**Readme information:**

This repository contains the corresponding Supplementary Movies and high-resolution Main and Supplementary Figures for “Evolutionary repurposing of a DNA segregation machinery into a cytoskeletal system controlling cyanobacterial cell shape” by Springstein et al. (2025). The movies supplied here are processed from the raw movies. Raw movies are available from the authors upon request. Due to size constraints during the submission process, we uploaded the Supplementary Movies in this repository. A preprint of the manuscript is available from:

https://www.biorxiv.org/content/10.1101/2025.06.27.661541v1

**Below, further details are given for each individual file:**

Supplementary Movie 1: Live cell time lapse microscopy of mNG-CorM

*Anabaena* cells expressing mNG-CorM were grown on 1.5% agar pads and illuminated with an in-house plant growth light. Images were acquired at 15-minute intervals. Scale bar: 5 µm.

Supplementary Movie 2: Dynamic instability of mNG-CorM *in vivo*

Polymerization and sudden shrinkage of mNG-CorM filaments in *Anabaena* cells recorded by TIRF microscopy. Images were acquired at 10-second intervals. Scale bar: 1 µm.

Supplementary Movie 3: Dynamic instability of mNG-Δ1-40CorM *in vivo*

Polymerization and sudden shrinkage of mNG-Δ1-40CorM filaments in *Anabaena* cells recorded by TIRF microscopy. Images were acquired at 5-second intervals. Scale bar: 1 µm.

Supplementary Movie 4: FRAP of CorM *in vitro*

FRAP experiment of Alexa488-CorM filaments *in vitro* on SLBs. Images were acquired at 1-second intervals. Scale bar: 10 µm.

Supplementary Movie 5: FRAP of Δ1-40CorM *in vitro*

FRAP experiment of Alexa488-Δ1-40CorM filaments *in vitro* on SLBs. Images were acquired at 1-second intervals. Scale bar: 10 µm.

Supplementary Movie 6: CorM polymerization dynamics *in vitro*

TIRF microscopy of Alexa488-CorM filaments on supported lipid bilayers. Individual filaments can only be tracked during the early stages of polymerization. Images were acquired at 0.15-second intervals. Scale bar: 2 µm.

Supplementary Movie 7:CorM dual color filament tracking *in vitro*

TIRF microscopy of Alexa488-CorM filaments with single Cy5-CorM monomers on supported lipid bilayers. Images were acquired at 1-second intervals. Scale bar: 1 µm.

Supplementary Movie 8: Live cell time lapse microscopy of mNG-MinC

*Anabaena* cells expressing mNG-MinC were grown on 1.5% agar pads and images were acquired at 1-minute intervals.

Supplementary Movie 9: CorM + MinC *in vitro*

TIRF microscopy of pre-assembled Alexa488-CorM filaments. MinC was added at equimolar concentration after 30 seconds. Images were acquired at 1-second intervals. Scale bar: 5 µm.

Supplementary Movie 10: CorM + MinCS50A+L55D *in vitro*

TIRF microscopy of pre-assembled Alexa488-CorM filaments. MinCS50A+L55D was added at equimolar concentration after 30 seconds. Images were acquired at 1-second intervals. Scale bar: 5 µm.

Supplementary Movie 11: CorM + Δ46-67MinC *in vitro*

TIRF microscopy of pre-assembled Alexa488-CorM filaments. Δ46-67MinC was added at equimolar concentration after 30 seconds. Images were acquired at 1-second intervals. Scale bar: 5 µm.

Supplementary Movie 12: CorM + buffer ctrl. *in vitro*

TIRF microscopy of pre-assembled Alexa488-CorM filaments. Buffer was added at a volume equivalent to that of MinC after 30 seconds. Images were acquired at 1-second intervals. Scale bar: 5 µm.

