



The Diversity of Filamentous Morphologies and Magnetic Sensitivity Modulated by Diverse MagR Expression in Bacteria*

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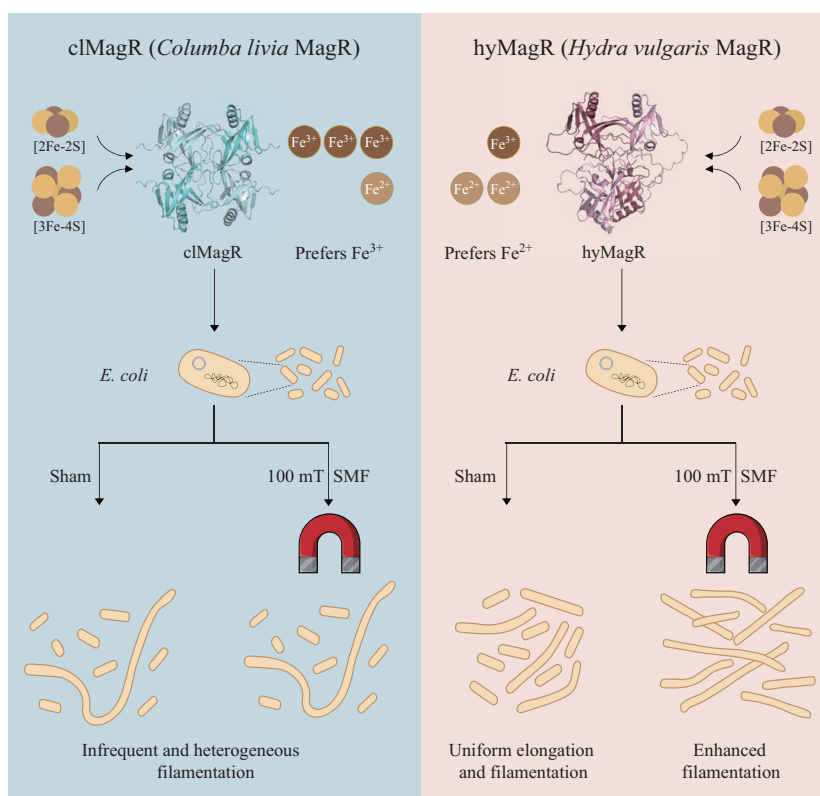
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Graphical abstract



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Abstract Objective Magnetoreception, the remarkable ability of diverse animals to sense and utilize the geomagnetic field for orientation and navigation, remains a molecularly unresolved mystery in sensory biology. The putative magnetoreceptor (MagR, previously known as IscA1) is a highly conserved iron-sulfur protein implicated in both magnetoreception and iron metabolism; however, the functional diversity among its cross-species homologs remains poorly understood. Cellular morphology is a key genetically determined trait that can be altered through genetic or environmental modifications—a process known as cell morphology engineering. Constructing engineered cells with specific morphological features and magnetic sensitivity to achieve remote, non-invasive magnetic modulation represents a crucial goal in this field with significant application potential. Therefore, this study aims to systematically investigate the effects of MagR heterologous expression on bacterial morphology and magnetic sensing capabilities, screen for MagR-based magnetically sensitive morphology engineering pathways, and reveal the underlying molecular mechanisms. **Methods** We systematically screened 28 MagR homologous genes from diverse prokaryotic and animal taxa to evaluate their expression and corresponding phenotypic effects in *Escherichia coli* (*E. coli*). To compare the differential magnetic responses among bacteria expressing various recombinant MagR proteins, we utilized high-throughput automated bright-field microscopic imaging and scanning electron microscopy (SEM). Furthermore, comprehensive biochemical and biophysical characterizations of iron and iron-sulfur cluster binding were performed using Ferrozine colorimetric assays, electron paramagnetic resonance (EPR) spectroscopy, ultraviolet-visible (UV-Vis) absorption, and circular dichroism (CD) spectroscopy. Additionally, 100 mT static magnetic field (SMF) exposure experiments were conducted to assess magnetically tunable phenotypes, while the intrinsic magnetic properties of purified MagR proteins were directly measured using a superconducting quantum interference device (SQUID) magnetometer. **Results** Our results demonstrated that the heterologous expression of MagR homologs induced varying degrees of bacterial filamentation. From this comprehensive screen, two distinct morphological patterns were identified: hydra (*Hydra vulgaris*) MagR (hyMagR) promoted uniform cell elongation and filamentation, exhibiting robust magnetic sensitivity manifested as significantly enhanced filamentation under the 100 mT SMF. In contrast, pigeon (*Columba livia*) MagR (clMagR) induced only low-frequency, extreme filamentation (sporadically exceeding 80 μm) with a relatively weaker magnetic morphological response. Mechanistically, our data unambiguously proved that these phenotypic differences are primarily driven by distinct iron redox preferences rather than total cellular iron accumulation. Specifically, hyMagR preferentially binds ferrous iron (Fe^{2+}), whereas clMagR favors ferric iron (Fe^{3+}) and forms more stable iron-sulfur clusters. Intriguingly, although SQUID magnetometry showed that purified clMagR exhibited approximately five-fold higher mass magnetic susceptibility than hyMagR, its cellular magnetic response was weaker. We hypothesize that the Fe^{2+} -preferred intracellular environment associated with hyMagR overexpression primes the cell for enhanced generation of reactive oxygen species (ROS) *via* the Fenton reaction. Exposure to an SMF synergizes with this primed redox state, triggering the bacterial SOS response and upregulating cell division inhibitors to efficiently induce uniform filamentation. **Conclusion** Our findings identify the $\text{Fe}^{2+}/\text{Fe}^{3+}$ redox state as a critical determinant of MagR-mediated morphological remodeling and magnetic responsiveness. This discovery suggests a potential strategy for engineering magnetically responsive cellular systems for synthetic biology applications, and provides a plausible framework, which potentially combines intrinsic protein magnetism with redox-state modulation, for further investigating the evolutionary mechanisms of MagR-mediated magnetoreception.

Key words MagR homologs, MagR overexpression, filamentous morphology, iron redox state, magnetic sensitivity

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The geomagnetic field, one of the fundamental physical fields generated by our planet, has persisted throughout the entire course of biological evolution. All living organisms on Earth including bacteria, algae, fungi, plants and animals, have evolved under its constant influence. Notably, some animals gradually developed the ability to detect the geomagnetic field—a sensory modality known as magnetoreception (MagR)—and utilize it for long-distance orientation and navigation^[1-13]. Despite compelling behavioral evidence, the underlying mechanism of MagR has intrigued scientists for over one century and remains one of the least understood mysteries in sensory biology to this day^[14-16].

Several biophysical mechanisms have been proposed to explain how animals sense magnetic field^[17-18]: (1) the Magnetite-based Model, where biogenic magnetite crystals act as magnetic sensors^[19-22]; (2) the Radical Pair Model, a light-dependent chemical process within cryptochrome (Cry) acts as quantum compass^[23-27]; (3) the Electromagnetic Induction Model, where animal's movement through the magnetic field may generate detectable currents, particularly in some aquatic species^[28-29] and probably also in the vestibular system in pigeons^[30]; (4) the MagR/Cry Biocompass Model, in which a MagR/Cry protein complex may sense the geomagnetic field *via* both intrinsic magnetic moment of the complex and the spin states of the Cry protein^[31-32].

As a highly conserved iron-sulfur protein, MagR (previously known as IscA1) has been implicated as a key player in both magnetoreception and iron metabolism. Over the past decade, accumulating experimental evidence has systematically characterized the magnetic properties and regulatory mechanisms of MagR, uncovering an unusual diversity in its biochemical and biophysical features. Specifically, studies indicate that the magnetic sensitivity of MagR can be modulated by factors such as the valence states of its bound iron-sulfur clusters^[33], the presence of mononuclear iron^[34], and its hierarchical protein assembly^[35]. Furthermore, small-angle scattering experiments have demonstrated that an external magnetic field can, in turn, directly induce the molecular assembly and aggregation of MagR^[36-37]. Despite its high sequence conservation, MagR demonstrated substantial molecular diversity across evolution from bacteria to humans^[38]. For

instance, a two-residue variation between pigeon MagR and European robin MagR results in remarkable differences in their iron and iron-sulfur binding capacity and protein conformation^[39]. In addition, some reports have also highlighted a vital role of MagR as regulator in iron metabolism and iron-sulfur biogenesis^[40-42].

