The significances of bacterial colony patterns

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Summary

Bacteria do many things as organized populations. We have recently learned much about the molecular basis of intercellular communication among prokaryotes. Colonies display bacterial capacities for multicellular coordination which can be useful in nature where bacteria predominantly grow as films, chains, mats and colonies. *E. coli* colonies are organized into differentiated non-clonal populations and undergo complex morphogenesis. Multicellularity regulates many aspects of bacterial physiology, including DNA rearrangement systems. In some bacterial species, colony development involves swarming (active migration of cell groups). Swarm colony development displays precise geometrical controls and periodic phenomena. Motile *E. coli* cells in semi-solid media form organized patterns due to chemotactic autoaggregation. On poor media, *B. subtilis* forms branched colonies using group motility and long-range chemical signalling. The significances of bacterial colony patterns thus reside in a deeper understanding of prokaryotic biology and evolution and in experimental systems for studying self-organization and morphogenesis.

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Introduction

Single-celled and multi-celled views of bacteria

Thinking of bacterial colonies as biological entities -- multicellular organisms – seems peculiar because we have grown so used to the idealization of bacteria as autonomous single-celled organisms. Certainly the single-celled paradigm, which has its origins in Koch's postulates, has been extraordinarily successful in our understanding of biochemistry, genetics and pathogenesis. Nonetheless, there are many phenomena where numerous bacterial cells communicate and act in a coordinated, organized fashion. The goal of this minireview is to convince the reader that the bacterial colony can serve as excellent experimental material to study these multicellular interactions and that the results will be significant in many realms of science, ranging from acquiring a deeper knowledge of prokaryotic cell biology to answering fundamental questions of genetics, evolution and morphogenesis.

The biological significance of multicellular organization

What adaptive advantages do bacteria derive from multicellularity? The same as other organisms: strength in numbers and the potential for specialization and cellular division of labor. Some examples of these advantages are differentiation into cell types with complementary but incompatible biochemistries, increased resistance of colonies and biofilms to antibacterial agents, and the ability to trap food resources in spatially restricted environments[1]. Because bacterial multicellularity is still an unconventional concept, its implications have not yet been explored in many promising areas. My expectation is that two areas will prove especially rewarding: (i) spatial organization of metabolically interacting populations as they carry out essential mineral cycles in the biosphere, and (ii) self-defense and specialization of symbiotic and pathogenic bacteria as they colonize eukaryotic hosts.

Table 1. *Some intercellular communication molecules and structures known to be used by bacteria*

Molecular class	Phenotype	Species	Reference
Amino acids	Chemotactic autoaggregation	*E. coli, S. typhimurium*	33, 36
	Aggregation, morphogenesis	*Myxococcus xanthus*	29
	Swarmer cell differentiation	*Proteus mirabilis*	57
'Autoinducers' (homoserine lactones and analogues)	Bioluminescence	*Vibrio harveyii, V. fischerii*	47 (and refs therein)
	Exoenzyme synthesis	*Pseudomonas aeruginosa*	47 (and refs therein)
	Plasmid transfer	*Agrobacterium tumefaciens*	47 (and refs therein)
	Antibiotic synthesis	*Streptomyces* spp., *Erwinia carotovora*	47 (and refs therein)
	Stationary phase sigma factor	*E. coli*	48
Oligopeptides	Agglutination, plasmid transfer	*Enterococcus faecalis*	49
	Sporulation	*Bacillus subtilis*	50, 51
	Competence for DNA uptake	*Bacillus subtilis*	52
	Aerial mycelium development	*Streptomyces coelicolor*	53
Lipopeptides	Swarming	*Serratia marcescens*	27
Polypeptides	Aggregation, morphogenesis	*Myxococcus xanthus*	29
Pili	Social motility	*Myxococcus xanthus*	54
	Aggregation in colonies	*Neisseria gonorrhoeae*	55
	Conjugal DNA transfer	Many species	56
Fibrils	Cohesion, morphogenesis	*Myxococcus xanthus*	29, 58

The molecular basis of intercellular communication among prokaryotes

A great deal of attention has been devoted in recent years to identifying intercellular communication molecules in bacteria[2]. These molecules range in size from amino acids to proteins, and the various phenotypes they influence include bioluminescence, metabolite and enzyme secretion, genetic exchange, cellular differentiation, autoaggregation, and multicellular morphogenesis (Table 1). Communication molecules represent some of the tools which bacteria use to organize their social life. Other tools include surface organelles, such as pili, and exopolymer matrix materials. We are discovering that bacteria, like eukaryotes, use a variety of molecular mechanisms to transfer information from cell to cell. In some cases, such as the C signal in *Myxococcus xanthus*, transfer involves oriented cell movements[3].

