

Bacterial Evolution: Rewiring Modules to Get in Shape

Bacterial species take on a wide variety of shapes, but the mechanisms by which specific shapes evolve have remained poorly understood. A recent study demonstrates that two *Asticcacaulis* species repurposed an ancestral regulatory protein to rewire the modules of stalk regulation, localization, and synthesis, thereby generating new shapes.

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Spheres, rods, commas, helices, branches, cubes, and stars are just some of the tremendous diversity of cell shapes found in the bacterial kingdom. To generate such a wide array of morphologies in a single cell, bacteria must organize growth in a tiny $\sim 1 \mu\text{m}^3$ space as the architecture of their rigid cell wall determines bacterial morphology. The biochemistry of cell wall synthesis is largely conserved across species with different shapes [1]. Consequently, adopting different shapes requires bacteria to organize and regulate the conserved cell wall synthesis machinery in space and time. Recent work on bacterial cell shape determination shows how species assemble specific shapes such as the straight rods of *Escherichia coli* or the curved rods of *Caulobacter crescentus* [1]. Here we highlight a new paper by Jiang *et al.* [2] that sheds light on a mechanism by which bacterial evolution enables related species to transition from one shape to another. Specifically, these authors demonstrated how rewiring a single protein to carry out multiple functions enabled morphological transitions between stalked bacteria known as prosthecae.

Prosthecae are a class of bacteria that share the common morphological characteristic of having stalks. These long, thin appendages to the cell body help bacteria take up nutrients in scarce environments [3]. In *C. crescentus*, stalks form by recruitment of the cell wall synthesis machinery to a specific location that becomes the base of the stalk. While *C. crescentus* forms a single polar stalk, two related species have different shapes: *Asticcacaulis excentricus* produces one stalk slightly off the cell pole (subpolar) and *A. biprosthecum* produces two stalks positioned bilaterally off from the mid-cell region. Jiang *et al.* [2] first

show that, despite their different shapes, these three species share the same mechanism for stalk formation. To generate different morphologies, they must therefore possess distinct components that differentially localize this common machinery.

To find the determinants of stalk localization, Jiang *et al.* [2] searched for conserved proteins that localize to the base of stalks in *C. crescentus*, *A. excentricus* and *A. biprosthecum*. Only two proteins fit these criteria: the cell-cycle regulator DivJ and its activation factor SpmX. In *C. crescentus*, SpmX regulates the timing of stalk synthesis by recruiting DivJ to the cell pole, but in the absence of SpmX cells still make stalks at the correct locations. Excitingly, genetic analysis showed that SpmX gained new functions in *Asticcacaulis*: in addition to recruiting DivJ, it also mediates stalk localization in both *A. excentricus* and *A. biprosthecum* and additionally mediates stalk initiation and elongation in *A. biprosthecum*.

So, how does a bacterial protein like SpmX acquire a new function? The hypothesis that emerges from the studies of Jiang *et al.* [2] is that stalk synthesis, localization, and regulation can be viewed as discrete modules that were originally separated, which is how they remain in *C. crescentus*. But in the *Asticcacaulis* species, these modules evolved to become coupled, thereby 'rewiring' the network governing stalk physiology. Support for this view initially came from expressing the *spmX* homolog from one species in each of the other species' backgrounds. The *spmX* homologs from the three species all possess similar muramidase and transmembrane domains, while the *Asticcacaulis* species have a distinctly extended carboxy-terminal region. The cross-species heterologous expression of *spmX* indicated that

the information determining the distinct stalk synthesis sites is encoded in this carboxy-terminal extended sequence. This suggests that SpmX diverged to bind to different targets in the different species. Cross-expression of chimeric forms of SpmX confirmed that the carboxy-terminal extension is responsible for the newly acquired functions of stalk formation and localization. These results, along with phylogenetic analysis of several newly sequenced related prosthecae, indicated that SpmX evolved from a localized protein with a single regulatory function to a regulatory protein that also serves to coordinate stalk localization and synthesis.

Consequently, three building blocks — regulation, localization and synthesis — seem to have assembled as three modules to spatially and temporally coordinate cell wall insertion and lead to diverse morphologies (Figure 1). Phylogenetic analysis suggests that these bacteria evolved their shapes through two major gain-of-function events. The first gain of function combined the three modules into a single protein — SpmX — and led to subpolar stalk localization in *A. excentricus*. Later, retargeting this combined-function SpmX enabled stalks to be redirected to new subcellular sites, such as the bilateral localization found in *A. biprosthecum*.

These findings suggest an important role in bacterial evolution for co-option — the acquisition of new functions by repurposing an ancestral protein. There are multiple examples of co-option in eukaryotic evolution. For example, heat shock proteins and metabolic enzymes have been repurposed as lens crystallins for animal eyes, as these need to remain highly stable in harsh environmental conditions to maintain sight [4]. Other mechanisms of co-option occur at the regulatory level, where mutations in regulatory sequences alter spatial or temporal expression of proteins to create selectable traits in new contexts, as is the case with bird feathers and plant shape [5].