Various applications of MagR have been developed recently based on either its magnetic properties or its regulatory role in cellular iron metabolism. Examples include protein purification using MagR as a magnetic tag^[43-45]; the development of MagR-based kits for coronavirus disease 2019 (COVID-19) detection^[46]; the construction of bionic magnetic sensors using MagR/Cry complex on a chip^[47-48] and its application as a contrast enhancer for magnetic resonance imaging^[49-51]; as well as the recent engineering of MagR as a novel magnetogenetic actuator for remote neuromodulation^[52]. In earlier work, we also observed that heterologous overexpression of pigeon (*Columba livia*) MagR (clMagR) altered cellular iron metabolism in *Escherichia coli* (*E. coli*), resulting in distinct filamentous morphological changes^[53], which may serve as the first step for morphology engineering through MagR-mediated modulation of cellular iron metabolism. However, the relatively low efficiency of clMagR-induced filamentation has constrained its broader application.

Considering the remarkable diversity of MagR's molecular properties across species, we sought to investigate whether the capacity to induce bacterial filamentation is a universal property of animal MagRs upon overexpression and whether a specific MagR variant could achieve more uniform and efficient filamentation. To this end, we conducted a cross-species screen of cell morphology by heterologously expressing 28 homologs from diverse animal phyla in *E. coli*. Distinctive filamentation patterns were observed, and one particular MagR, hydra (*Hydra vulgaris*) MagR (hyMagR), consistently induced uniform cell elongation and filamentation. This significantly contrasted with the low-frequency filamentation previously observed with clMagR, providing a potential candidate for further investigating whether such engineered bacteria could exhibit a controllable response to an external magnetic field.

To elucidate the underlying mechanism and the

differences between cIMagR and hyMagR, comprehensive biochemical and biophysical characterization was performed. Comparative analyses revealed that the filamentous morphological change was not simply correlated with total intracellular iron accumulation, but rather depended on iron valence states, where an increase in ferrous iron proportion, rather than ferric iron, specifically triggered cell filamentation upon MagR expression. Based on these observations, we further explored the potential for MagR-based morphological engineering to create cell lines with controllable response to external magnetic fields. Experiments were designed to determine whether hyMagR-expressing filamentous cells confer magnetic sensitivity (defined here as a magnetically tunable morphological phenotype). Our results demonstrated that filamentation in hyMagR-expressing bacteria was markedly enhanced under a 100 mT static magnetic field (SMF), whereas the effect on the cIMagR-expressing strain was minimal. Intriguingly, direct magnetic measurements revealed that purified cIMagR possessed higher magnetic susceptibility than hyMagR. This implies that MagR-mediated magnetic sensitivity may not solely depend on the intrinsic protein magnetism, but may also be affected by the iron redox preference.

Taken together, our data unambiguously proved that the increased ferrous iron triggered MagR-dependent cell filamentation upon MagR expression. The overexpression of hyMagR induced uniform filamentation and conferred controllable magnetic response, providing a practical platform for morphological engineering. These findings repositioned MagR as a multifunctional module for magnetoreception and iron metabolism. Further investigation of MagR's physiological roles in migratory animals and across broader phylogenetic groups will be essential to elucidate its evolutionary and functional diversity.

1 Materials and methods

1.1 Multiple sequence alignment

Based on our previously compiled large-scale dataset of MagR protein sequences^[38], MagR sequences from 28 representative species spanning prokaryotic and animal taxa were selected for in-depth analysis in this study (Table S1). All sequences were aligned using ClustalW ([megasoftware.net/citations\) and visualized with ESPript v.3.0 \(<https://esprict.ibcp.fr/ESPript/ESPript/index.php>\) based on sequence identity \(Figure 1a\).](https://www.</p></div><div data-bbox=)

1.2 Bacterial strains, culture conditions, and growth analysis

All cellular experiments were performed using *E. coli* strain BL21 (DE3). To screen for MagR proteins inducing filamentation, recombinant plasmids harboring MagR genes from 28 different species (constructed as described previously^[31]) were individually transformed into this strain. Transformants were selected on lysogeny broth (LB) agar plates containing kanamycin. A single colony for each construct was inoculated into 30 ml of LB liquid medium containing the same antibiotic and grown at 37°C with shaking at 220 r/min until the absorbance at 600 nm (A_{600}) reached approximately 0.6. Protein expression was then induced by adding isopropyl β -D-1-thiogalactopyranoside (IPTG) to a final concentration of 20 μ mol/L, and cultures were incubated at 15°C with shaking at 160 r/min for 20–24 h to promote soluble expression and facilitate the observation of filamentation phenotypes.

To analyze the effect of SMF, the experimental setup was based on previously reported methods^[53]. During the cultivation and induction phase, cultures were placed on top of neodymium iron boron (NdFeB) N38 permanent magnets (10 cm×10 cm×2 cm, Hefei Gaoshu Magnetic Materials, China), with the surface magnetic field intensity calibrated to 100 mT. To ensure maximum consistency, culture vessels were placed at fixed positions directly centered on the magnet surface; thus, all experimental groups (plasmid with N-terminal Strep-tag (pNS) (control group), cIMagR, and hyMagR) were exposed to identical spatial configurations and field distributions. A non-magnetic aluminum 6061 (Al 6061) alloy block of identical dimensions (Hefei Gaoshu, China) served as the sham control, representing the ambient geomagnetic field condition (approximately 50 μ T) without the application of an external SMF.

To analyze growth kinetics in liquid culture, the above strains were cultivated with or without SMF, and A_{600} was monitored periodically using a NanoDrop spectrophotometer (NanoDrop OneC, Thermo Scientific, USA) to generate growth curves. To assess growth on solid medium, equal volumes of

bacterial culture were spread on LB agar plates containing antibiotics. After incubation under identical magnetic conditions, colony diameters were measured using ImageJ, employing an analysis pipeline consistent with that used for cell length quantification.

1.3 Protein expression and purification

The codon-optimized coding sequences for the selected 28 MagR homologs were synthesized by Sangon Biotech (Shanghai, China). Expression vectors were constructed as described previously^[31]. All MagR proteins were expressed with an N-terminal Strep-II tag. To rapidly verify the expression of each MagR, bacterial cells from equal A_{600} volumes were collected and analyzed by SDS-PAGE.

For subsequent biochemical and biophysical characterization, the selected pigeon (*Columba livia*) MagR (clMagR) and hydra (*Hydra vulgaris*) MagR (hyMagR) were purified following the method as described previously^[38]. Briefly, induced cells were lysed by sonication, and the supernatant was applied to a Strep-Tactin affinity column (IBA GmbH, Germany). The target proteins were eluted with elution buffer containing 5 mmol/L desthiobiotin. Protein purity was verified by SDS-PAGE, and concentration was determined by measuring absorbance at 280 nm using a NanoDrop spectrophotometer.

1.4 Scanning electron microscopy sample preparation and imaging

Samples for SEM were prepared as follows. Bacterial cultures overexpressing MagR proteins or containing the empty vector (pNS) were harvested by centrifugation. The cell pellet was resuspended in 2.5% glutaraldehyde fixative and fixed at room temperature for 4 h on a decolorizing shaker. Fixed samples were washed three times (10 min each) with 0.1 mol/L phosphate buffer (pH 7.2). Subsequently, the samples were dehydrated through a graded ethanol series (30%, 50%, 70%, 85%, and 95%, 10 min per step), followed by two 10-min treatments with 100% ethanol. The dehydrated samples were dried for 24 h using a freeze dryer (Christ, Alpha 2-4 LSCbasic, Germany). The dried bacterial powder was evenly

dispersed on a sample stub with conductive tape, and loose particles were removed using a blower bulb. A thin gold coating was applied using a high-vacuum sputter coater (QUORUM, Q150T ES Plus, UK) for 2 min. Finally, the prepared samples were observed under a field-emission scanning electron microscope (Hitachi SU-70, Japan). Imaging was performed at an accelerating voltage of 8.0 kV in secondary electron detection mode. Multiple random fields of view were captured for each sample at various magnifications. Bacterial cell lengths were quantified from the SEM micrographs using ImageJ.

1.5 Bright-field microscopy imaging and filamentation quantification

To quantitatively assess the filamentation phenotype, bacterial cultures expressing different MagR proteins or the empty vector (pNS) were normalized to the same A_{600} , washed, and prepared on slides for observation. Bright-field images were acquired using an inverted fluorescence microscope (Nikon Ts2-FL, Japan), with multiple random fields of view captured per sample.