Colonies as dynamic, organized and differentiated structures

One of the best arenas for viewing bacterial multicellularity in action is the Petri dish. The colonies of virtually all prokaryotic species display capacities for cellular differentiation and multicellular organization. These capacities are, of course, also available to bacteria in natural settings, where biofilms, chains, mats and microcolonies are the predominant modes of existence[4,5]. Thus, the first significance of bacterial colony patterns is that they expand our understanding of the biological capabilities which bacteria employ in their essential biosphere roles as biogeochemists, recyclers, symbionts and pathogens.

My own work has emphasized pattern formation in *E. coli* colonies because phenomena displayed by that species are widely accepted in the scientific community as general properties of bacteria rather than uniquely evolved specializations. A number of different technologies have been applied to investigating *E. coli* colony structure and development. These technologies include genetic engineering and histochemical staining (Fig. 1A)[6], macrophotography (Fig. 1B)[7], scanning electron microscopy[8,9] and time-lapse video recordings[10]. These methods reveal the colony as a dynamic, organized and differentiated structure which goes through a complex sequence of morphogenetic events[9,11,12]. Indeed, as soon as a single cell on agar has divided, a population of interacting cells has been created such that the geometry of growth involves responses between the two sister progeny[10]. After division of an isolated bacterium, the two daughter cells elongate by extending their inside poles alongside each other, while the outside poles remain fixed in place. This growth pattern produces a symmetric quartet of grandaughters because septation of each daughter accompanies elongation. It could be shown that this characteristic elongation mode involved cell-cell interaction rather than intracellular regulation by examining what happened when two cells were located within a few microns of each other. In such cases, one of the daughters frequently deviated from the usual pattern by extending from its outside pole to make contact with a neighboring cell and elongating alongside it rather than alongside its sister. Neighboring microcolonies invariably fuse together, and one has the impression from viewing videotapes that microcolony growth often accelerates in the direction of

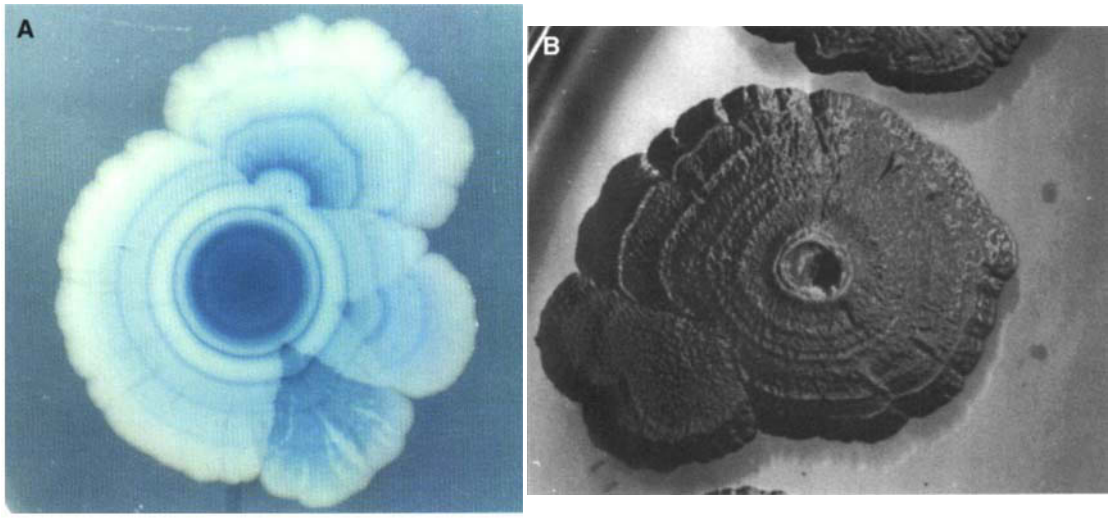

Fig. 1. Structure and pattern in *E. coli* colonies. The colony on the left (A) was grown on β-galactosidase indicator medium and had a differentially expressed *lacZ* fusion at 18.2 minutes on the bacterial chromosome. The colony on the right (B) was grown on standard medium and photographed by reflected light to visualize surface structure. Note the clonal sectors displaying different growth controls and the non-clonal concentric rings in both colonies.

