News & views

Synthetic biology

Sticky logic toolkit programs cells to pattern

Luis Ángel Fernández

The engineering of cells to express synthetic adhesion molecules creates a simple logic for patterning cell populations with visible boundaries. The approach paves the way for smart living materials and programmable biosensors. **See p.324**

The emergence of multicellular organisms from individual cells probably occurred several times during evolution. These steps would have required the development of a certain type of 'glue', in the form of adhesion molecules (adhesins) that bind cells together, and that can recognize cells of the same type. Early adhesins would simply have detected like and unlike cells. But as they evolved, these molecules would have become capable of recognizing specialized cells in the organism that had distinct biological functions, and of spatially organizing tissues that had defined boundaries. On page 324, Kim *et al.*[1] report a way of using synthetic adhesins to form multicellular patterns that are visible to the naked eye — shedding light on the molecular mechanisms that might have driven the evolution of multicellular organisms.

The authors built their cell-adhesion tools by engineering *Escherichia coli* bacteria to express two pairs of complementary synthetic adhesins on their surfaces. These synthetic adhesins had previously been reported to display single-domain antibodies, known as nanobodies, that recognize molecules (antigens) on the surface of some mammalian cells, thus driving the adhesion of the bacteria to these cells[2]. Members of the same group as Kim *et al.* expanded this idea to pair complementary nanobodies and antigens in two strains of *E. coli* cell (Fig. 1a), enabling them to control adhesion between different types of bacterium to form multicellular assemblies[3].

The team's present work was motivated by the observation that the interface between colonies of these strains is visible to the naked eye. When two distinct bacterial colonies are seeded a few millimetres apart on a plate covered with agar, the cells multiply and migrate towards each other by swarming (a type of rapid collective movement). Swarming cells that have complementary nanobody–antigen pairs interact when they meet, forming large clusters that make it difficult for the cells to migrate further. This means of hindering migration increases the cell density in a narrow area between the two colonies, creating a clear line at the interface.

Kim *et al.* found that the interface was around 250 times wider than the typical length of an *E. coli* cell, because individual cells from each colony could still 'invade' the region occupied by the other strain. Cells with incompatible adhesins did not form clusters, allowing individual cells to migrate into the other colony and thereby expand the interface boundary. Intriguingly, the clusters formed by nanobody–antigen pairings filled only a fraction (around 20–25%) of the available space, becoming a porous mesh for cells that lacked complementary adhesins. These boundaries therefore behaved as selective barriers for the cells that interacted with each other, while still allowing the passage of non-interacting cells.

Having established the formation of this macroscopic boundary, Kim *et al.* explored the possibilities that emerge from seeding combinations of colonies expressing two pairs of complementary adhesins in ordered, repeating arrays. The library of these pairs can be expressed in terms of a simple adhesion logic toolbox comprising four 'bits' of information. By defining the system in this way, the authors showed that general interface patterns could

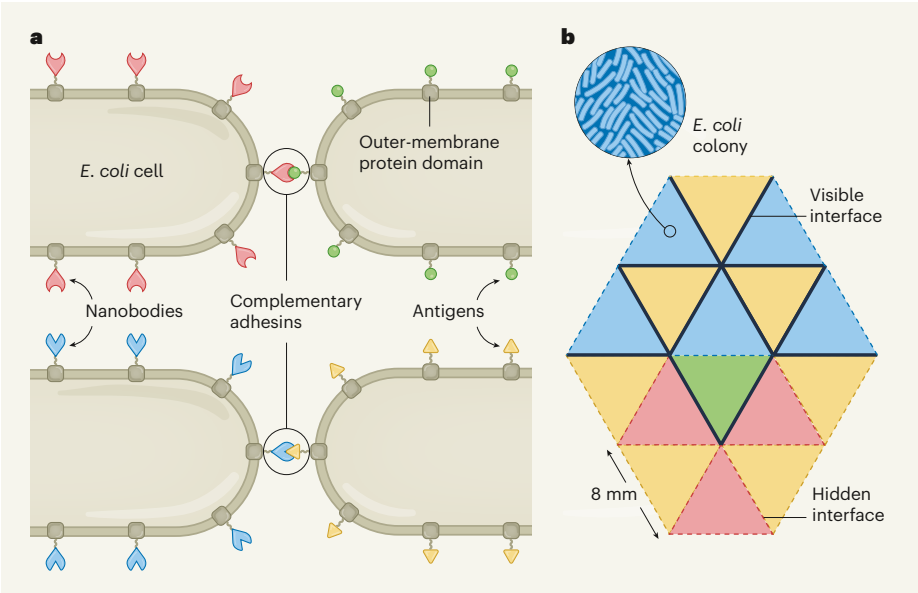

Figure 1 | Programmed cell–cell adhesion forms multicellular interface patterns. a, Kim *et al.*[1] engineered cells of the bacterium *Escherichia coli* to express two pairs of complementary synthetic adhesion molecules (adhesins). These adhesins display a single-domain antibody (nanobody) and its complementary antigen molecule, fused to an outer-membrane protein domain on the surface of the cells. **b**, When seeded on soft agar (not shown), the cells (coloured here to indicate the type of adhesin they express) multiply and migrate towards each other. Visible interfaces form between colonies expressing compatible adhesin pairs, because the two cell types cluster when they meet, and interact. By seeding the colonies in repeating arrays, the authors showed that they could produce simple patterns, such as triangles, with the interfaces. Because cells with incompatible adhesins moved past each other without interacting, they formed 'hidden' boundaries. Arrays of colonies forming combinations of visible and hidden interfaces enabled the authors to program complex patterns.

be generated in a programmable manner, from regular tilings of triangles, cubes and hexagons to mosaic-like tessellations involving combinations of shapes (Fig. 1b). The fact that such complex patterns could be formed on the basis of a simple logic suggests that there might have been a low threshold for the evolution of single cells into multicellular systems.