Image analysis was performed using a custom Python (version 3.11.4) pipeline. To ensure reproducibility and transparency, the complete source code and processing scripts have been deposited in a public repository (GitHub:<https://github.com/Cya-hub/Count.git>). Original images were converted to 8-bit grayscale. Binary masks were then generated *via* thresholding and morphological denoising to isolate individual bacterial cells. Following spatial calibration using an embedded micrometer scale bar, a contour detection algorithm was employed to identify bacterial objects, count cell numbers, and calculate cell length based on the arc length of their contours. Filamentation efficiency was quantified as the percentage of filamentous cells (length $\geq 15 \mu\text{m}$; ≈ 7 –8 times the typical cell length). For reference, cells with a length $\geq 5 \mu\text{m}$ (≈ 2 –3 times the typical length) were categorized as elongated. To avoid the possible effects from variations in MagR protein abundance, the filamentation efficiency was normalized to the relative MagR expression level. The normalized value was calculated as follows:

$$\text{Normalized filamentation efficiency} = \frac{\text{Percentage of filamentous cells}}{\text{Intensity of MagR band/Total intensity of the lane}}$$

where the denominator represents the relative MagR expression level, determined by densitometric

analysis of SDS-PAGE using ImageJ. This total protein normalization method has been demonstrated

to be a more reliable loading control than the use of single housekeeping proteins^[54-55]. These metrics, along with bacterial length distribution and scatter plots, were used to assess the extent of filamentation. Colony diameter measurements on solid agar plates were performed using the similar image analysis pipeline.

1.6 Ferrozine assay for iron quantification

Intracellular and purified protein iron content was determined using the Ferrozine colorimetric assay, a robust and time-efficient technique for iron analysis in biological samples^[56]. This method relies on the formation of a purplish-red complex between Fe^{2+} and Ferrozine, which exhibits a characteristic absorption peak at 562 nm^[57]. Total iron was measured after reducing Fe^{3+} to Fe^{2+} with hydroxylamine hydrochloride. To measure intracellular iron in bacteria, cell pellets expressing cIMagR, hyMagR, or the empty vector (pNS) post-induction were harvested, extensively washed with ddH₂O, and lyophilized. Twenty milligrams of dried powder was extracted with 0.5 mol/L HCl and diluted for analysis. Purified cIMagR and hyMagR proteins were analyzed directly.

A standard curve was generated using a series of FeCl_3 solutions (0–1.0 mmol/L) dissolved in 1 mol/L HCl. In a 96-well plate, reactions for Fe^{2+} measurement (80 μL of 1 mol/L HCl) and total iron measurement (80 μL of 10% hydroxylamine hydrochloride solution) were set up, with 20 μL of sample or standard added to each well. The plates for total iron measurement were incubated at 37°C in the dark for 30 min. Subsequently, 100 μL of Ferrozine working solution (0.1% in 50% ammonium acetate) was added to all wells, followed by incubation at 37°C in the dark for 15 min. Absorbance was measured at 562 nm using a microplate reader (Thermo Scientific, Varioskan Lux). The concentrations of Fe^{2+} and total iron were calculated based on the standard curve, and Fe^{3+} content was derived by subtraction. Each experiment was performed with 6 independent replicates.

1.7 Electron paramagnetic resonance (EPR) spectroscopy

Electron paramagnetic resonance (EPR) spectroscopy was employed to analyze the iron-binding status in whole bacterial cells and purified

proteins, as well as the oxidation states of iron-sulfur clusters. For whole-cell samples, bacterial pellets expressing cIMagR, hyMagR, or the empty vector (pNS) were harvested and resuspended in LB medium containing 20 mmol/L deferoxamine, followed by incubation at 37°C for 15 min as previously described^[58]. The cells were then washed once with 10 mmol/L diethylenetriaminepentaacetic acid and twice with 20 mmol/L cold Tris-HCl (pH 7.4), and finally resuspended in 20 mmol/L cold Tris-HCl (pH 7.4). A 200 μL aliquot of the bacterial suspension or purified protein sample was mixed with 50 μL glycerol, loaded into a quartz EPR tube (Wilma, 707-SQ-250 M, USA), and flash-frozen in liquid nitrogen. X-band EPR spectra were acquired using a spectrometer equipped with a liquid helium cryostat. For iron-binding analysis, measurements were performed at 10 K over a magnetic field range of 0–300 mT with the following parameters: microwave frequency 9.40 GHz, microwave power 2 mW, modulation amplitude 0.2 mT, and receiver gain 1.0×10^4 . To characterize the oxidation states and thermal stability of the iron-sulfur clusters, purified cIMagR and hyMagR proteins were measured at multiple temperatures (10 K, 25 K, 45 K, and 60 K) over a magnetic field range of 300–380 mT. This specific magnetic field range was chosen to encompass the characteristic $g \approx 2.0$ signals (including [3Fe-4S], [2Fe-2S] and [4Fe-4S] cluster), which is typical for MagR/IscA family proteins under X-band frequency based on previous reports^[31, 33, 59]. All measurements were performed under identical spectrometer settings to ensure comparability.

1.8 Ultraviolet-visible (UV-Vis) absorption analysis and circular dichroism (CD) spectroscopy

Both ultraviolet-visible (UV-Vis) absorption and circular dichroism (CD) spectral measurements were performed at room temperature. The cIMagR and hyMagR proteins for analysis were prepared in TBS buffer (20 mmol/L Tris, 150 mmol/L NaCl, pH 8.0) at a concentration of 0.1 mmol/L. UV-Vis absorption spectra were recorded from 300 to 600 nm using a NanoDrop OneC spectrophotometer (Thermo Scientific, USA). CD spectra were acquired on a MOS-500 spectropolarimeter (BioLogic, France) for the analysis of optical activity associated with their metal cofactors and iron-sulfur clusters.

1.9 Superconducting quantum interference device (SQUID) magnetometry

The magnetic properties of purified clMagR and hyMagR proteins were characterized using a SQUID magnetometer (MPMS-XL-5, Quantum Design, USA). Protein samples were prepared in Buffer E (20 mmol/L Tris, 150 mmol/L NaCl, 5 mmol/L D-dethiobiotin, pH 8.0) and sealed in a sample holder for measurement. Magnetization versus applied field (M-H) curves were collected at 300 K over a field range of -100 to +100 mT utilizing the instrument's reciprocating sample option. The diamagnetic background signal from the buffer and sample holder was subtracted from the raw data to obtain the net protein magnetization curves.

1.10 Statistical analysis

All statistical analyses were performed using GraphPad Prism software (version 10.4.1). Data normality was assessed using the Shapiro-Wilk test. To account for the non-normal distribution of single-cell lengths and avoid artificial inflation of statistical power from large sample sizes, quantitative group comparisons were strictly performed using the mean values of three independent biological replicates ($n=3$, with >500 cells analyzed per replicate). Data are presented as the mean $\pm$ standard error of the mean (SEM). Two-tailed Student's *t*-test was used for two-group comparisons (iron content). Multi-group comparisons for the percentage of filamentous cells were analyzed by two-way ANOVA with Dunnett's or Šidák's multiple comparisons tests. Colony diameters were analyzed using a Mixed-effects model followed by Šidák's multiple comparisons test, where "strain type" and "magnetic field exposure" were defined as fixed effects, and "experimental batch" was treated as a random effect to control for biological and technical replicates. Statistical significance was set at $P<0.05$.

2 Results

2.1 MagR overexpression induces diverse filamentous morphologies in *Escherichia coli*

In a previous study, we found that heterologous overexpression of pigeon (*Columba livia*) MagR (clMagR) in *E. coli* elongates bacterial cells, converting the typical rod-shaped morphology (1.0–3.0 μm in length)^[60] into filaments exceeding 15 μm by modulating cellular iron metabolism. However, this occurred at a reproducible but low efficiency^[53].

This phenotype suggests MagR could be a valuable tool for bacterial morphology engineering if its efficiency and the uniformity of filamentation could be enhanced. We therefore performed a cross-species screen of MagR homologs to systematically evaluate their engineering capacity and to identify homologs with enhanced or unique phenotypic effects.

