is vital for many fundamental cellular processes. This raises two intriguing questions: Can such intrinsically complex processes be reduced to certain core principles and, if so, what roles do the molecular details play in individual systems? A prototypical example for protein patterning is the bacterial Min system, in which self-organized pole-to-pole oscillations of MinCDE proteins guide the cell division machinery to midcell. These oscillations are based on cycling of the ATPase MinD and its activating protein MinE between the membrane and the cytoplasm. Recent biochemical evidence suggests that MinE undergoes a reversible, MinD-dependent conformational switch from a latent to a reactive state. However, the functional relevance of this switch for the Min network and pattern formation remains unclear. By combining mathematical modeling and in vitro reconstitution of mutant proteins, we dissect the two aspects of MinE's switch, persistent membrane binding and a change in MinE's affinity for MinD. Our study shows that the MinD-dependent change in MinE's binding affinity for MinD is essential for patterns to emerge over a broad and physiological range of protein concentrations. Mechanistically, our results suggest that conformational switching of an ATPase-activating protein can lead to the spatial separation of its distinct functional states and thereby confer robustness on an intracellular protein network with vital roles in bacterial cell division.

Min system | pattern formation | protein reaction–diffusion networks | conformational switching | in vitro reconstitution

Self-organized pattern formation by proteins is vital for many fundamental cellular processes, ranging from cell division (1) and chromosome segregation (2) to chemotaxis (3). To what extent then do these intrinsically complex processes depend on common, core principles and, conversely, what role do specific molecular details play in these biochemical reaction networks? In this context, it is particularly interesting to ask how robust network function is against changes in network structure and system parameters such as protein concentrations.

Among intracellular pattern-forming networks, the *Escherichia coli* Min system has become a paradigmatic model for both experimental (4–9) and theoretical (4, 6, 10–16) studies of protein pattern formation over the last 15 years.

Here, MinD and MinE self-organize to generate pole-to-pole oscillations that establish a time-averaged concentration minimum of MinC at midcell. Since MinC acts as an inhibitor of the cell division protein FtsZ, the Min system thereby confines the division machinery to midcell to ensure division into equally sized daughter cells (17). The Min system is a particularly instructive example, because its components are well characterized and it can be reconstituted in lipid bilayer assays in vitro (1, 4, 5). In the presence of ATP, MinD and MinE self-organize into surface waves on a flat, supported membrane (4). Experimental (4, 5, 9, 18) and theoretical (4, 6, 10–16) studies have yielded insights into the reaction network underlying this self-organization process. However, the relationships between pattern formation and the molecular properties of the proteins involved are a matter of ongoing interest, as they bridge the molecular and cellular scales.

The oscillatory dynamics of the Min system are driven by the stimulation of MinD's ATPase activity by MinE. ATP-bound MinD dimerizes and binds to the plasma membrane (9, 19, 20). It then recruits further MinD-ATP, as well as its ATPase-activating protein MinE, which together form membrane-bound MinDE complexes (19). MinE stimulates MinD's ATPase activity, thereby initiating disintegration of MinDE complexes and subsequent release of MinE and ADP-bound MinD into the cytosol (19, 21). After detachment, MinD exchanges ADP for ATP, before the ATP-bound form rebinds to the membrane (19, 20). This biochemical reaction network, which we refer to as the skeleton network (Fig. 1*A*), is in agreement with various experimental studies (4, 5, 18, 22) and has formed the basis for a number of theoretical models (10, 11, 13) that recapitulate various aspects of Min pattern formation.

Experimental studies have established the crucial role of MinE's stimulation of MinD's ATPase activity in Min protein pattern formation (4, 21). Moreover, mathematical models centered around the conversion of MinD from the ADP- to the ATP-bound state suggest that this step is critical for efficient localization of the FtsZ ring to midcell (11), formation of multistable patterns, and adaptation to cell geometry (6, 11, 23). Recently, it has been shown that the skeleton network captures the in vitro phenomenology of Min protein patterns on flat

Significance

Many fundamental cellular processes are spatially regulated by self-organized protein patterns, which are often based on nucleotide-binding proteins that switch their nucleotide state upon interaction with a second, activating protein. For reliable function, these protein patterns must be robust against parameter changes, although the basis for such robustness is generally elusive. Here we take a combined theoretical and experimental approach to the *Escherichia coli* Min system, a paradigmatic system for protein self-organization. By mathematical modeling and in vitro reconstitution of mutant proteins, we demonstrate that the robustness of pattern formation is dramatically enhanced by an interlinked functional switching of both proteins, rather than one. Such interlinked functional switching could be a generic means of obtaining robustness in biological pattern-forming systems.