nearby neighbors. The molecular basis of these behaviors is not known, but they are displayed by bacteria deleted for the chemotactic sensory apparatus[12]. Surprisingly, these observations indicate that the tendency of *E. coli* cells is to maximize cell-to-cell contact rather than individual cell access to substrate. Since crowded bacteria on agar divide as rapidly as their siblings in well-aerated liquid medium, it appears that *E. coli* (and, by inference, most bacteria) have evolved to live efficiently under dense multicellular conditions[13].

The predominant modes of organization in *E. coli* colonies are clonal sectors and non-clonal concentric rings (Fig. 1)[6]. Patterns of rings and sectors were first observed with histochemical staining for β-galactosidase activity of genetically engineered strains, but they can also be visualized in standard cultures by scanning electron microscopy[9] and reflected light photography (Fig. 1)[12,13]. Sectioning of colonies revealed further differentiation perpendicular to the agar surface in a series of strata displaying distinct patterns of cellular morphology and gene expression. For example, sections of an *E. coli* colony 60 μm thick were stained with toluidine blue and displayed a series of clearly distinguished layers: 6 μm of mostly well-stained cells just above the substrate; 16 μm containing a few well-stained cells amidst a vast majority of empty cells; a sharp layer 1-3 cells thick of well-stained bacteria; and an upper zone about 40 μm thick composed mostly of well-stained bacteria[12]. The empty cells observed in the bottom half of the colony presumably correspond to non-viable subpopulations detectable with cell-sorter technology that appear between 8 and 16 hours of colony growth (B. Hauer, H. Eippel and J. A. Shapiro, unpublished observations). When colonies containing the *lacZ* fusion seen in Fig. 1 were sectioned and examined for XGal staining, β-galactosidase expression was also seen to be organized in strata: a monolayer of expressing cells next to the agar substrate covered by a zone of non-expressing cells half the colony deep, two sharply defined layers of expressing cells at mid-colony, and a series of vertical 'flares' of expression in the uppermost zone[12]. This kind of stratified colony organization was documented 75 years ago in sectioned and stained colonies of *V. cholerae*[14]. The observation of spatially organized cells distinguished in terms of morphology, biochemical composition and gene expression leads one to conceive of the colony as composed of different 'tissues', each with its own physiological characters.

Pattern formation could be probed by viewing what happened when colonies encountered obstacles during development, such as glass fibers or other colonies[13]. In fields of colonies that developed synchronously from a strain carrying a differentially regulated *lacZ* fusion, the rings of β-galactosidase expression always merged when two colonies grew together (Fig. 2A). This result was to be expected simply on geometrical grounds. However, β-galactosidase rings also aligned when colonies of **different** ages grew together, and the pattern of the younger colony skipped early stages of expression to come into register with the older colony (Fig. 2B). This result was not so readily predictable and indicated that a diffusible chemical field in the substrate surrounding the older colony prematurely induced the corresponding phase of colony development in the younger colony. Since the bacteria both generate and react to these chemical fields, many