A total of 28 representative MagR sequences from the NCBI database, spanning seven major taxonomic groups including prokaryotes, mollusks, arthropods, teleost fish, reptiles, birds, and mammals, were selected for this study (Figure 1a, Table S1). Each sequence was heterologously overexpressed in *E. coli* BL21 (DE3) cells. Successful expression was confirmed by SDS-PAGE analysis for all but the golden mole MagR, which was barely detectable (Figure 1c). To evaluate their capacity to induce filamentation, we quantified the filamentation efficiency, defined as the percentage of "filamentous cells" (length $\geq 15 \mu\text{m}$, vs. "elongated cells" at $\geq 5 \mu\text{m}$), and normalized this value to the corresponding MagR expression level. Our screen identified five MagR homologs from *Hydra vulgaris* ($P<0.0001$), *Dermochelys coriacea* ($P<0.0001$), *Homo sapiens* ($P<0.0001$), *Columba livia* ($P=0.0014$), and *Salmo salar* ($P=0.0014$), that exhibited significantly higher filamentation induction efficiency per unit of protein compared to the others (Figure 1b). It was worth pointing out that the most uniform filamentation was observed in bacteria overexpressing the hydra (*Hydra vulgaris*) MagR (hyMagR). Therefore, hyMagR was selected for further characterization, with the well-studied clMagR included for comparison.

2.2 Distinct filamentation morphologies induced by clMagR and hyMagR

To characterize the diverse filamentous morphologies induced by clMagR and hyMagR overexpression in *E. coli*, field-emission SEM was applied for high-resolution imaging. Control cells transfected with an empty vector (pNS) retained the typical short-rod shape of *E. coli* (Figure 2a). In contrast, clMagR overexpression produced a heterogeneous population, as previously reported^[53] (Figure 2b). While most cells remained near the normal length or showed only modest elongation, rare but striking examples of extreme filamentation (occasionally $>80 \mu\text{m}$) were consistently observed. In sharp contrast, hyMagR overexpression induced a

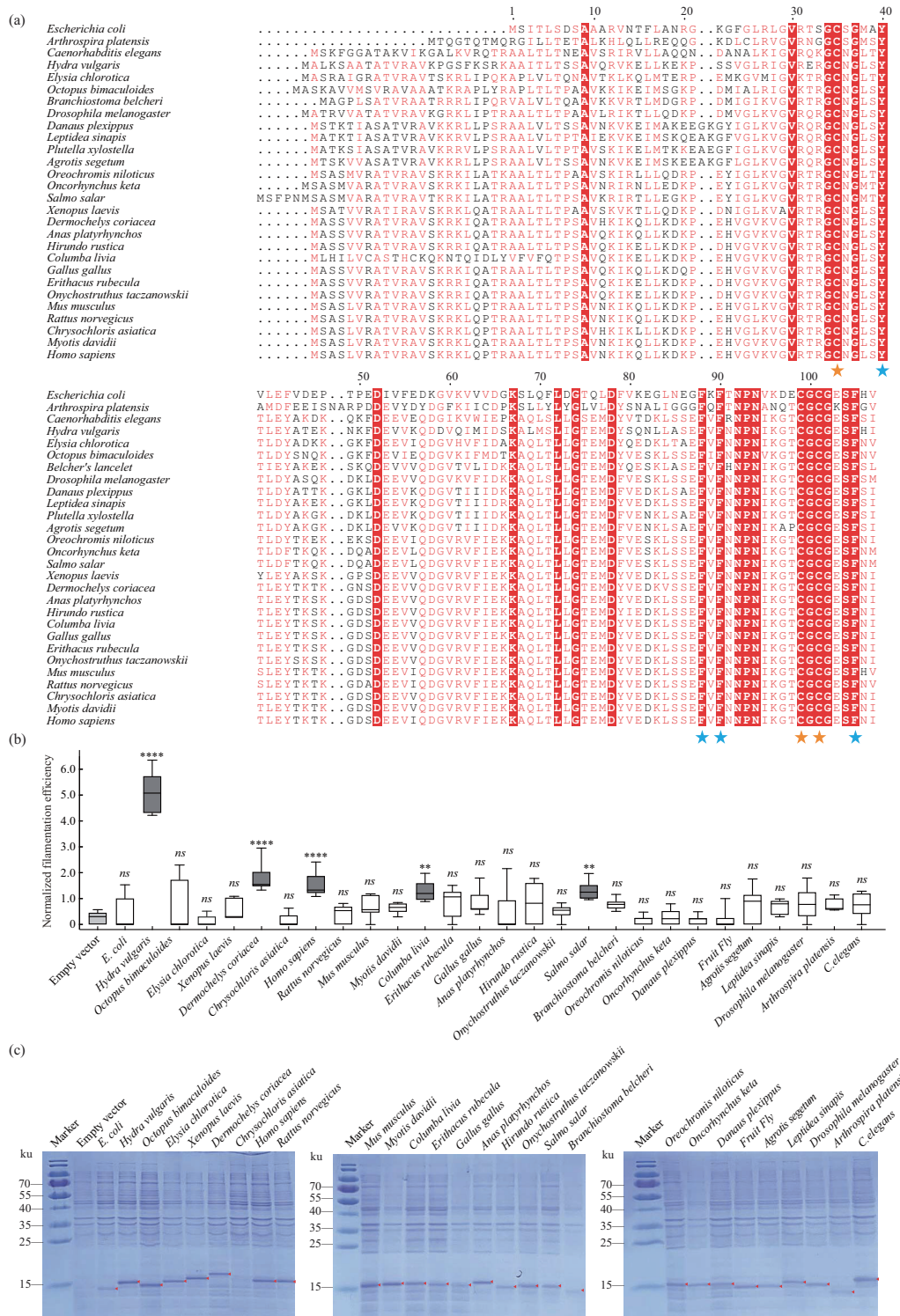


Fig. 1 Screening of MagR homologs identifies variants with filamentation-inducing activity

(a) Sequence alignment of MagR from 28 representative species (Latin names are used for taxonomic accuracy; see Table S1 for full details). Residues conserved across all species are shown in white font on a red background; other conserved residues are highlighted in red. Residues critical for iron-sulfur cluster binding are indicated by orange asterisks. Residues involved in electron transfer are marked by blue asterisks^[38].

(b) Filamentation-inducing capacity of MagR homologs in *E. coli*. For each homolog, the percentage of filamentous cells ($\geq 15 \mu\text{m}$) was quantified and normalized to its expression level (calculated as the ratio of the MagR band intensity to the total protein intensity of the entire SDS-PAGE lane). The bar graph (with overlaid individual data points) shows the normalized filamentation efficiency. The five homologs inducing statistically significant filamentation compared to the empty-vector control are indicated with solid bars. Data are mean $\pm$ standard error of the mean (SEM) ($n=7$). Statistical significance: ordinary one-way ANOVA with Dunnett's multiple comparisons test (** $P < 0.01$, **** $P < 0.0001$; ns, not significant).

(c) SDS-PAGE analysis of recombinant MagR proteins expressed in *E. coli*. Whole-cell lysates from induced cultures were separated by gel electrophoresis. The red arrows indicate the expected position of MagR proteins (~15 ku). The empty vector lane serves as a negative control.

uniform and synchronous shift of the population toward elongated morphologies (Figure 2c). Cell lengths were predominantly distributed between 5 and 20 μm , representing a consistent elongation with frequent filament formation, in clear contrast to the sporadic extreme filaments observed in cIMagR-expressing cells. Notably, cell width remained consistently unchanged under all conditions, indicating a specific defect in cell division (septation) rather than general cell enlargement. However, it should be noted that the multi-step SEM preparation—involving chemical fixation, graded dehydration, and freeze-drying—can impose significant mechanical stress on bacterial cells. These rigorous treatments are

prone to causing entanglement, fragmentation, or selective loss of extremely long filaments (tens of micrometers), which are inherently more fragile than shorter cells. Furthermore, cell stacking in dried samples introduces significant artifacts for automated quantification. Despite these constraints, we performed quantification of filamentation ratios using ImageJ from SEM micrographs (Figure S1), which generally aligns with the observed trends. Therefore, SEM was primarily employed for high-resolution structural characterization, while population-wide statistical analysis was reserved for more robust imaging modalities.