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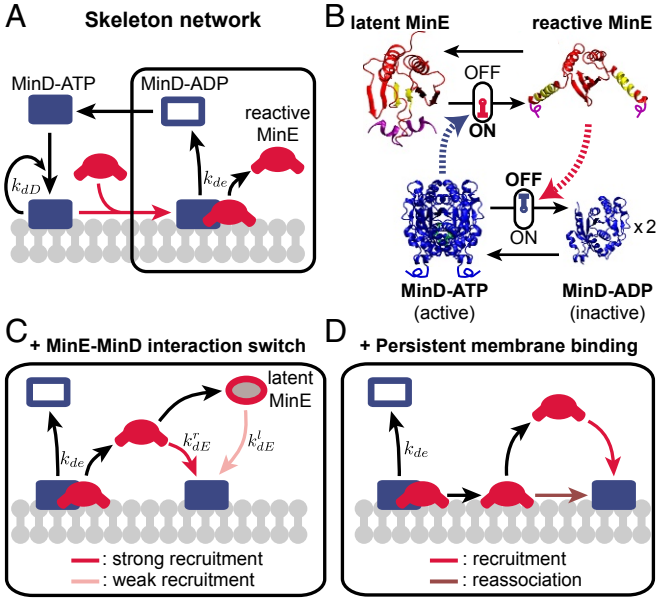

Fig. 1. MinE's conformational switch suggests distinct ways to form MinDE complexes. (*A*) The skeleton model accounts for only one MinE conformation. (*B*) Scheme of interlinked MinDE protein switches. While reactive MinE is known to trigger MinD's ATPase activity, membrane-bound (active) MinD induces the switching of MinE from a latent to a reactive state in which the previously inaccessible MinD interaction region (yellow) and MTS (purple) are exposed [PDB files 3R9J (27), 2KXO (51), and 3Q9L (52) are used to illustrate reactive and latent MinE and MinD, respectively]. (*C*) The extension to the skeleton network includes a MinE–MinD interaction switch for interconversion between latent and reactive states of MinE, which are weakly or strongly recruited to MinD with rates k^l_{dE} or k^r_{dE}, respectively. (*D*) Persistent MinE membrane binding allows MinDE complexes to form either by recruitment of cytosolic MinE or by reassociation of already membrane-bound MinE with membrane-bound MinD. For both extensions the MinD reaction dynamics remain unchanged.

lipid bilayers (16). The theory predicts chemical turbulence (spatiotemporal chaos) at the onset of the pattern-forming instability, e.g., at low MinE/MinD ratios. Moreover, previous theoretical analyses (10, 11, 13), based solely on the interactions in the skeleton network, found that patterns can form only if MinE is less abundant than MinD (*The Skeleton Model Cannot Explain Pattern Formation When MinE Exceeds MinD in Concentration*). However, reconstitution experiments clearly show that patterns emerge for MinE/MinD ratios ranging between 0.125 and 5 (4, 7, 24–26). This contradiction prompts a reconsideration of the current perspective on the Min reaction network and raises the general question of how pattern-forming networks become robust against variations in protein concentrations. Furthermore, over the course of evolution, the robustness of a network's function, such as protein patterning, against alterations in protein number is essential to enable the system's characteristics to adapt without disrupting its core function.