In contrast to the examples in the previous paragraph where existing proteins were co-opted for new functions but not dramatically altered at the domain level, the rewiring of stalk regulation, localization, and synthesis

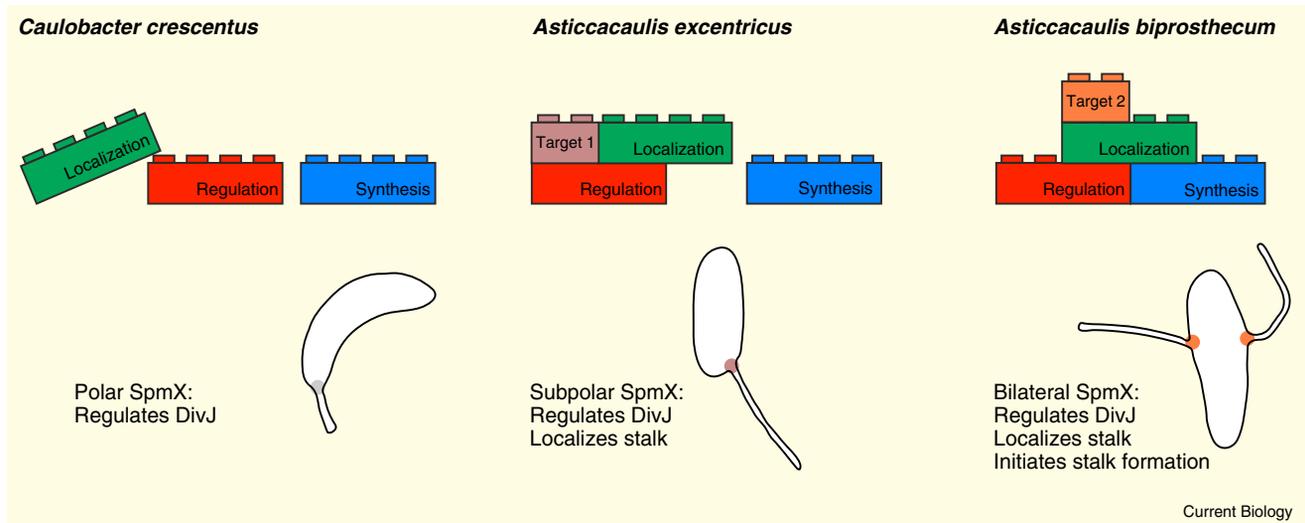


Figure 1. The modularity of genetic elements drives bacterial cell shape evolution.

In *Caulobacter* and *Asticcacaulis*, stalk physiology is mediated by three distinct modules of regulation, localization, and synthesis. Here, we depict these modules as bricks and show how they have been reorganized and assembled in a single protein, SpmX. In the ancestral state that persists in *Caulobacter* (left), the modules are separate and SpmX only contributes to the regulatory module by recruiting DivJ. In *A. excentricus* (middle) and *A. biprosthecum* (right), SpmX acquired a new subdomain that mediates stalk localization (*A. excentricus*) and synthesis (*A. biprosthecum*). By associating with different target proteins (Target 1 and Target 2), the combined-function SpmX could have been retargeted to new subcellular destinations to achieve subpolar (*A. excentricus*) or bilateral (*A. biprosthecum*) stalk localization. The alternative localizations along with the new stalk synthesis function explain the morphological transitions between species.

involved adding a whole new domain to the carboxy-terminal region of SpmX. It appears that such a drastic change could not be achieved by point mutations alone. Indeed, just as SpmX drastically evolved by insertion of sequences encoding a new protein domain, earlier work from the Lenski group suggested that drastic evolution of *E. coli*, such as acquiring the ability to metabolize citrate, cannot be achieved by point mutations and requires a larger-scale deletion or transposition even to bring a key gene under the control of a different promoter [6]. Thus, while more evidence will be needed before broad conclusions can be made, current examples suggest that co-option and modularity are major drivers of evolution across all systems. Whereas point mutations can dramatically alter animal evolution, larger genomic rearrangements seem to be necessary for bacteria to acquire significantly divergent traits. This difference may be due to the simplicity and robustness of bacterial gene regulation that enables rapid growth and division.

Another mystery remains: what are the selective pressures that pushed *Asticcacaulis* to generate stalks at different locations on the cell? What benefits do these species derive from this morphological transition? In

C. crescentus, stalks have been proposed to promote nutrient uptake [3]. But it is not obvious how moving the stalk would improve nutrient uptake, and, if multiple stalks function better than one, why did so few of the prosthecae evolve more than one stalk? *Asticcacaulis* evolved mechanisms to stably localize stalks, showing that there must be pressure(s) that maintain specific localization and morphology in the wild. Several observations may be useful to identify these environmental factors. First, while a highly adhesive holdfast is found at the tip of the stalk of *C. crescentus*, the holdfast remains polarly localized in *Asticcacaulis*, separated from the stalk. In their natural environment, we can thus expect to find stalked *Asticcacaulis* attached to a surface with their stalks remaining free. One possibility is that an ancestral function of the stalk is to extend and function as a spacer, so that in stressful conditions, like phosphate starvation, *C. crescentus* can attempt to improve its environment by growing away from the surface to which it is irreversibly attached. This function may have become obsolete for *Asticcacaulis*, which repurposed the stalk for distinct functions like mechanical interactions with their fluid environment or with other cells.