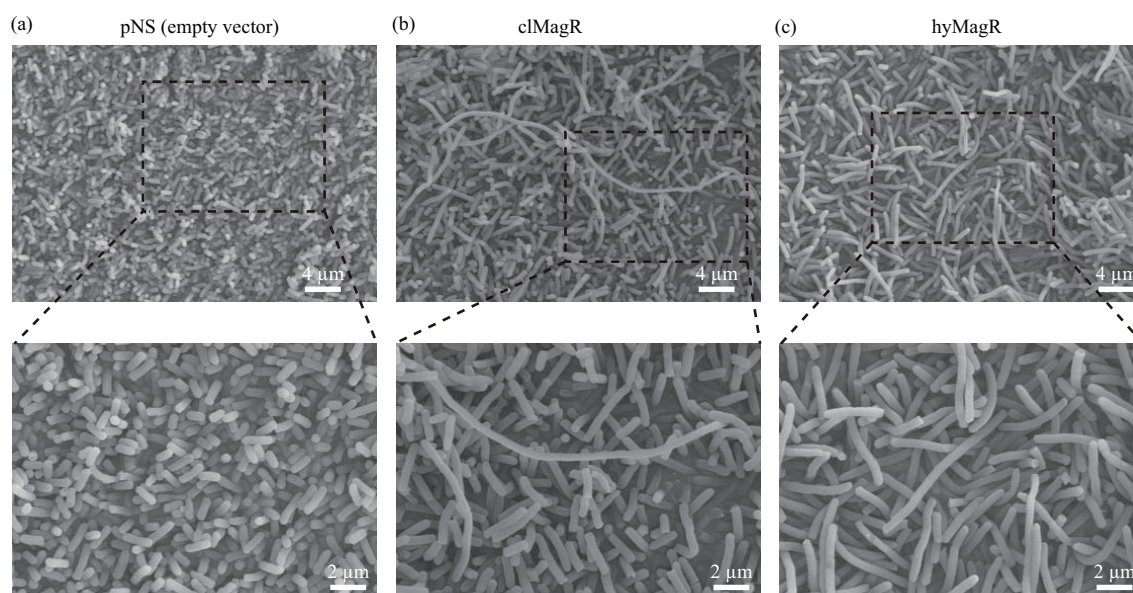


Fig. 2 Distinct filamentous morphologies induced by hyMagR and cIMagR overexpression in *E. coli*

Representative scanning electron microscopy (SEM) images of *E. coli* cells under different expression conditions. (a) Control cells transformed with an empty vector (pNS) retain the typical short-rod morphology. (b) cIMagR-overexpressing cells show a heterogeneous population, characterized by the sporadic presence of rare, extreme filaments against a background of cells of near-normal length. (c) hyMagR-overexpressing cells exhibit a uniform phenotype of prevalent cellular elongation and frequent filament formation. Lower panels present magnified views of the boxed regions. Quantitative analysis of SEM images is provided in Figure S1.

To further quantitatively analyze the distinct filamentation patterns, we performed high-throughput quantitative analysis of bright-field microscopy images using a custom Python pipeline for automated cell identification and length measurement (Figure 3a–c, e–g). The results firmly confirmed the observed morphological differences. Kernel density estimation of cell lengths revealed a narrow distribution for the control cells (pNS), with a peak at 1.7 μm and a mean length of 1.721 μm (Figure 3d). In contrast, the cIMagR-expressing population showed moderate

elongation in most cells, accompanied by some rare but extreme filamentous forms. The corresponding kernel density plot showed a broader distribution, with the peak shifted to 2.3 μm , and a higher mean length of 2.637 μm , compared with the regular 1.7 μm of *E. coli*. Although the density plot for this group showed a single broadened peak, the scatter plot of individual cell lengths clearly revealed sporadic outliers exceeding 100 μm —a subpopulation too rare to form a distinct secondary peak (Figure 3h). Notably, the hyMagR-expressing population exhibited

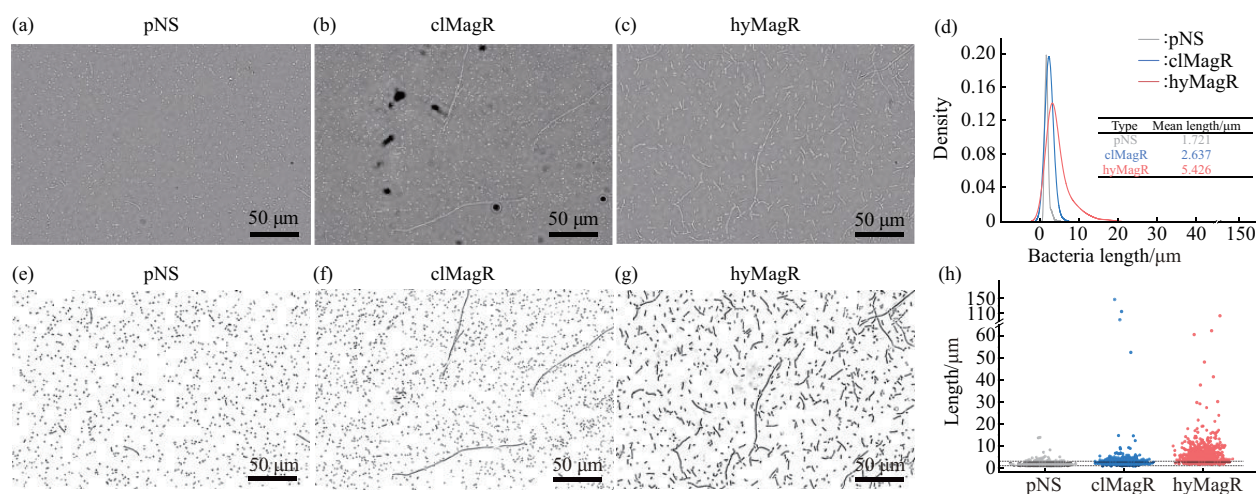


Fig. 3 Quantification of clMagR- and hyMagR-induced filamentation

(a–c) Representative bright-field microscopy images of *E. coli* cells expressing none with an empty vector as control (a), clMagR (b), or hyMagR (c). (d) Kernel density estimation plots of cell length distributions for the three groups. The mean length for each group is indicated in the inset table. (e–g) Corresponding binary images generated by the automated analysis pipeline for cell identification and length measurement for images in (a–c). (h) Scatter plot of individual cell lengths. Each dot represents one measured cell. Dashed lines at 1 and 3 μm indicate the typical length range of wild-type *E. coli*.

a markedly different profile, characterized by a significantly broader and right-shifted distribution with a peak at 3.2 μm, a substantially higher mean length of 5.426 μm, and a near absence of such extreme outliers as observed in clMagR-expressing cells (Figure 3d, h). Given the non-normal, right-skewed nature of these single-cell length distributions (Shapiro-Wilk test, $P < 0.05$), the statistical significance of these morphological shifts was validated using the mean cell lengths across three independent biological replicates (Figure S2a).

In summary, these data demonstrated that overexpression of clMagR and hyMagR induced fundamentally distinct filamentation phenotypes. clMagR expression triggered extreme but relatively rare filamentation, whereas hyMagR overexpression produced uniform cell elongation and filamentation.

2.3 Divergent iron redox preference correlates with distinct filamentous patterns

To elucidate the biochemical basis underlying the contrasting filamentation patterns, we first quantified cellular iron content using the Ferrozine assay^[56, 61]. Measurements of total iron in processed bacterial pellets showed that expression of either clMagR or hyMagR increased intracellular iron levels relative to the control cells, with clMagR producing a more pronounced elevation. The results nonetheless revealed significant increases in ferrous iron in both MagR-expressing strains compared to the control

(pNS), with a more substantial increase observed in hyMagR-expressing cells (clMagR: $P = 0.090$ 1, hyMagR: $P = 0.045$ 1; Figure 4a).

It is worth pointing out that the harsh sample preparation required for whole-cell analysis, specifically lyophilization and acid extraction, may introduce a risk of partial oxidation of labile Fe^{2+} . To avoid the possible artifacts caused by cellular sample preparation, we next analyzed the iron redox preference with freshly purified proteins, which better preserve the native redox states. This analysis uncovered a definitive difference: clMagR bound more total iron per monomer and displayed a strong preference for Fe^{3+} ($P < 0.000$ 1), yielding a high $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio. By contrast, hyMagR incorporated a substantially greater proportion of Fe^{2+} ($P = 0.004$ 2) under identical conditions (Figure 4b). This inherent propensity of hyMagR to bind ferrous iron provides a direct molecular correlate for its distinct and uniform filamentation phenotype.