Indeed, recent biochemical findings (27, 28) suggest a possible extension of the skeleton network. In addition to the MinE-induced switch in MinD's nucleotide state, MinE itself is now believed to undergo a MinD-dependent conformational switch. This conformational switch causes cytosolic MinE to unmask its buried MinD- and membrane-interacting regions, i.e., its anti-MinCD helix and membrane-targeting sequence (MTS), respectively (Fig. 1*B*) (27). Importantly, to expose its anti-MinCD helix, MinE must first "sense" membrane-bound MinD (27), a process that was proposed to involve formation of an "encounter complex" of MinE with MinD, which then triggers the conformational change (28, 29). Once the anti-MinCD domain is released, MinE is assumed to form a tighter complex with membrane-bound MinD and stimulate its ATPase activity (27–29). In addition, after dissociation of the MinDE complex and the release of MinD-ADP into the cytosol, MinE's MTS enables it to remain bound to the membrane, and the protein may reassociate repeatedly with other membrane-bound MinD molecules or (eventually) return to the cytosol (5, 27, 28). This membrane-bound cycling of MinE has been dubbed the "Tarzan of the jungle" mechanism (27) or persistent MinE membrane binding (24) in the literature. Upon detachment from the membrane, MinE quickly reassumes its latent conformation with its MinD- and membrane-interaction regions buried (Fig. 1*B*). In vivo studies have suggested that the MinD-dependent conformational switch of MinE is important for correct cell division, as a mutation that locks MinE into the reactive state was not able to restore the WT phenotype when expressed with MinC and MinD in a Δmin strain of *E. coli* (30). Despite recent experimental research on the molecular interaction steps involved in this switch (28, 29), the functional role of MinE's conformational switch in the Min reaction–diffusion network and its effect on pattern formation remain unclear. In vivo, this question is difficult to address systematically due to the disruptive effects on cell morphology and viability caused by mutations and changes in protein concentration (27, 31). In contrast, in silico and in vitro approaches both allow highly comparable conditions and the precise variation of parameters. Therefore, we addressed the function of MinE's conformational switch in pattern formation by combining mathematical modeling and cell-free reconstitution experiments.

The two novel properties of MinE's reactive conformation—facilitation of the MinE–MinD interaction and persistent membrane binding of MinE—could independently affect the formation of patterns. To disentangle these two aspects and analyze their respective impacts on pattern robustness to variations in the MinE/MinD ratio, we first numerically studied the dynamics of reaction–diffusion networks that exhibit either aspect of the switch by a linear stability analysis, which predicts the parameter regime within which patterns form (*Reaction–Diffusion Equations Accounting for a MinD-Dependent Switch of MinE*). Then we tested the theoretical predictions by reconstituting the networks using suitable MinE mutants (*Materials and Methods*, *Relation of MinE Mutant Proteins to Model Extensions*, and *Detailed Experimental Materials and Methods*). Our combined theoretical and experimental results demonstrate that the MinE–MinD interaction switch of MinE is critical for the emergence of patterns over a broad and physiological range of protein concentrations. Furthermore, we experimentally show that, unlike the MinE–MinD interaction switch, persistent membrane binding of MinE does not markedly affect the protein concentration range compatible with pattern formation.

Results

The MinE–MinD Interaction Switch Is Critical for the Robustness of Min Patterns Against Variations in Protein Concentration. First, we addressed the functional relevance of the MinD-induced exposure of MinE's buried MinD interaction region alone. Upon recruitment of MinE by MinD, a membrane-bound MinDE complex is formed, in which MinE is assumed to be present in its reactive state. After disintegration of a MinDE complex, both partners are released into the cytosol. We assume that switching of reactive MinE to its latent form occurs rapidly, but not simultaneously with the disintegration of a MinDE complex and release of MinE into the cytosol (*On the Assumption of Dissociation of Reactive MinE from MinD and Its Switch to Latent MinE as a Two-Step Process*). The timescale for reversion of reactive MinE to its latent conformation is taken to be of the order of 0.01 s, the upper bound for a typical conformational switch (32). To account for the alternative conformations of MinE, we extended the skeleton network (11, 13) to include both a latent MinE conformation and a reactive form with recruitment rates to membrane-bound MinD, k^l_{dE} and k^r_{dE}, respectively (Fig. 1*C*). Reactive MinE no longer requires the MinD-dependent release

of its anti-MinCD helix, as this structure is already exposed. Thus, it is reasonable to assume the recruitment rate of reactive MinE to be higher than that of latent MinE. Note that for equal recruitment rates, i.e., $k^l_{dE} = k^r_{dE}$, the two MinE conformations are identical and the original skeleton network with only one MinE recruitment rate is recovered.

Our mathematical analysis shows that, in a broad regime of low k^l_{dE} and high k^r_{dE}, patterns are formed over a wide range of MinE/MinD ratios, including those where MinE is present in excess (Fig. 2 *A* and *B*). To test these theoretical predictions experimentally, we made use of the MinE L3E mutant, which is impaired in membrane interaction (27). It should therefore be capable of undergoing the MinD-induced interaction switch, but unable to remain attached to the membrane in the absence of MinD (*Relation of MinE Mutant Proteins to Model Extensions*). Thus, we expect this mutant to mimic MinE in our extended model that includes a MinE–MinD interaction switch without persistent membrane binding (Fig. 1*C* and *Relation of MinE Mutant Proteins to Model Extensions*). When reconstituted together with MinD on flat membranes, MinE L3E promoted pattern formation over a wide range of MinE/MinD ratios, just like WT MinE (Fig. 3). In agreement with our theoretical predictions (Fig. 2 *A* and *B*), experiments showed that patterns formed even when MinE was present in excess over MinD (Fig. 3).