**Figure legends:**

**Figure 1: A ParMR system functioning in cell shape control**

(**A**) Schematic representation of the chromosomal *parMR* operon from *Anabaena*. The promoter region is predicted to be about 400 bp long. Numbers below indicate genomic locus position. (**B**) Sequence identity and similarity (in brackets) in percent of chrParMR from *Anabaena* with chromosome-encoded KpParMR from *K. pneumoniae* and plasmid-encoded ParMR from *E. coli* R1 plasmid. (**C**) Schematic representation of the chromosome and plasmid-encoded ParM and ParR proteins from *Anabaena* and *E. coli*, respectively. chrParM contains a N-terminal 40 amino acid (aa) long intrinsically disordered region (IDR) in addition to globular domain observed in both, chrParM and EcParM. chrParR is longer than EcParR and lacks helix H3 and H4. Instead, helix H5 appears to be split into one large helix (H5a) and one small helix (H5b). Numbers indicate aa positions. (**D**) Maximum-likelihood phylogeny of bacterial ParM. All cyanobacterial sequences (outlined with a dashed line) form a monophyletic clade. Chromosome-encoded sequences are marked with a green background for Cyanobacteria, and with a black dot for other bacterial phyla. The scale bar represents the average number of substitutions per site. For the detailed tree, see Source Data. ParM proteins from the yellow clade structurally resemble previously characterized plasmid segregation systems, whereas the functions of ParM proteins from the green and pink clades remain unknown. (**E**) Micrographs of *Anabaena* WT and Δ*parMR* cells showing an overlay of Direct Illumination Acquisition (DIA) with chlorophyll a (chlA) autofluorescence images and DAPI staining from the same cells. (**F-H**) Scatter plots showing the mean cell property values ± standard deviation (SD) for (**F**) cell width (WT: 4.8 ± 0.96 µm; Δ*parMR*: 4.53 ± 0.47 µm), (**G**) cell length (WT: 2.89 ± 0.35 µm; Δ*parMR*: 3.88 ± 0.56 µm) and (**H**) cell aspect ratio (WT: 0.62 ± 0.13; Δ*parMR*: 0.86 ± 0.09) of *Anabaena* WT and Δ*parMR* cells. WT: n=222 cells; Δ*parMR*: n=259 cells. (**I**) Mean DAPI fluorescence intensity from individual cells plotted against the normalized cell length (0 = one cell pole, 1 = opposite cell pole). Values for WT are shown in cyan and values for Δ*parMR* are shown in grey. Shaded areas correspond to the SD around the mean. The intensity profiles show a peak near the center of the cell, indicating midcell enrichment of DAPI signal, with reduced signal toward the poles. WT: n=46 cells; Δ*parMR*: n=49 cells.

**Figure 2: CorM forms cortical filaments transverse to the long cell axis**

(**A**) Maximum intensity projection micrographs of *Anabaena* cells expressing mNG-ParM from its native locus as the sole copy within the cell. Left: mNG-ParM fluorescence signal, middle: overlap of mNG-ParM and chlorophyll A (chlA) autofluorescence (magenta), right: side view of the left image. (**B**) Cross section view of the same cells as in (**A**) showing the middle plane of the cells, highlighting that mNG-ParM and chlA signals do not overlap. Beige bar indicates region used to measure the intensity plot profile from mNG-ParM and chlA signal as shown in (C). (**D**) Angles of mNG-ParM filaments (n=386 filaments) in respect to the long cell axis. Cartoon indicates process of angle measurement. (**E**) Dynamic localization of mNG-ParM during the cell division cycle (see Supplementary Movie 1). The kymograph (center) shows the mean fluorescence intensity of mNG-ParM over time from 56 dividing cells. Top and bottom cell sketches indicate the chrParM patterns before and after cell division, respectively. Horizontal dashed lines indicate specific time points (~2.5 h, ~7.5 h, ~12.5 h), corresponding to intensity profiles shown on the right. These profiles display the average distribution of mNG-ParM intensities at three key division stages: during growth, before constriction, and after division. Each line profile is the average signal from 3 micrographs taken within a 45-minute time window (images were taken every 15 min). Intensity profiles correspond the normalized mean fluorescence intensity signal from mNG-ParM, while shaded purple regions indicate the cell boundaries. The SD of the cell boundaries is shaded in light purple (n=56). (**F**) Time series from Supplementary Movie 2 displaying TIRF micrographs of individual mNG-ParM filaments over a period of 180 seconds. White triangle marks the initial position of a mNG-ParM filament, and the red triangle indicates its furthest observed position during the time series. (**G**) Kymograph of individual mNG-ParM filaments from TIRF microscopy. White arrows indicate growing filaments whereas magenta arrows indicate filament disassembly/shrinkage. (**H**) Scatter plot showing the growth velocities (mean 18.96 ± 3.61 nm/s) of mNG-ParM filaments as recorded by TIRF microscopy. Error bars represent the mean ± SD of 80 independent filaments measured. (**I**) Micrograph showing merged DIA and mNG-ParM fluorescence images of Δ*parMR* carrying a low copy number plasmid heterologously expressing mNG-ParM from a synthetic *ntcA* promoter integrated into the neutral *thrS2* site on the *Anabaena* chromosome74 resulting in medium expression levels. Unlike in the WT, mNG-ParM loses its transverse localization in the absence of ParR.