Kim *et al*. developed a mathematical model to simulate the interface formed between four strains that were initially spatially localized, allowing the authors to examine how seeding conditions affected the geometry of this interface. For example, they looked at how changing the binding affinity of the nanobody–antigen pair affected the width of the interface, and how different bacterial growth rates changed the interface's curvature.

The boundaries separated different cell types that had distinct biophysical properties, and that behaved in different ways. Kim *et al*. showed that these differences affected the wettability of the areas delineated by the boundaries. Likewise, the formation of interfaces could be controlled by introducing adhesion-inhibiting molecules into the medium. This mechanism allowed the colonies to be engineered into letters forming legible words, for instance.

The study is an elegant demonstration that a small number of adhesins suffices to organize cell collectives within well-defined boundaries at the macroscopic scale. The team's four-bit logic could be used to engineer synthetic tissues from single cells, or to develop zones with distinct biophysical properties in cell-based biomaterials. The approach could even form the basis of biosensing devices that can respond to external signals by producing visible interfaces, similar to digital displays, that can be read by the human eye.

Although Kim and colleagues' study stops short of realizing these practical applications, the potential of their adhesion toolkit is clear. The biophysical principles uncovered by the authors could be used to engineer microbial consortia with defined metabolic capabilities, as well as smart living materials[4,5] and organoids[6]. Some of these applications will require similar adhesion tools for other microorganisms, such as yeast, and for mammalian cells. But existing methods for expressing nanobodies and antigens on the surfaces of these cells are a good starting point for such developments.

Kim *et al*. have established a set of general principles for engineering programmable biosensors, biomaterials and artificial tissues with predictable patterns, based on a simple adhesion toolkit. And although further work will be needed to demonstrate the utility of the approach, the study shows that synthetic biology can help to answer complex biological questions, such as how tissue boundaries form during development.

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Food

The mystery of early milk consumption in Europe

Shevan Wilkin

What underpins how humans evolved the capacity to consume milk during adulthood? A look at the connection between health and the genetic changes needed to break down milk offers a surprising new perspective. **See p.336**

For decades, it was assumed that the beginnings of human consumption of animal milk occurred in tandem with the spread of the genetic changes needed for people to digest milk into adulthood. However, studies of ancient milk-drinking populations — using methods such as the analysis of ancient DNA, lipids and proteins — indicate that the relationship between these developments is more complex than that. Examination[1] of ancient dairy fat and protein residues shows that consumption of animal milk began in Anatolia (a region corresponding to the bulk of what is Turkey today) in the seventh millennium BC. By 5000 BC, this behaviour had spread across Europe[2] and the Eurasian steppe[3,4], and into northern Africa[5]. Although milk use was widespread in each of these regions by at least 5000 BC, the genetic underpinnings that enable adults to digest milk were extremely rare. Such genetic changes enable expression of the milk-digesting enzyme lactase to be retained beyond childhood, a state termed lactase persistence (LP). On page 336, Evershed *et al*.[6] offer a fresh perspective on the origins of lactase persistence.

"The ability to consume large amounts of dairy might have boosted the odds of both reproduction and survival."

Today, about one-third of the world's population can be categorized as being lactase persistent, yet early milk consumers in Eurasia (between approximately 9,000 and 2,000 years ago) lacked a version (allele) of the gene needed for LP, and this adaptation did not start to become common in Europe until roughly 3,000 years ago[7,8]. Numerous possibilities to explain the spread of LP alleles are often discussed, but little quantitative work has been done so far to explore the evolutionary reasons that might reveal the pattern of prevalence of these alleles.

To address this gap, Evershed and colleagues began to unravel the complexities behind the spread of a particular allele associated with LP in modern European populations. The authors demonstrate, through their analysis of present-day UK health data, that the ability to digest milk thanks to LP alleles does not seem to offer any benefit in terms of evolutionary fitness (as assessed through characteristics such as lifespan or having children). These data, combined with extensive archaeological data supporting the consumption of milk fats (lipids) from ceramic vessels (Fig. 1), have enabled Evershed and colleagues to present two exciting hypotheses for how and why LP spread across Europe over the past two millennia.

Infants and small children produce lactase naturally; the enzyme breaks down the molecule lactose into two digestible sugars, enabling infants to consume breast milk. However, until about 3,000 years ago, this ability was typically mainly 'switched off' after weaning. People lacking lactase in adulthood are described as lactase non-persistent (LNP). After consuming milk, LNP individuals can experience mild to severe symptoms that might include bloating, cramps and diarrhoea. Although up to 95% of modern Europeans[9], in certain regions, are lactase persistent, this has not always been the case.

One challenge when trying to investigate dietary health as it relates to populations