Based on our theoretical observations, which showed a strong increase in pattern robustness upon incorporation of a MinE–MinD interaction switch, we propose that MinE's ability to switch between conformations with high or low affinity for MinD is responsible for the experimental observation that high MinE/MinD concentration ratios are compatible with Min

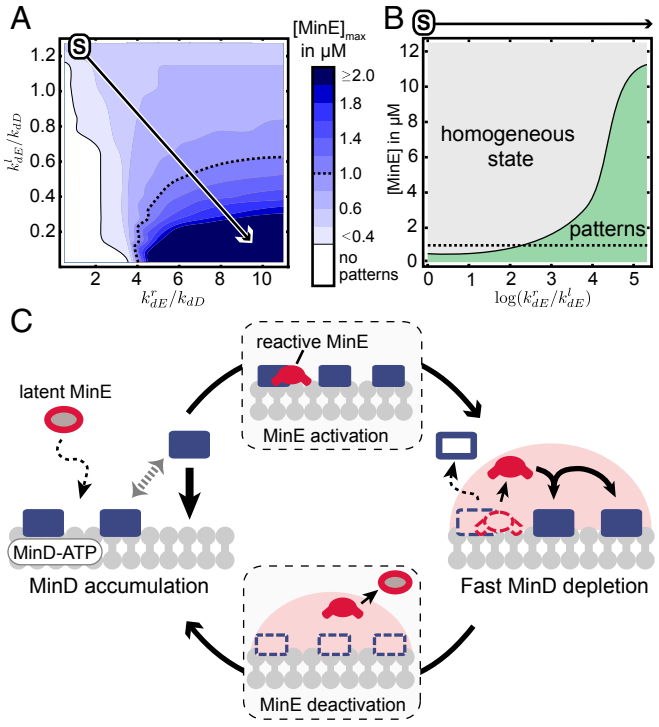

Fig. 2. The MinE–MinD interaction switch is essential for the robustness of Min patterns in silico. (*A*) For high k^r_{dE} and low k^l_{dE} (compared with the MinD recruitment rate k_{dD}), linear stability analysis predicts an increase in the maximal MinE concentration compatible with patterns ($[MinE]_{max}$) relative to the skeleton network where $k^r_{dE} = k^l_{dE}$ (the case $k^r_{dE} = k^l_{dE} = 1.25k_{dD}$, indicated by S, is given as an example). [MinD] is fixed at 1 μM. (*B*) Along the arrow in *A* the range of [MinE] compatible with patterns dramatically increases with k^r_{dE}/k^l_{dE}. For k^l_{dE} close to zero, MinE eventually ceases to cycle between the bulk and the membrane, and pattern formation is suppressed (*In the Limit Case of Vanishing Recruitment of Latent MinE by Membrane-Bound MinD, the Ability to Form Patterns Is Lost*). (*C*) MinD-induced switching of MinE facilitates alternation of MinD accumulation and MinD depletion on the membrane. For kinetic rates see Table S1.