**Figure 3: CorR binds to lipid membranes and recruits CorM**

(**A**) EMSA assay with increasing concentrations of CorR or *E. coli* ParR using the promoter region of *corMR* (400 bp) or *parC* (380 bp), respectively, as DNA bait. Concentrations in µM are given above the individual lanes. Blot shows a representative example of six individual experiments. (**B**) Left shows an AlphaFold3 prediction of a CorR dimer with C-terminal CorM interaction site and N-terminal amphipathic helix (AH) indicated. The AH within the first 10 aa was identified using Amphipaseek51. Notably, AlphaFold3 does not predict the N-terminal AH as a helical structure but rather as an unstructured region. This may be because AHs often exist as nascent helices that only adopt their proper fold upon contact with the membrane. Right shows the helical wheel projection of the first 11 aa of CorR and *E. coli* ParR. Arrows indicate the hydrophobic moment of the predicted helix indicating the amphipathicity of the respective proteins. The high hydrophobic moment of CorR indicates the potential to bind to lipid membranes at the interface between the lipid bilayer and the cytosolic phase. (**C**) Representative Coomassie-stained SDS-PAGE gel of a pelleting assay with indicated protein combinations with or without MLVs. 30 % of the total pellet [P] and 12.5 % of the total supernatant [S] samples were separated on the gel. The respective quantifications are shown in Fig. S3B. CorR is enriched in the pellet fraction in the presence of MLVs. CorM alone is not efficiently pelleted at 21,000 x *g* but gets enriched in the pellet when co-incubated with CorR, indicating that CorR recruits CorM to the MLV-containing pellet. CorR lacking the N-terminal AH (Δ1–10CorR) fails to bind MLVs and is thus not enriched in the MLV-containing pellet fraction. In contrast, CorRΔ150–162 retains the N-terminal AH and can be recruited to the pellet via MLV interactions but cannot recruit CorM to the pellet due to the absence of the C-terminal peptide required for CorM binding. Dotted lines separate individual gels stiched together for better visualization. (**D**) Maximum-likelihood phylogeny based on the concatenation of CorM/ParM and CorR/ParR sequences from Cyanobacteria. The presence of a predicted AH in CorR/ParR cyanobacterial homologs is indicated with a red dot. *Anabaena* CorR is indicated with a red circle. The scale bar represents the average number of substitutions per site. For the detailed tree, see Source Data.

**Figure 4: Cryo-EM structure of *in vitro* reconstituted CorM filaments**

(**A**) Cryo-EM micrograph showing CorMR filaments formed in vitro at a protein concentration of 5 µM for each CorM and CorR. (**B**) Cryo-EM density map of *in vitro* reconstituted wild type CorM filament in the presence of CorR (CorMR) at 3.9 Å. The details of the cryo-EM processing pipeline for all the three obtained structures are provided in Fig. S6.1-S6.3. (**C-D**) Refined model of the CorM filament built by fitting the Alphafold-predicted CorM monomer into CorM+CorR cryo-EM density map. ADP molecules are shown. (**E**) The two antiparallel protofilaments are annotated as n and n\*. Rectangles outline the enlarged lateral and longitudinal interaction interfaces in **F**. Both of the interface interactions were determined using PDBePISA (Proteins, Interfaces, Structure and Assemblies)52. (**G**) Comparison of surface representations of CorM (this study), with previously published *E. coli* ParM (PDB entry 5AEY) and *C. crescentus* MreB (PDB entry 4CZJ) models, respectively. Individual protofilaments are colored in dark or light green to illustrate their relative arrangement. Similarities and differences in polarity, subunit arrangement and helical parameters are given below the models.

**Figure 5: *In vitro* reconstitution of CorMR polymerization on supported membranes**

(**A**) Illustration of the experimental assay to reconstitute CorM polymerization with CorR on supported lipid bilayers (SLBs). CorR binds to the SLB through its AH and recruits CorM filaments to the membrane. CorM filaments grow from both sites through bidirectional addition of monomers to an existing filament. (**B**) TIRF micrographs of purified and Alexa488-labeled CorM (0.25 µM) together with unlabeled CorM (0.75 µM) recruited to the SLB through interaction with CorR (0.25 µM). Initially, CorM forms individual filaments (left image) that then eventually form a denser network of filaments (middle image), which ultimately merge into a dense, seemingly highly interconnected meshwork of thin filaments (right image). (**C**) Time series from Supplementary Movie 6 displaying TIRF micrographs of individual Alexa488-labeled CorM filaments showing dynamic instability. Blue triangles mark the initial position of an individual CorM filament that continues to grow throughout the whole time series. Orange triangles mark the initial position of an individual CorM filament that grows initially and then rapidly collapses before regrowing. The purple triangles indicate the furthest observed position of another filament which ultimately undergoes rapid and complete depolymerization during the time series. (**D**) Kymographs of the respective filaments marked with blue, orange and purple triangles in (C) showing growth, catastrophe and rescue events. The filament indicated with blue triangles only grows during the 120 sec time series shown in (C) and eventually also undergoes depolymerization. The filament indicated with purple triangles grows bidirectionally and then completely collapses. (**E**) CorM filament undergoing an initial polymerization phase followed by rapid depolymerization. Growth is represented by the increase in distance between the two poles of the filament over time. The filament polymerizes steadily at a rate of 96.95 nm/s (green line) followed by a rapid depolymerization phase with a rate of -958.07 nm/s (orange line). (**F**) Time series from Supplementary Movie 7 displaying TIRF micrographs of an individual Alexa488-labeled CorM filament supplemented with Cy5-CorM over a period of 32 seconds. Alexa488-CorM: 0.5 µM; Cy5-CorM: 4.48 pM; CorR: 0.25 µM. The Cy5-CorM monomer within the Alexa488-CorM filament remains static while Alexa488-CorM monomers are added to both filament poles. (**G**) Kymograph of CorM filament depicted in (F), confirming the immobile nature of the Cy5-CorM monomer and the bipolar extension of the CorM filament. (**H**) Start (triangle) and end (cross) positions of the two ends of the filament ends (light and dark green) shown in (F) as well as the position of the Cy5-CorM monomer (orange) during the time lapse. (**I**) Growth and shrinkage properties of CorM filaments from *in vitro* time-lapse TIRF microscopy. Scatter plots show the mean ± SD filament velocities of Alexa488-CorM during bipolar growth (76.63 ± 19.23 nm/s; n=13), unipolar growth (29.69 ± 8.28 nm/s; n=10), and shrinkage (-1313.42 ± 671.54 nm/s; n=5), as determined by single-filament tracking from TIRF microscopy. Bipolar growth and shrinkage were measured from single and dual color time-lapse imaging series using Alexa488-CorM or Alexa488-CorM with low levels of Cy5-CorM, while unipolar growth was extracted from dual color time-lapse imaging series only.