We then applied a suite of spectroscopic techniques to characterize both mononuclear iron and iron-sulfur clusters bound iron species associated with the MagR proteins. EPR analysis of deferoxamine-treated whole-cell lysates yielded complex background signals that hindered direct comparison (Figure 4c). However, EPR spectra of purified proteins revealed that clMagR exhibited stronger signal amplitudes at $g = 4.3$ and $g \approx 6$ ^[62], which are

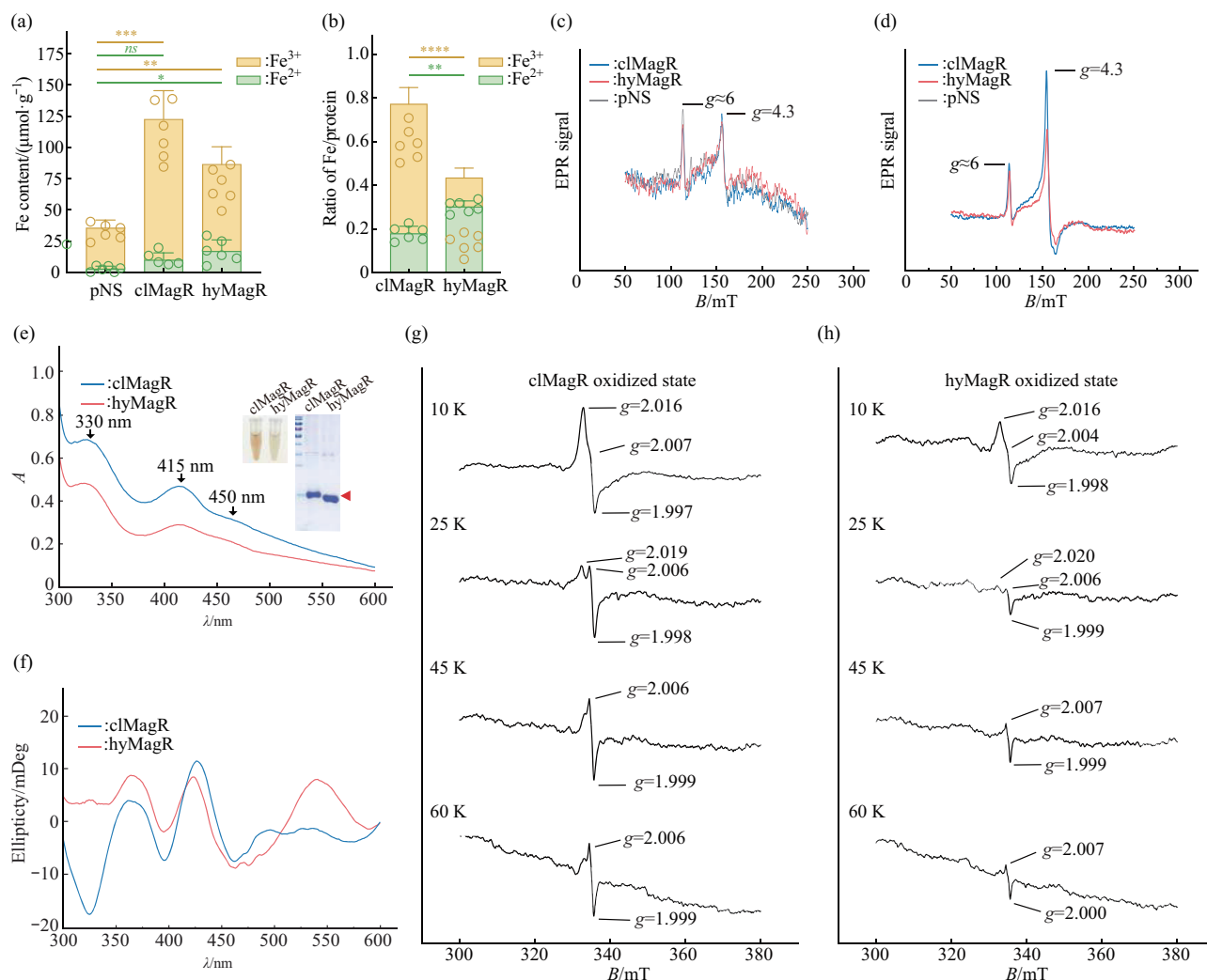


Fig. 4 Divergent iron redox states and cluster properties of cIMagR and hyMagR

(a, b) Quantitative analysis of iron content by Ferrozine assay. (a) Ferrous (Fe^{2+}), ferric (Fe^{3+}), and total iron in dried pellets of *E. coli* cells expressing cIMagR, hyMagR, or none with empty vector as control (pNS). Data are mean $\pm$ SEM ($n=6$). Statistical significance: two-way ANOVA followed by Dunnett's multiple comparisons test (* $P<0.05$, ** $P<0.01$, *** $P<0.001$; ns, not significant). (b) Iron content per monomer in freshly purified cIMagR and hyMagR proteins. Data are mean $\pm$ SEM ($n=6$). Statistical significance: two-way ANOVA followed by Šidák's multiple comparisons test (** $P<0.01$, *** $P<0.0001$). (c, d) EPR spectroscopic analysis of iron coordination. (c) Spectra of *E. coli* cells expressing cIMagR, hyMagR, or none with an empty vector (pNS) following treatment with the membrane-permeable chelator deferoxamine. (d) Spectra of the corresponding purified proteins. Spectra were recorded at 10 K. Signal at $g=4.3$ and $g=6$ are characteristic of high-spin Fe^{3+} , and their amplitudes reflect the relative content of such species. (e) UV-Vis absorption spectra of purified cIMagR (blue) and hyMagR (red). Arrows indicate characteristic absorption peaks at 330 nm and 415 nm, with a shoulder at ~450 nm. Insets: (top left) photograph of 0.1 mmol/L protein solutions showing the darker brown color of cIMagR (left) compared to hyMagR (right). Inset (top right): SDS-PAGE analysis confirming the purity and approximate molecular weight (~15 ku) of the proteins. (f) Circular dichroism (CD) spectra of cIMagR (blue) and hyMagR (red). The spectrum of cIMagR displays characteristic peaks of a [2Fe-2S] cluster. (g, h) X-band EPR spectra of purified cIMagR (g) and hyMagR (h) recorded at different temperatures (15 K, 25 K, 45 K, and 60 K). Signals consistent with a $[\text{3Fe-4S}]^+$ cluster are observed in both proteins.

characteristic of high-spin Fe^{3+} , consistent with its higher Fe^{3+} content compared to hyMagR (Figure 4d). UV-Vis absorption spectra confirmed the presence of iron-sulfur clusters in both proteins, with characteristic peaks at ~330 nm, ~415 nm, and a shoulder at ~450 nm; cIMagR consistently showed higher absorbance across these wavelengths (Figure 4e). Together with its darker solution color and higher

total iron content, these results suggested a greater cluster content or enhanced stability in cIMagR.

CD spectroscopy further revealed that cIMagR displayed a canonical [2Fe-2S] cluster signature^[63], with positive peaks at 360 and 430 nm and negative peaks at 325, 395, and 460 nm. In contrast, hyMagR exhibited a non-canonical CD spectrum characterized by an additional positive peak at 540 nm, indicating a

distinct microenvironment around its [2Fe-2S] cluster or the possible presence of other iron-sulfur clusters such as [4Fe-4S]^[64] (Figure 4f). Low-temperature X-band EPR analysis of the oxidized samples further confirmed the presence of a [3Fe-4S]⁺ cluster ($g \approx 2.016$, 2.007, and 1.997)^[65-66] in both proteins, with the signal being substantially stronger and more temperature-stable in cIMagR than in hyMagR (Figure 4g, h).

Collectively, these spectroscopic data indicate that although cIMagR and hyMagR are capable of binding similar types of iron-sulfur clusters, cIMagR exhibits a higher iron-binding capacity and confers greater stability to its associated clusters. This functional divergence likely originates from subtle structural differences between the two proteins.