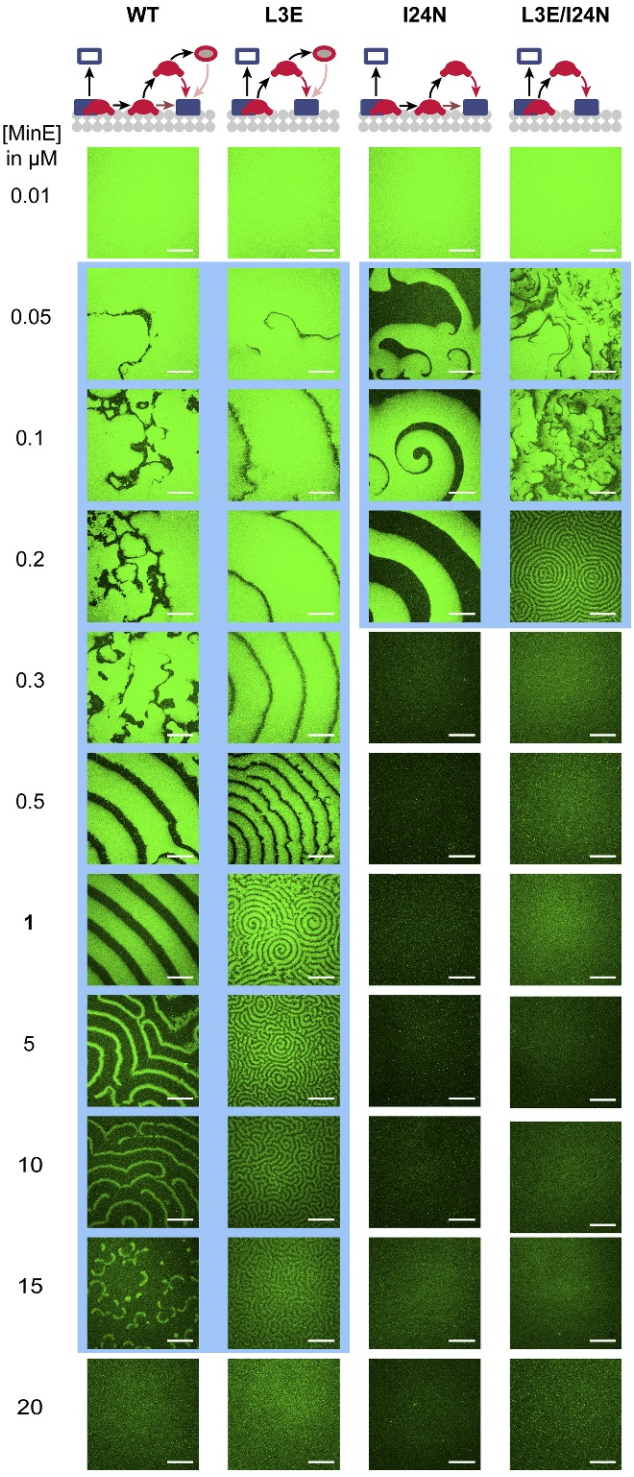

Fig. 3. Impairment of MinE's MinE–MinD interaction switch dramatically decreases the robustness of Min protein patterns in vitro. Reconstitution assays were performed on flat supported lipid bilayers in the presence of 1 μM MinD with 20% eGFP-MinD. The L3E mutation, which impairs MinE membrane binding, permits pattern formation (blue background) over a similar range of MinE concentrations as WT MinE. In contrast, the I24N mutation, which locks MinE into its reactive conformation, dramatically decreases the maximal MinE concentration at which patterns can form. (Scale bar: 50 μm.)

protein patterns. If this hypothesis is true, experiments with MinE mutants that lack the ability to switch between a reactive and a latent state should display a strongly decreased maximal MinE/MinD concentration ratio compatible with patterns. To test this hypothesis, we took advantage of the I24N mutation, which was previously shown to lock MinE into the reactive state (ref. 27 and *Relation of MinE Mutant Proteins to Model Extensions*). Strikingly, introducing this mutation into either WT MinE or MinE L3E dramatically reduced the concentration range within which protein patterns formed (Figs. 3 and 4). Indeed, in agreement with the above hypothesis based on our theory (11), patterns prevailed only in a very narrow range and only for MinE/MinD concentration ratios far below one (Figs. 3 and 4). In particular, MinE I24N formed patterns only outside the physiological concentration range (33). This is consistent with in vivo experiments in which the I24N mutant failed to restore midcell division when expressed together with MinD and MinC in an *E. coli* Δmin strain (30), most probably due to the fact that MinC is not recruited to the membrane by MinD. This agrees with our observations that (*i*) MinD cannot effectively accumulate on the membrane to initiate pattern formation above a certain threshold MinE/MinD ratio and (*ii*) this threshold is strongly decreased for MinE I24N relative to WT MinE (Fig. 3). In summary, our analyses demonstrate that mutually interlinked protein switching is critical for the robustness of an exemplary pattern-forming system against variations in protein concentrations.