**Figure 6: Control of CorMR filaments by the Min system**

(**A**) *in silico* pulldown to identify interactions of CorM (bait) against the whole *Anabaena* proteome (5326 open reading frames included in the analysis). Accuracy of the predicted interaction is given as interface predicted template modelling (ipTM) score as described by AlphaFold275. ipTM scores for CorM, CorR and MinC are highlighted in the plot. (**B**) Scatter plots of MinC sequence lengths (in aa) from all bacteria, cyanobacteria and *Nostocales* cyanobacteria. (**C**) (top) AlphaFold3 prediction of an *Anabaena* MinC dimer. (bottom) A zoom-in into a well-conserved pocket of CorM predicted to bind to the C-terminal a-helix of CorR (blue) and the N-terminal a-helix of MinC (orange), suggesting a competition of CorR and MinC for binding to CorM. Conservation is based on a multiple sequence alignment from all identified cyanobacterial chromosomal CorMs (green clade). Coloring by sequence conservation is based on the entropy-based measure AL2CO from ChimeraX76 using the default coloring scheme (cyan: poor conservation; white: intermediate conservation; red: highly conserved; grey: no conservation). (**D**) *in vivo* time lapse microscopy of *Anabaena* cells expressing mNG-MinC from a synthetic *ntcA* promoter integrated into the neutral *thrS2* site on the *Anabaena* chromosome. Orange arrows indicate the positions of mNG-MinC fluorescence maxima in the uppermost cell over time, highlighting its pole to pole oscillatory behavior throughout the time-lapse analysis. Grey bar spanning the cell filament indicates region used to generate the kymograph show in (E). (**F**) Representative Coomassie-stained SDS-PAGE gel from a high-speed ultracentrifugation-based pelleting assay of 5 µM CorM with or without indicated MinC concentrations in the presence of 2.5 mM ATP (i.e., under polymerizing conditions). 40 % of the total pellet [P] and 11.1 % of the total supernatant [S] samples were separated on the gel. CorM runs at around 45 kDa while MinC runs at ~55 kDa. The respective quantifications are shown in Fig. S8G. (**G**) Representative Coomassie-stained SDS-PAGE gel from a high-speed ultracentrifugation-based pelleting assay of 5 µM CorM with or without 5 µM MinC WT, MinCS50A+L55D, Δ46-67MinC or a synthesized peptide mimicking MinC’s N-terminal a-helix in the presence of 2.5 mM ATP (i.e., under polymerizing conditions). 40 % of the total pellet [P] and 11.1 % of the total supernatant [S] samples were separated on the gel. The respective quantifications are shown in Fig. S10A. (**H**) Snapshots from TIRF microscopy time lapse series extracted from Supplementary Movies 8-11 of pre-formed CorMR filaments (0.75 µM Alexa488-CorM, 0.25 µM CorM and 0.25 µM CorR) on SLBs supplemented with 1 µM MinC WT, MinCS50A+L55D, Δ46-67MinC or buffer. Only MinC WT can depolymerize CorMR filaments on the SLBs. We note that CorMR filaments re-assemble several minutes after addition of MinC WT, indicating that CorM depolymerization by MinC is a transient effect. (**I**) Normalized fluorescence intensity over time from the time lapse series shown in (H), with the initial value set arbitrarily to 1. A decrease in fluorescence signal is observed only upon addition of MinC WT, while both MinC mutants and the buffer control samples cause an increase in fluorescence signal, likely due to mixing after addition, which replenishes the local concentration of CorM monomers near the membrane.