Strikingly, the change in stalk localization from *C. crescentus* to *Asticcacaulis* seems to correlate with a loss of curved cell shape, perhaps indicating that polar stalk localization provides a preferential benefit to curved bacteria.

To better understand both the function and evolution of bacterial cell shape, we need to improve both our understanding of the natural ecologies of bacteria and our ability to model natural habitats in the lab. Achieving these goals will require a multidisciplinary approach combining imaging, genetics, and genomics with ecology, evolution, and engineering. There is a particularly acute need for engineering new cell culture platforms that better mimic the natural chemical and mechanical environments of specific habitats. For example, the mechanical environment of microbes, such as fluid flow, has been largely underestimated as a driver of the evolution of bacterial shape.

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Ecology: Honey Bee Foraging in Human-Modified Landscapes

Comprehensive information on the spatial resource use of honey bees is rare, but highly relevant to assess the consequences of habitat loss and fragmentation, agricultural intensification or extensification on colony fitness, pesticide exposure risks and pollination functions.

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European landscapes have been highly modified by human impact with habitat fragmentation, habitat conversion and agricultural land use intensification as major processes [1]. As a result most landscapes are characterised by a mix of different habitat types with varying proportions of natural, semi-natural, agricultural and urban areas. The transformation of landscapes and the increase of human land use have severe negative impacts on biodiversity in landscapes dominated by intensive agriculture [2,3] and threaten important ecosystem functions such as pest control and pollination of crops [4–6]. European agri-environmental schemes such as organic farming have been implemented to counteract biodiversity loss and maintain ecosystem services, but their effectiveness depends on more elaborated conservation measures, selection of organism groups and landscape structure [7,8]. An important aspect here is the provision of additional resources such as pollen and nectar for pollinators in a landscape and the way organisms are able to locate and use it for reproduction [9,10].

In the case of the honey bee with an estimated foraging range of 100 km² [11], it is simply impossible to gain a detailed spatial picture of floral food resources and their use by bee foragers by direct observations. In particular, it is difficult to link flower-visiting bees to a certain colony and the location of nesting sites in the

countryside. However, honey bees offer the opportunity to ‘eavesdrop’ on the internal communication system to understand food recruitment and spatial resource use in more detail. A successful forager communicates rewarding food locations via the well-known waggle dance to her nest mates. This behaviour can be regarded as one of the most fascinating phenomena in the insect kingdom.

Pioneering experiments conducted by von Frisch (1965) [12] and Seeley (1995) [11] opened the door to a unique perspective from the bee colony to the surrounding landscape and its resources. In the last decades, the dance language has been intensively studied as a fascinating mode of communication to understand basic mechanisms of in-hive organisation and behavioural physiology [11]. However, surprisingly few studies use the dance language to address ecological questions, and in particular landscape-wide analyses of foraging patterns are almost absent (but see [13–15]). As a consequence, foraging and resource use of honey bees on a broader landscape scale is simply under-investigated. Several studies used the capacity of honey bees to monitor environmental pollution [16], but the idea to take advantage of waggle dance information to evaluate agri-environmental schemes on a landscape scale has not been developed so far. In this issue of *Current Biology*, Margaret Couvillon and co-workers [17] applied this approach in a year-round analysis of the spatial resource use of three honey

bee colonies in a mixed agricultural landscape in England.

Over two years they decoded more than 5,600 bee dances and plotted the location of used flower resources on geographical maps; they show how different habitat types with or without different agri-environmental schemes are preferred or avoided in a foraging area of close to 100 km². They find a significant preference of foraging honey bees for nature conservation areas under high level stewardship and, more surprisingly, low preference values for organically managed farm land. These results are novel due to two innovative methodological achievements. Firstly, the authors corrected for distance to take into account the higher energy expenditure of more distant floral resources when they calculated habitat preferences. Secondly, Couvillon *et al.* included naturally occurring variations within the dance communication in their models. These tools will significantly improve future research on spatial and temporal patterns of resource use by honey bees. While we applaud the achievements made in this study, we are also excited about the future perspectives and so far unexplored aspects of honey bee foraging in agricultural landscapes. The revealed preference of foraging honey bees for nature reserves not only underlines the resource richness of these habitats and their value for honey bees, it also indicates that the spatial overlap in resource use with threatened wild bee species might be higher than previously assumed [18]. Using honey bee colonies as bioindicators to identify the location of high-resource patches in a landscape, as suggest by Couvillon *et al.* [17], might thus also help to quantify the competitive pressure by honey bees on other flower-visiting insects in conservation areas [19].

Human-dominated landscapes are characterised by high spatial and temporal dynamics of cropping