The relationship between the MinE/MinD ratio and the ability to generate patterns can be understood by considering the roles of the two proteins in the establishment of Min oscillations. Min oscillations are essentially the result of alternating dominance of MinE and MinD (11, 24). In membrane regions depleted of Min proteins, cooperative binding of MinD first facilitates its own accumulation on the membrane (MinD dominance). Then, recruitment of MinE and MinE-induced detachment of MinD together outpace further MinD accumulation and progressively deplete the latter from the membrane (MinE dominance). But MinE-induced detachment can outpace MinD accumulation only if the released MinE is recruited more rapidly to membrane-bound MinD than is MinD itself. Thus, the rate of recruitment of MinE must be higher than that of MinD. Since the skeleton network incorporates only a single, rapidly recruited MinE conformation, initial dominance of MinD accumulation is feasible only if MinD exceeds MinE in concentration. In contrast, if MinE can exist in both a latent and a reactive conformation, dominance of MinD over MinE becomes possible even if MinE exceeds MinD in concentration (Fig. 2*C*). This is because initially most MinE is in the latent form, whose recruitment rate is low. If MinE was always present in its latent form only, MinD would accumulate on the membrane and Min dynamics would cease, because the recruitment of latent MinE will never dominate MinD recruitment. However, after inducing ATP hydrolysis by MinD, MinE is assumed to be released into the cytosol in its reactive conformation. As this state is short-lived, the reactive species is effectively restricted to a thin boundary layer close to the membrane (Fig. 2*C*, red shaded region) and will be preferentially recruited (over cytosolic MinD) to membrane-bound MinD. Once the membrane is depleted of MinD, reactive MinE cannot rebind promptly and rapidly switches to its latent cytosolic form. This enables a transient dominance of MinE, which displaces MinD from the membrane. Remarkably, our theoretical analysis predicts an extended range of MinE/MinD ratios that support patterns even for very rapid MinE switching, i.e., when the layer of reactive MinE (~0.7 μm) is orders of magnitude thinner than the depth of the cytosol (~5,000 μm) (*Thickness of Reactive MinE Layer*). Note that any effective ad hoc reduction of the cytosol to two dimensions would, by neglecting the protein distribution perpendicular to the membrane (34, 35), fail to uncover such subtle but crucial effects, as the emergence of the thin layer of reactive MinE would be entirely lost. This further emphasizes the importance of accounting for the extended bulk in (3D) quantitative theoretical models (11, 16, 36).

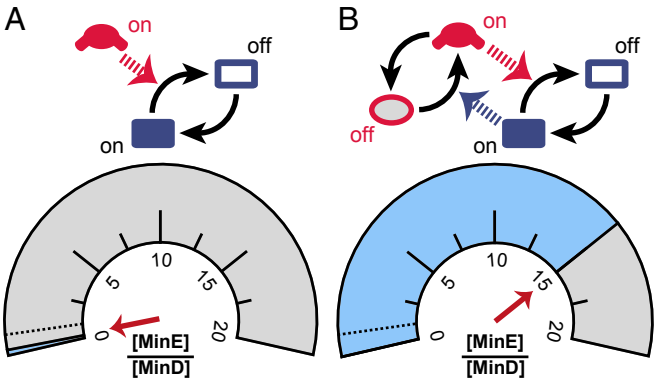

Fig. 4. Mutually interlinked switching dramatically increases the robustness of protein pattern formation. (*A*) The MinE variants in which the MinE–MinD interaction switch is disabled (I24N and L3E/I24N) display patterns only within a narrow range (blue region) of MinE/MinD ratios below one (dotted line). (*B*) In contrast, variants that retain the interaction switch (WT and L3E) also form patterns even when MinE is present in great excess. The schematic networks highlight the roles of MinE and MinD in dynamically switching the activity of their respective interaction partner.

Persistent MinE Membrane Binding Is Not a Major Determinant of the Concentration Range of Min Patterns. As MinE's conformational switch affects its affinity for both MinD and the membrane (27), we independently explored the impact of persistent MinE membrane binding mediated by its MTS (Fig. 1*D*, *Reaction–Diffusion Equations Accounting for a MinD-Dependent Switch of MinE*, *Effect of Persistent MinE Membrane Binding on the Concentration Range of Pattern Formation*, and *Direct Attachment of MinE to the Membrane Does Not Affect the MinE Concentration Range Compatible with Pattern Formation*). This was previously shown to influence Min patterns (22, 24, 25, 35, 37) and was implied to be required for Min protein pattern formation per se (35), although the validity of the underlying theoretical analysis (35) is controversial (36). Recent experimental studies have confirmed that Min protein patterns can indeed form without direct MinE membrane interaction, although with altered in vitro characteristics (22, 25).

As hypothesized previously (11), persistent membrane binding might also affect the concentration range compatible with pattern formation. Assume that "free" membrane-bound MinE has a weak affinity for membrane-bound MinD, such that membrane-bound MinE is more likely to detach after lingering on the membrane than to reassociate with membrane-bound MinD. Then persistent MinE membrane binding will reduce the overall efficacy of MinE-mediated removal of MinD from the membrane, because free MinE lingering on the membrane does not participate in the depletion process. As a consequence, the maximal MinE/MinD concentration ratio compatible with patterns should increase in this case. On the other hand, if free membrane-bound MinE interacts very strongly with membrane-bound MinD—hypothetically even more strongly than the MinE in the bulk—persistent MinE membrane binding will enhance MinD depletion and patterns should form for even lower MinE/MinD concentration ratios.