**Figure 7: A quadruple evolutionary event regulated cell shape in *Anabaena***

(**A**) Cyanobacterial phylogenetic species tree with annotations indicating the presence of a chromosomal CorMR system, an amphipathic helix (AH) in CorR homologs, an N-terminal intrinsically disordered region (IDR) in CorM homologs, and a long MinC protein (>300 aa). Monophyletic families were collapsed into single branches. Presence of the respective feature within a family is assumed if at least 50 % of the species within the clade display the feature. The complete tree is available in the Supplementary Figure 5 and Source Data, and detailed information is provided in Supplementary File 1 and 3. (**B**) Proposed model for the regulation of the chromosomal CorMR system in *Anabaena*. CorM assembles into cortical-like filaments oriented transverse to the cell’s long axis. These filaments are dynamically unstable and are recruited to the cytoplasmic membrane by CorR, which has acquired membrane-binding affinity through its N-terminal AH. CorM polymers are excluded from the cell poles and division sites due to the regulatory actions of MinC, which localizes to these regions and actively depolymerizes CorM filaments. (**C**) A model for the evolutionary diversification and cytoskeletal innovation of an existing plasmid segregation system (ParMR), which was functionally repurposed and evolved into CorMR, thereby contributing to the emergence of complex multicellular (cyano)bacterial species.

**Supplementary figure legends:**

**Figure S1: Properties of the chromosomal ParMR**

(**A**) AlphaFold3-predicted structure of chromosomal ParM (chrParM) from *Anabaena* (pTM score: 0.83) and the resolved X-ray structure of plasmid-encoded ParM from *E. coli* (PDB ID: 4A62). (**B**) AlphaFold3-predicted structure of a chromosomal ParR (chrParR) dimer from *Anabaena* (pTM score: 0.34; ipTM score: 0.34) and the resolved X-ray structure of the plasmid-encoded ParR dimer from *E. coli* (PDB ID: 2JD3). For A and B, predicted aligned error (PAE) maps and plDDT score-colored structure predictions for AlphaFold3 predictions are also shown. (**C**) Mean amino acid residues ± SD within the N-terminal IDR from ParM homologs from the green (42.05 ± 11.03), yellow (9.66 ± 11.32) and pink (0.71 ± 2.149) clades of cyanobacterial ParM’s showing that only the green clade has any substantial N-terminal IDR. (**D**) Representative growth curve analysis of *Anabaena* WT and Δ*parMR* revealing that the mutant lacking ParMR has a slight growth defect. OD750 values were recorded every day or every other day for a total of 11 days. Afterwards, cultures became too sticky to properly measure the OD750 value. Error bars represent the mean ± SD of three technical replicates. The experiment was performed twice with similar results. (**E**) Scanning electron microscopy images of (left) *Anabaena* WT and Δ*parMR* revealing cell division defects in 30 % of actively dividing Δ*parMR* cells (6 of 20 cells). These cells displayed irregular or incomplete septa, being partially constricted but not fully separated, a phenotype not observed in the WT (16 cells). Left Δ*parMR* image shows defects in proper constrictions, which are not observed in another subset of Δ*parMR* cells as indicated on the right Δ*parMR* image. (**F**) Complementation attempts of the Δ*parMR* phenotype. DIA micrograph of Δ*parMR* carrying a replicative plasmid expressing *parM*-*parR* from a medium level, unregulated synthetic promoter (PntcA) derived from the promoter of the global regulator of nitrogen homeostasis NtcA from *Anabaena*77. For comparison Δ*parMR* is shown. The swollen phenotype of Δ*parMR* is moderated in the complementation strain but expression of *parM-parR* from PntcA is not sufficient to provide the same cellular levels of ParMR as the native promoter can. However, attempts to clone such construct failed because *E. coli* can employ the PparMR promoter, and we found that excess levels of ParMR are toxic for *E. coli*. (**G**) Micrographs of *E. coli* C41 (DE3) co-expressing mNG-ParM from PT7 and ParR-mScarlet-I from PBAD grown over night in TB medium supplemented with 0.02 % L-Ara and 0.25 mM IPTG at 37 °C. Cells containing ParR-mScarlet-I signal are strikingly malformed, forming huge balloon-like structures. These cells lack any discernible mNG-ParM signal, indicating that they cannot tolerate both proteins together for extended periods. Micrograph with enhanced contrast reveals red fluorescent cell debris, likely originating from burst cells expressing ParR-mScarlet-I. mNG-ParM localizes as filamentous bundles in cells with WT-like morphology that lacked ParR-mScarlet-I signal, suggesting that specifically ParR is responsible for the aberrant cell phenotype.