2.4 hyMagR overexpression confers enhanced magnetic sensitivity to bacterial filamentation

Considering MagR as a putative magnetoreceptor, we then investigated whether the distinct filamentation phenotypes correlated with differential sensitivity to external magnetic fields—an essential aspect for potential applications. Here in this study, we refer to the magnetic sensitivity as a “morphological phenotype affected by magnetic field”, representing a field-dependent modulation of the cellular morphological changes. Quantitative analysis of cells exposed to a 100 mT SMF revealed a striking specificity. The filamentation of hyMagR-expressing cells was markedly enhanced under SMF, as indicated by a rightward shift of and broadening of the cell length distribution in the Kernel density estimation plots, together with a significant increase in the mean length and percentage of filamentous cells ($P < 0.0001$, Figure 5a–j). By contrast, neither the empty vector control ($P = 0.9309$) nor the cIMagR-expressing strain ($P = 0.7489$) exhibited a significant morphological response to the same treatment (Figure 5c, f, i, j). The specific enhancement of filamentation by SMF in hyMagR-expressing cells was consistently reproduced and statistically validated across three independent biological replicates (Figure S2b). Crucially, this SMF-induced morphological change enhancement in the hyMagR group was observed without any change in MagR protein expression levels (Figure 5m). While SMF treatment also exerted distinct effects on bacterial growth across different strains (Figure 5k, l), these growth variations are independent of the filamentation phenotype. For

instance, SMF significantly altered the growth speed of the empty vector control ($P < 0.0001$; Figure 5l) without triggering any morphological elongation. This observation effectively decouples the morphological response to magnetic field treatment from growth kinetics, confirming that the enhanced filamentation in hyMagR-expressing cells is a specific hyMagR-mediated response to the magnetic field, rather than a change related to the growth kinetics caused by magnetic field treatment.

To explore the possible biophysical basis of this differential response, we characterized the intrinsic magnetic properties of the purified proteins. Magnetization curves measured at 300 K confirmed that both MagR variants are paramagnetic (Figure 5n). The paramagnetic or superparamagnetic property of cIMagR is consistent with previous reports^[33-35, 39]. Interestingly, cIMagR exhibited approximately 5-fold higher mass magnetic susceptibility than hyMagR within the field range we measured (from -100 to $+100$ mT, Figure 5n). The relatively higher magnetic susceptibility of cIMagR is in agreement with the biochemical characterization results—cIMagR has higher Fe³⁺ content and more stable iron-sulfur clusters.

3 Discussion

The present study reveals that bacterial filamentous morphologies and magnetic sensitivity can be differentially modulated through the expression of diverse MagR homologs. MagR, an ancient and highly conserved iron-sulfur protein, originally identified for its role in iron-sulfur cluster biogenesis and iron metabolism^[67-69], was later recognized as a putative magnetoreceptor^[31-32]. Its multifunctional nature has since become evident. The remarkable diversity in its biochemical and biophysical level^[38, 52, 70] properties across homologs, has underscored its potential for both mechanistic investigation and practical applications. Building on our previous finding that cIMagR overexpression perturbs iron metabolism and induces bacterial filamentation though at very low frequency^[53], this study systematically explores phenotypic variations within the MagR family to identify homologs with enhanced or distinctive morphological engineering capabilities and evaluate their potential as magnetically responsive biological modules.

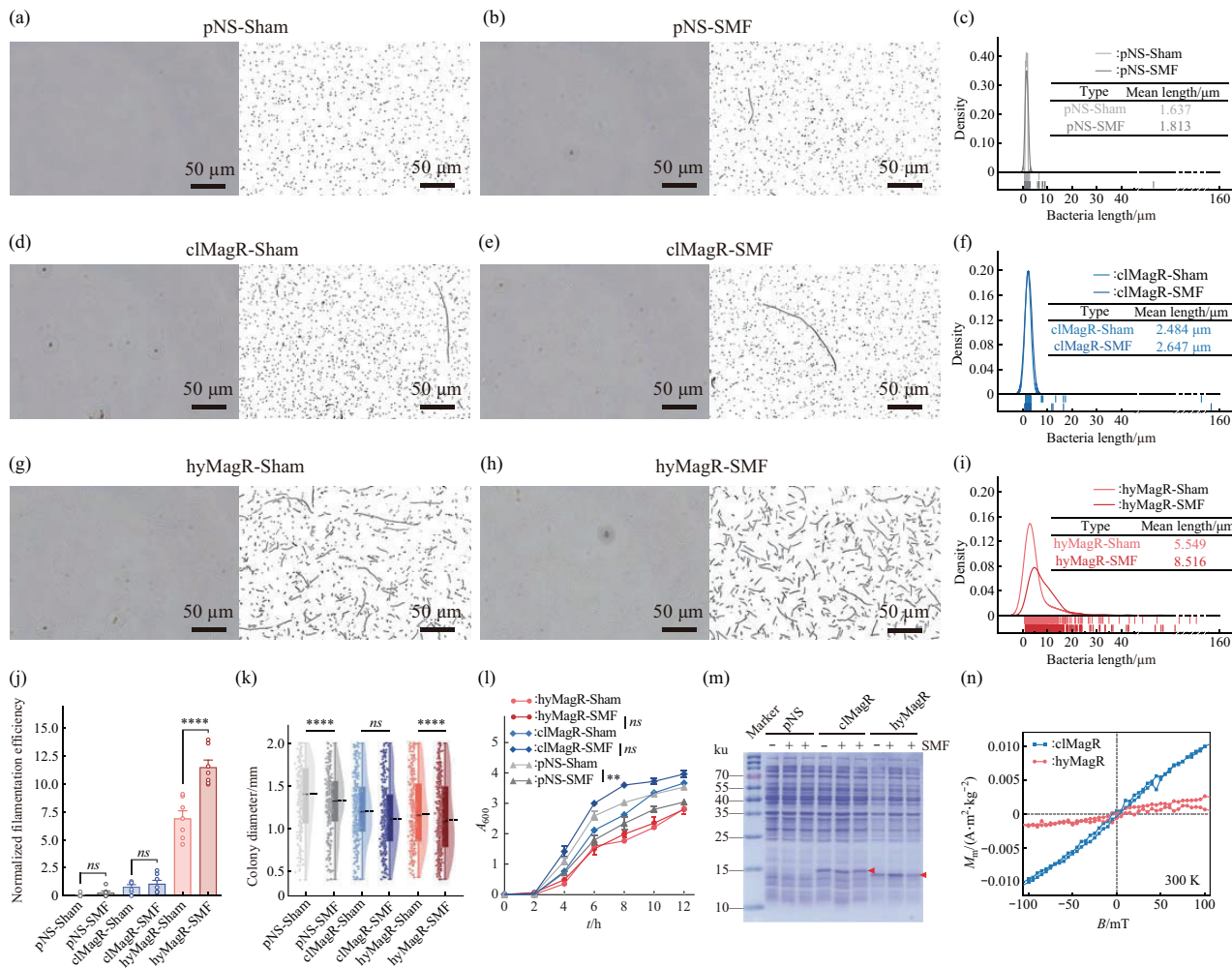


Fig. 5 MagR-mediated differential magnetic sensitivity

(a–i) Effects of a 100 mT SMF on bacterial filamentation. Cells were cultured in LB medium at 15°C under either ambient geomagnetic field (sham) or SMF conditions. Representative bright-field microscopy images (left) and corresponding binary masks (right) generated by custom Python pipeline for automated cell length analysis. *E. coli* expressing empty vector (a, b), cIMagR (d, e), or hyMagR (g, h) were grown without (a, d, g) or with (b, e, h) 100 mT SMF. (c, f, i) Kernel density estimation plots of bacterial cell length distributions, representing the probability density of the population. (c) pNS under sham (light gray) or 100 mT SMF (dark gray). (f) cIMagR under sham (light blue) or 100 mT SMF (dark blue). (i) hyMagR under sham (light red) or 100 mT SMF (dark red). A minimum of 500 individual cells were analyzed per condition for each replicate. Mean cell lengths for each condition are shown in the adjacent tables. (j) Quantification of the percentage of filamentous cells (defined as cells with length $\geq 15 \mu\text{m}$) under each condition, normalized to corresponding MagR expression levels (relative to total protein loading per lane to account for potential variation in expression efficiency). Data are mean $\pm$ SEM ($n=6$, independent biological replicates). Statistical significance: two-way ANOVA followed by Šidák's multiple comparisons test (**** $P<0.0001$; ns, not significant). (k) Colony diameter analysis of the three strains on LB agar under sham or 100 mT SMF. Data are mean $\pm$ SEM ($n>100$). Statistical significance: two-way ANOVA followed by Šidák's multiple comparisons test (**** $P<0.0001$; ns, not significant). (l) Growth curves of pNS, cIMagR, and hyMagR strains in liquid LB medium under sham or 100 mT SMF. A_{600} was monitored at 2-h intervals with continuous shaking. Color coding: pNS-Sham (light gray) vs. SMF (dark gray); cIMagR-Sham (light blue) vs. SMF (dark blue); hyMagR-Sham (light red) vs. SMF (dark red). Statistical significance: two-way ANOVA followed by Tukey's multiple comparisons test (** $P<0.01$; ns, not significant). (m) SDS-PAGE analysis showing that expression of cIMagR and hyMagR is not altered by SMF exposure. The symbols “–” and “+” denote the sham control (ambient geomagnetic field) and the SMF-exposed (100 mT) groups, respectively. (n) Field-dependent magnetization (m – h) curves at 300 K for cIMagR (blue) and hyMagR (red). Measured mass magnetic susceptibilities were 1.31×10^{-7} and 2.45×10^{-8} , respectively. M_m : mass magnetization.