We quantitatively studied a reaction network in which MinE can persistently bind to the membrane but is permanently locked into its reactive state (Fig. 1*D*). We expect this model to be best realized by our experiments with the I24N mutant, which lacks the MinE–MinD interaction switch while retaining the ability to bind persistently to the membrane (*Relation of MinE Mutant Proteins to Model Extensions*). With this mathematical model, we were able to confirm the above intuition regarding the two theoretical scenarios involving weak and strong interaction between free MinE and MinD on the membrane (*Effect of Persistent MinE Membrane Binding on the Concentration Range of Pattern*

Formation, *Direct Attachment of MinE to the Membrane Does Not Affect the MinE Concentration Range Compatible with Pattern Formation*, and Figs. S4 and S5). As reported above, our reconstitution experiments with MinE mutants that lack the ability to persistently bind to the membrane show no change in the range of MinE/MinD concentrations permissive for patterns compared with experiments with the respective MinE types without this mutation. In summary, we infer that, unlike the MinE–MinD switch, persistent membrane binding does not markedly affect the range of concentrations compatible with in vitro Min patterns (Figs. 3 and 4).

The Skeleton Network Suffices to Reproduce in Vitro Min Patterns. In the MinE L3E/I24N double mutant, both membrane interactions and the MinE–MinD interaction switch are disabled, mimicking the MinE dynamics in the original skeleton network (11, 13). This mutant still self-organized into dynamic protein patterns, albeit only in a narrow range of MinE/MinD ratios (Figs. 3 and 4), and—notably—only if MinD exceeds MinE in concentration, confirming previous theoretical predictions (ref. 11 and *The Skeleton Model Cannot Explain Pattern Formation When MinE Exceeds MinD in Concentration*). This result shows that, given a suitable choice of low MinE/MinD ratios, neither persistent membrane binding nor the MinE–MinD switch is required to generate patterns, and it confirms the skeleton network as a valid and useful basis for the investigation of pattern-forming mechanisms in the Min system. A recent theoretical analysis of in vitro Min protein pattern formation based on the skeleton model (16) predicted chemical turbulence (disordered patterns) at the onset of instability (low MinE/MinD ratios). Interestingly, our experiments confirm this prediction (Fig. 3) and show that this characteristic is preserved for all mutants.

Discussion

Based on recent experimental insights into the molecular structure of MinE and its ability to undergo MinD-dependent conformational changes (27–29), we studied the role of this conformational switch in the context of the Min reaction network. Our combined theoretical and experimental investigation reveals that this switch is essential for the robustness of the key function of the Min reaction network—the formation of spatiotemporal protein patterns.

Previous experiments (27, 28) strongly suggested that the different conformations of MinE are not in chemical equilibrium with each other; i.e., MinE does not switch between the two states independently of external triggers. Instead, MinE's conformational switch from latent to reactive critically depends on the "sensing" of membrane-bound MinD (27). In the context of a reaction–diffusion network, the spatial confinement of MinE's switched state to the immediate vicinity of membrane-bound MinD leads to a spatial separation of reactive MinE close to the membrane and latent MinE in the bulk. With regard to the change in MinE's binding affinity for MinD, this spatial separation provides for dynamic control of MinE's two distinct modes of action: In its latent form, MinE allows MinD to accumulate on the membrane even if the total MinE concentration exceeds that of MinD. Accumulation of MinD on the membrane in turn facilitates the formation of a thin reactive layer of MinE above the membrane, which eventually depletes MinD from the membrane (Fig. 2*C*). In contrast to networks with only one MinE conformation (10, 11, 13), the dynamic switching of MinE enables patterns to form even when MinE is much more abundant than MinD.