**Fig. S2: ParM’s N-terminal IDR is not involved in cellular ParM localization or dynamics**

(**A**) Scatter plots showing the mean cell property values ± SD for cell width (WT: 4.8 ± 0.96 µm; Δ*parMR*: 4.53 ± 0.47 µm; mNG-ParM: 3.73 ± 0.65 µm; mNG-D1-40ParM: 3.42 ± 0.49 µm), cell length (WT: 2.89 ± 0.35 µm; Δ*parMR*: 3.88 ± 0.56 µm; mNG-ParM: 4.91 ± 0.56 µm; mNG-D1-40ParM: 4.06 ± 0.29 µm) and cell aspect ratio (WT: 0.62 ± 0.13; Δ*parMR*: 0.86 ± 0.09; mNG-ParM: 0.76 ± 0.09; mNG-D1-40ParM: 0.84 ± 0.09) of *Anabaena* WT, Δ*parMR*, mNG-ParM or mNG-D1-40ParM expressing cells. WT: n=222 cells; Δ*parMR*: n=259 cells; mNG-ParM: n=67 cells; mNG-D1-40ParM: n=144 cells. Values for WT and Δ*parMR* are the same as in Fig. 1F-H. (**B**) mNG-D1-40ParM and merged mNG-D1-40ParM and chlA autofluorescence micrographs of *Anabaena* cells expressing mNG-D1-40ParM as the sole copy obtained with a Nikon SoRa spinning disk confocal microscope. (**C**) Time series from Supplementary Movie 3 displaying TIRF micrographs of individual mNG-D1-40ParM filaments over a period of 230 seconds. White triangles mark the initial position of a single mNG-D1-40ParM filament and red triangles mark the furthest observed position of this filament during the time series. Blue triangles mark the initial position of another single mNG-D1-40ParM filament, yellow triangles mark the furthest observed position of this filament during the time series and purple triangles indicate the position this filament retracted to upon a dynamically instable shrinkage event. (**D**) Scatter plot showing the mean ± SD of the growth velocities of mNG-ParM (18.96 ± 3.61 nm/s; n=80) and mNG-D1-40ParM (35.75 ± 8.19 nm/s; n=58) filaments, revealing the N-terminal IDR is responsible to limit the growth kinetics *in vivo*. (**E**) ParR-mNG, DIA and merged ParR-mNG and chlA autofluorescence micrographs of *Anabaena* expressing ParR-mNG as the sole copy. This strain’s cellular morphology is markedly altered, resembling the Δ*parMR* strain. This suggests that the ParR-mNG fusion is not fully functional, likely because ParR’s C-terminal peptide is essential for interaction with ParM and is sterically hindered by the C-terminal mNG fusion. Note that membrane localization of proteins in *Anabaena* looks mostly diffuse at the periphery as is the case for ParR-mNG. For comparison, *Anabaena* cells expressing soluble mNG are shown on the right side. Micrographs were obtained by a standard Nikon TI2 epi-fluorescence microscope.

**Fig. S3: Biochemical properties of CorM reveal functional divergence from EcParM and regulation by the N-terminal IDR**

(**A**) NTPase activity assay using the Malachite Green Phosphate Assay Kit (Sigma Aldrich) shows that CorM readily hydrolyzes ATP but, unlike the EcParM, is not able to meaningfully hydrolyze GTP. NTPase activity is quantified by the degree of phosphate release during the conversion of NTP to NDP during NTPase activity. Error bars represent the mean ± SD of two independent replicates (n=2). (**B**) Representative Coomassie-stained SDS-PAGE gel from a high-speed ultracentrifugation-based pelleting assay comparing CorM with EcParM (both 5 µM) in the presence of buffer (ctrl.), ATP, ADP, GTP and a non-hydrolyzable ATP analog (AMP-PNP). Nucleotides were provided at 2.5 mM each. 40 % of the total pellet [P] and 11.1 % of the total supernatant [S] samples were separated on the gel (**C, D**) Quantification of the pelleting efficiency as displayed in (B), confirming that CorM only polymerizes in the presence of ATP but not with GTP or AMP-PNP. Error bars represent the mean ± SD of three independent replicates (n=3). (**E**) ATPase activity assay showing that CorM ATPase activity is a concentration dependent process that seemingly begins to plateau at higher concentrations. Error bars represent the mean ± SD of three independent replicates (n=3). (**F**) The degree of CorM polymerization is not a concentration-dependent process and is not altered upon high-levels of ParM (n=1). (**G**) ATPase activity assay comparing CorM with a mutant lacking the first 40 aa (D1-40CorM) reveals that the N-terminal IDR limits CorM’s ATPase activity. Error bars represent the mean ± SD of two independent replicates with 3 technical replicates each. (**H**)