The cross-species screen of 28 MagR homologs revealed significant diversity in their capacity to remodel bacterial morphology. Among them, hyMagR emerged as a highly efficient and uniform inducer of

filamentation in *E. coli*, in sharp contrast to the low-frequency, heterogeneous, and extreme filamentation triggered by cIMagR. This phenotypic divergence is not a simple consequence of total cellular iron

accumulation but rather reflects a fundamental difference in iron redox preference. Specifically, hyMagR displayed a pronounced affinity for ferrous iron (Fe^{2+}), whereas clMagR preferentially bound ferric iron (Fe^{3+}) and formed more stable iron-sulfur clusters. The Fe^{2+} -preferred cellular environment associated with hyMagR overexpression likely enhances redox reactivity and facilitates dynamic interactions with cellular division machinery and producing uniform filamentous morphologies. Furthermore, the structural homology modeling of the clMagR and hyMagR proteins (Figure S3) also suggested that the differences in iron-sulfur binding pockets, such as N131 in clMagR being replaced by H129 in hyMagR, may remodel the local electrostatic and spatial microenvironment and form the structural basis for the distinct iron redox preference in these two proteins.

More importantly, hyMagR-expressing bacteria displayed clear magnetic sensitivity, here defined as a morphological phenotype affected by magnetic field (*i.e.*, a statistically significant field-dependent increase in the proportion of cells with cell length $\geq 15 \mu\text{m}$). In our study, exposure to a 100 mT SMF markedly increased both the mean cell length and the proportion of filamentous cells, whereas clMagR-expressing or control strains showed no significant response. Intriguingly, direct magnetic measurements revealed that clMagR exhibited approximately fivefold higher mass magnetic susceptibility than hyMagR. This apparent paradox indicates that the cellular magnetic response cannot be explained solely by intrinsic magnetization (*e.g.*, the classical biocompass model). Instead, MagR-mediated magnetic response likely arises from a synergistic interplay between the protein's intrinsic magnetic properties of the protein and cellular redox dynamics. The data presented here strongly suggest that redox modulation plays a fundamental role in this process.

It has been previously reported that ferrous iron (Fe^{2+}) acts as a potent one-electron reductant, driving the formation of hydroxyl radicals ($\bullet\text{OH}$) *via* the classical Fenton reaction^[71-72]. Given that expression of hyMagR significantly increased the intracellular Fe^{2+} pool, this condition likely establishes a primed redox environment within the cell. Moreover, static magnetic fields have been shown to modulate intracellular reactive oxygen species (ROS) levels by influencing the spin-state interconversion of transient

radical pairs generated during Fenton-type reactions^[73]. Thus, it is reasonable to hypothesize that exposure to a static magnetic field may synergize with the Fe^{2+} -enriched state characteristic of hyMagR-expressing cells, leading to enhanced ROS generation and subsequently triggering the bacterial SOS response. As a consequence of elevated ROS levels, upregulated cell division inhibitors such as SulA^[74] may sequester FtsZ monomers, thereby blocking Z-ring assembly and ultimately inducing cell filamentation^[75-76].

Importantly, the biological effects of magnetic fields are highly complex and strictly dependent on multiple physical parameters. A substantial body of literature indicates that variations in field intensity, frequency, exposure duration, and spatial orientation can result in divergent, or even opposing cellular redox outcomes^[73]. In the current study, a 100 mT SMF was utilized as a robust proof-of-concept experimental condition to demonstrate how cell morphology can be modulated in MagR-expressing cells. A comprehensive parameter screening, including multi-gradient field intensities (*e.g.*, 10–500 mT), alternating current (AC) magnetic fields, and distinct directional setups, are certainly required in the future to precisely define the response thresholds and dose-dependent kinetics of the hyMagR-based actuator.

Ultimately, our findings redefine MagR as more than a putative magnetoreceptor for animal navigation—it also serves as a versatile, environmentally responsive molecular toolkit that integrates chemical (iron valence) and physical (magnetic field) cues to modulate cellular morphology. The uniform and magnetically controllable filamentation induced by hyMagR establishes a framework for morphology engineering in microbes and offers a new tool for synthetic biology and biomaterials research. Such systems could enable magnetically guided bacterial filaments for targeted delivery^[77], shape-shifting cell factories for biosensing^[78], or platforms to investigate how physical force modulates cell division^[79]. However, it is important to note that the MagR-mediated magnetic response observed in engineered MagR-expressing cells, particularly under 100 mT SMF exposure may differ fundamentally from the innate magnetoreception or compass-like navigation behavior seen in migratory animals.

4 Conclusion

In summary, this study demonstrates that variation in iron redox preference among MagR homologs governs both the extent of bacterial filamentation and the degree of magnetic sensitivity. The hyMagR functions as a highly efficient, Fe^{2+} -dependent magnetic actuator, which we hypothesize may couple redox state modulation to morphological and magnetic responses. These findings not only provide new insights into how MagR might integrate magnetic and metabolic signals but also provide a foundation for engineering magnetically responsive cellular systems, offering broad implications for synthetic biology, biophysics, and biomaterials design.

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Data Availability The data underlying this article are available in the Science Data Bank (ScienceDB) repository, and can be accessed at <https://www.scidb.cn/s/ZFb2Qj>.

Supplementary Available online (<http://www.pibb.ac.cn>, <http://www.cnki.net>):

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磁受体介导的细菌丝状形态多样性与磁敏感性研究*

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摘要 **目的** 细胞形态是遗传决定的关键特征, 也可通过基因改造或环境因子调控而发生改变, 这一过程称为细胞形态工程。通过基因修饰构建具有特定形态且对磁场敏感的细胞, 进而实现远程、无创的磁场调控生长, 是细胞形态工程的重要目标之一, 具有显著的应用潜力。动物磁感应依赖磁受体 (magnetoreceptor, MagR) 感知地磁场并指导定向导航。本研究旨在探究 MagR 异源表达对细菌形态及磁感应能力的影响, 筛选基于 MagR 的磁敏感形态工程途径, 并揭示其潜在分子机制。**方法** 系统筛选了 28 个物种的 MagR 同源基因在大肠杆菌中的表达和相应的表型效应, 通过显微成像结合 MagR 铁硫簇辅基的铁氧化还原态分析, 以及静磁场 (100 mT) 暴露实验, 比较不同 MagR 重组蛋白质表达菌的磁场响应差异, 从而解析其分子机制。**结果** 不同来源的 MagR 同源基因表达可诱导细菌出现不同程度的丝状化表型。从中筛选出两类典型形态: 水螅来源的 hyMagR 偏好结合 Fe^{2+} , 其表达促进细胞均一伸长与丝状化, 且表现出较高的磁场敏感性 (即在静磁场下丝状化显著增强); 鸽子来源的 clMagR 偏好结合 Fe^{3+} , 仅诱发低频出现的极端长度的丝状化, 磁场响应较弱。表型差异主要由 MagR 对不同铁价态的偏好性主导, 而与总铁含量无关。**结论** MagR 的铁硫簇价态 (特别是对亚铁的偏好性) 是调节细菌形态和磁敏感性的关键因素。本研究为构建磁响应细胞系统提供了理论依据, 也为理解 MagR 介导的磁感应机制提供了新视角。

关键词 磁受体同源蛋白, 磁受体过表达, 菌丝形态, 铁氧还原状态, 磁敏感性

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