Furthermore, it was proposed that the exposure of MinE's MTS not only leads to persistent membrane binding of MinE but also might even enable direct attachment of MinE to the membrane (25, 28), enable the stabilization of MinD by membrane-bound MinE (25), and be involved in the release of the anti-MinCD helix (28, 29). While MinE membrane interaction is evidently relevant for regulating the detailed characteristics of Min patterns, such as the wavelength (Fig. 3) (22, 25) and proper function of the Min system in vivo (27, 38), our analyses show that the concentration range of Min patterns is not markedly affected by this factor, in terms of either persistent membrane binding or direct MinE attachment to the membrane (Fig. 3 and *Direct Attachment of MinE to the Membrane Does Not Affect the MinE Concentration Range Compatible with Pattern Formation*). Instead, this concentration range is primarily determined by the MinD-dependent switch in MinE's affinity for MinD. This emphasizes that interlinked switching of the mutual binding affinities of MinD and MinE plays an important role in regulating the ability to form patterns.

The bacterial Min system is a prominent example of a class of intracellular pattern-forming networks that are based on the self-organization of nucleoside triphosphatases (NTPases). NTPases function as molecular switches, which upon interaction with a cognate NTPase-activating protein transition from an NTP-bound to a nucleoside diphosphate (NDP)-bound form. In the context of network motifs, we identify a reciprocal switch—triggered in the ATPase-activating protein MinE by the cognate ATPase MinD—as a critical factor in the robustness of patterns over a broad range of protein concentrations. In view of the ubiquity of structurally switchable proteins, including NTPases and possibly further NTPase-activating proteins (39, 40), our study highlights the role of alternative conformations and mutual switches for robust pattern formation.

From a structural perspective, MinE can be seen as a "metamorphic" protein, a type of protein that can reversibly switch between alternative conformations with distinct functions (41). Among various examples for such proteins (41, 42), it has recently been discovered that fold switching of the metamorphic protein KaiB plays an important role in the KaiABC system, a prototypical protein oscillator that serves as a circadian clock in cyanobacteria (43–45). The observation of fold switching for both KaiB and MinE suggests that metamorphic proteins may be widespread in dynamical systems with important roles in cell physiology.

Intracellular pattern-forming systems are often based on reaction–diffusion networks (46, 47). Their underlying nonlinearities render these networks sensitive to even small variations in system parameters, such as reaction rates and the (approximately constant) concentrations. However, such sensitivity can also provide evolutionary benefits, as changes in protein concentrations can be harnessed to adjust features of the patterns, such as the oscillation period or characteristic wavelength (22). In this context, it is essential that the ability to generate patterns in the first place (regardless of their quantitative characteristics) is robust against variations in the concentrations of the relevant components. This provides a large parameter regime within which pattern formation is possible, thus facilitating the emergence of patterns with different spatiotemporal characteristics that may lead to evolutionary adaptations in cell morphology or other favorable phenotypic features. Furthermore, retention of the ability to form patterns in the face of alterations in protein numbers is essential for evolution, since this allows for protein mutations while the key functions of the organism, such as protein patterning, remain intact (48).

We propose that mutually interlinked switching is likely to be a general design principle that enhances the robustness of important regulatory patterns to variations in protein concentrations in many biological reaction–diffusion systems. In particular, interlinked switches have been reported for the widely conserved F1hF–F1hG circuit (39, 49), which is essential for flagellar patterning (39). Pattern robustness due to functional switching may also be relevant in other pattern-forming systems, such as for chemotaxis patterns (3) or chromosome segregation (2), and may even play a major role in eukaryotic systems, for instance in the process of cell polarization in budding yeast (50).

Materials and Methods

Theoretical Prediction of MinE/MinD Ratios That Permit the Formation of Patterns. Our theoretical analyses are based on different biochemical reaction networks that incorporate either a MinE–MinD interaction switch or persistent MinE membrane binding. These networks extend a previous

theoretical model (11, 13) for the Min system, which accounts for the molecular interactions that are believed to be essential for Min protein dynamics, to include a MinE–MinD interaction switch and persistent MinE membrane binding, respectively, as additional features (*Reaction–Diffusion Equations Accounting for a MinD-Dependent Switch of MinE*). The data presented in Fig. 2 *A* and *B* and Figs. S2–S5 have been obtained numerically by performing stability analyses over an extensive range of reaction rates and protein concentrations (*Reaction–Diffusion Equations Accounting for a MinD-Dependent Switch of MinE*).

Experimental Methods. Model predictions were tested with an in vitro self-organization assay (4). For this, His-MinD, His-eGFP-MinD, WT, and mutant His-MinE were purified and reconstituted with ATP on flat supported lipid bilayers, essentially as described previously (4, 22). Pattern formation was then tested for with fluorescence imaging using confocal laser scanning microscopy. The experimental methods are described in more detail in *Detailed Experimental Materials and Methods*.

Data Availability. All relevant data are within this paper and its *Supporting Information* files.

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