AlphaFold3-predicted interaction between CorM and a CorR dimer, together with the corresponding PAE map, showing interaction of CorR’s C-terminal α-helix H5b with CorM, as well as a plDDT score-colored CorM-CorR complex (pTM score: 0.59; ipTM score: 0.51). (**I**) Representative Coomassie-stained SDS-PAGE gel from a high-speed ultracentrifugation-based pelleting assay comparing ParM and ParR, alone or in combination (5 µM each), incubated with 2.5 mM ATP or without nucleotide (ctrl.) (n=2). 34 % of the total pellet [P] and 9.7 % of the total supernatant [S] samples were separated on the gel (**J**) Quantification of the pelleting efficiency from (C), showing pellet and supernatant values for ParM (left graph) and ParR (right graph) individually. CorR is recruited to the pellet through polymerized CorM (ATP-containing sample). Data represent the mean ± SD from two independent experiments (n=2) (**K**) B2H assays testing for the interaction of chrParM with chrParR from Anabaena fused to either the T25 or T18 subunit, including a ParR variant lacking the predicted C-terminal a-helix, which is known to facilitate the interaction of plasmid-encoded ParR with ParM in *E. coli*. chrParR lacking amino acids 150-162 is no longer able to interact with ParM. Negative control: ParM-T18 with empty pKNT25 plasmid. Positive control: Zip/Zip control. Error bars represent the mean ± SD of three independent replicates (n=3).

**Fig. S4: CorR binds to lipid membranes**

(**A**) Quantification analysis of the CorM with CorR pelleting assay from Fig. 3C showing the values for CorM and CorR separately. P indicates values for pellet samples and S indicates values for supernatant samples. Error bars represent the mean ± SD from at least three independent replicates. (**B**) Representative Coomassie-stained SDS-PAGE gel from a pelleting assay with indicated protein combinations with or without MLVs (proteins are provided as 5 µM each). 30 % of the total pellet [P] and 12.5 % of the total supernatant [S] samples were separated on the gel. (**C**) Quantifications from pelleting assay shown in (B), showing values for CorM and CorR separately. Error bars represent the mean ± SD from three independent replicates (n=3). (**D**) Amino acid sequence logo (using WebLogo78) from the multiple sequence alignment shown in Fig. S5, showing only the first 20 aa of CorR’s N-terminus. To enhance readability, CorR sequences from *Oscillatoriales* and *Nostocales* are shown independently, which also highlights that the N-terminal CorR region is strongly conserved among *Nostocales* species and less so among *Oscillatoriales* species.

**Figure S5: Conservation of CorR’s amphipathic helix**

Cyanobacterial phylogenetic species tree with multiple sequence alignment of CorR homologs containing a N-terminal AH. Since AHs were only found among chromosomal CorMR homologs in *Nostocales* and *Oscillatoriales*, only sequences from these species are shown. The N-terminal region comprising the AH (roughly 10 aa) is highly conserved among *Nostocales* species and less among *Oscillatoriales* species.

**Fig. S6: Cryo-EM image processing strategy applied to obtain structure of CorM filament (CorMR filaments (6.1), D1-40CorM (6.2), and CorM (6.3)) and the final cryo-EM maps (6.4)**

Schematics of the image processing strategy used for determining all the three obtained CorM filament structures using cryoSPARC v4.4. Representative micrographs, 2D class averages, 3D class averages, and final electron microscopy density maps are shown. For helical refinement jobs, the initial estimates of helical rise and helical twist were obtained using online helical indexing tool (HI3D- Helical Indexing using cylindrical projection of a 3D map79). These values are marked with \* under each helical refinement job. Two side views of local resolution filtered EM maps and Fourier Shell Correlation (FSC) curves are displayed. The surface representation of the final cryo-EM maps of CorM, D1-40CorM, and CorMRand their estimated resolutions (using FSC 0.143 criteria) are shown in 6.4.

**Fig. S7: The N-terminal IDR prevents filament bundling and increases protein dynamics**

(**A**) Representative FRAP snapshots from TIRF microscopy time series (Supplementary Movies 4-5) of pre-formed CorMR or D1-40CorMR filaments (0.75 µM unlabeled CorM WT or D1-40CorM, 0.25 µM Alexa488-CorM or Alexa488-D1-40CorM and 0.125 µM unlabeled CorR) on SLBs subjected to photobleaching. (**B**) Dependency of the half-recovery time on CorR concentration. Plot showing the half-recovery time (T1/2) in seconds as a function of CorR concentration (0.125 µM, 0.25 µM, and 0.75 µM). Increasing concentrations of CorR lead to a dose-dependent decrease in T1/2, suggesting higher CorM dynamics with higher CorR levels. Error bars represent the mean ± SD from individual measurements: 0.125 µM: 10.64 ± 0.87 nm/s (n=9); 0.25 µM: 6.97 ± 0.24 nm/s (n=6); 0.75 µM: 4.94 ± 0.14 nm/s (n=5). Half-recovery times comparing CorM WT and D1-40CorM in the presence of 0.125 µM CorR, showing that the N-terminal IDR in CorM modulates dynamics and reaction kinetics. Error bars represent the mean ± SD from individual measurements: CorM values are the same as in (B);  D1-40CorM: 24.65 ± 1.22 nm/s (n=7). (**D**) Representative recovery curves from FRAP experiments comparing CorM WT and D1-40CorM in the presence of 0.125 µM CorR. (**E**) Representative snapshots of D1-40CorM (protein concentrations same as in A) filaments in the course of an imaging session. Like CorM WT, D1-40CorM initially forms individual filaments. These filaments then form thick bundles but eventually assemble into a well-defined network of filaments, which appears much more ordered compared to CorM WT (see Fig. 5B for comparison).

**Fig. S8: Properties of the *Anabaena* Min system and it’s modulating function on CorM**

(A) (top) AlphaFold3-predicted interaction between CorM and MinC, together with the corresponding PAE map, showing interaction of MinC’s N-terminal α-helix with CorM, as well as a plDDT score-colored CorM-MinC complex (pTM score: 0.51; ipTM score: 0.76). (bottom) AlphaFold3-predicted MinC dimer (pTM score: 0.46; ipTM score: 0.47), including the corresponding PAE map and a plDDT score-colored dimer. (**B-D**) B2H assays testing for the interaction of CorMR with (**B**) MinC, (**C**) Cdv3 or (**D**) MinD and MinE fused to either the T25 or T18 subunit. Both, CorM and CorR interact with MinC but only CorR interacts with Cdv3. Neither CorM nor CorR interacts with MinD or MinE. Negative control: (B, D) CorM-T25 with empty pUT18C plasmid or (C) CorM-T18 with empty pKNT25 plasmid. Positive control: Zip/Zip control Error bars represent the mean ± SD of three independent replicates (n=3). (**E**) B2H assays testing for the interaction of MinC with MinD, MinE and Cdv3 fused to either the T25 or T18 subunit. Results show that MinC interacts with all three proteins. Negative control: MinC-T25 with empty pUT18C plasmid. Positive control: Zip/Zip control. Error bars represent the mean ± SD of three independent replicates (n=3).

**Fig. S9: In *Nostocales* cyanobacteria MinC exhibits an extended N-terminal domain**

MinC phylogenetic tree, including cyanobacteria and other bacterial species. Blue-shaded cubes next to the tree indicates MinC sequence lengths. Note that specifically *Nostocales* cyanobacteria contain long MinC sequences (>300 aa), which is represented by an N-terminal IDR with an a-helix in between. On the right, a multiple sequence alignment of all listed MinCs is shown with two different conservation patterns. Orange shades indicate conservation exclusively among the N-terminal IDR of *Nostocales* MinCs whereas grey shades indicate conservation exclusively among the globular domain of MinC. Black dots indicate Ultrafast Bootstrap supports (UFB) >= 85. The scale bar represents the average number of substitutions per site.

**Fig. S10: MinC’s N-terminal a-helix mediates CorM depolymerization**

(**A**) AlphaFold3-predicted structures of (top) plasmid-encoded ParM (#BAB78165.1; All7081) from the large low copy number Alpha plasmid from *Anabaena* or (bottom) plasmid-encoded EcParM (WP\_000959884.1) from the R1 plasmid. Conservation is based on multiple sequence alignments from all identified cyanobacterial plasmid-encoded ParMs or all identified other bacterial plasmid-encoded ParMs. Coloring by sequence conservation is based on the entropy-based measure AL2CO from ChimeraX76 using the default coloring scheme (cyan: poor conservation; white: intermediate conservation; red: highly conserved). (**B**) mNG-MinC fluorescence, chlA autofluorescence and merged micrographs of *Anabaena* expressing mNG-MinC from PntcA integrated into the neutral chromosomal *thrS2* sites. Fluorescence intensity profiles show high levels of MinC at the cell poles and midcell in diving cells and only at the cell poles in vegetative cells. (**C**) Quantifications from pelleting assay shown in Fig. 6F showing values for CorM and MinC separately. Different MinC protein concentrations are indicated below. Error bars represent the mean ± SD of two independent replicates (n=2). (**D**) Sequence logo showing the conservation of aa residues within the N-terminal a-helix of *Nostocales* cyanobacteria. A multiple sequence alignment of all tested MinC sequences from cyanobacteria and other bacteria is shown in Fig. S9. (**E**) Quantifications from pelleting assay shown in Fig. 6G showing values for CorM. Both MinC helix mutants, MinCS50A+L55D and D46-67MinC lost the ability to depolymerize CorM filaments. A synthesized peptide mimicking MinC’s N-terminal a-helix had no influence on CorM polymerization, indicating that other aspects of the interaction of CorM with MinC are essential for active depolymerization. Error bars represent the mean ± SD of three independent replicates (n=3). (**F**) CorM ATPase activity assay with or without indicated MinC concentrations resolved over time, showing that MinC acts on CorM by decreasing its enzymatic activity. Data represents the mean values for each data point from three independent replicates (n=3). (**G**) B2H assays testing for the interaction of CorM with MinC helix mutants (MinCS50A+L55D and D46-67MinC) fused to either the T25 or T18 subunit. Results show that CorM interaction with MinC is lost in both MinC helix mutants. Negative control: CorM-T25 with empty pUT18C plasmid. Positive control: Zip/Zip control. Error bars represent the mean ± SD of three independent replicates (n=3).