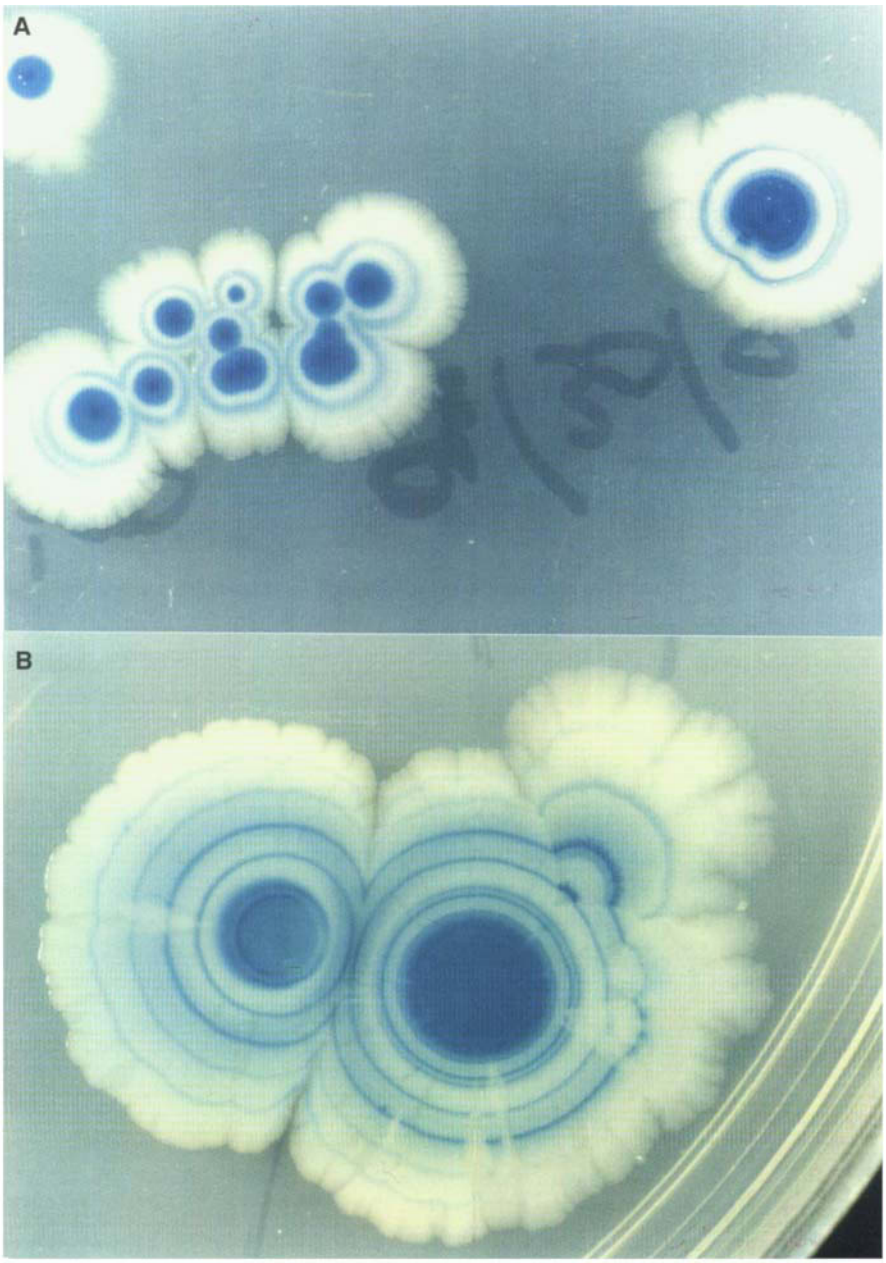

Fig. 2. Alignment of ring patterns in *E. coli* with a differentially expressed *lacZ* fusion. These colonies had the same *lacZ* fusion as in Fig. 1. The colonies in the top panel (A) were formed from bacteria streaked over the agar surface and so developed synchronously. The large colony in the bottom panel (B) was inoculated 36 hours before the adjacent smaller colony, and development had proceeded for a further 6 days when this photograph was taken. Note how the ring patterns of the two colonies were aligned despite their age differences. Also note that the smaller colony lacked a pair of closely spaced rings outside the dark central area which were present in the larger colony.

feedback situations can be set up creating possibilities for non-linear responses, such as the abrupt regulatory changes underlying the sharp boundaries of concentric rings (Figs 1 and 2). Even the synthesis of a basic cellular activity like DNA polymerase I responds differentially to bacterially created chemical fields as colony development proceeds[15]. Expression of *polA* is required in the first few hours of colony development to prevent SOS induction, but is not needed at later stages. Using *polA-lacZ* fusion constructs, the spatial consequences of this differential expression can be visualized on XGal indicator agar, and it can further be seen that old colonies produce some diffusible substance which inhibits *polA* expression in young colonies over distances of 1 cm or more. Comparable results demonstrating the effects of chemical fields on non-clonal patterns of differential *lacZ* fusion expression in *B. subtilis* have recently been published[16].

Using transposable Mu*dlac* elements, it has become clear that biochemical complexes which rearrange the genome also respond to the changing conditions that occur during colony development. Mu*dlac* was the original genetic engineering tool that facilitated observations of

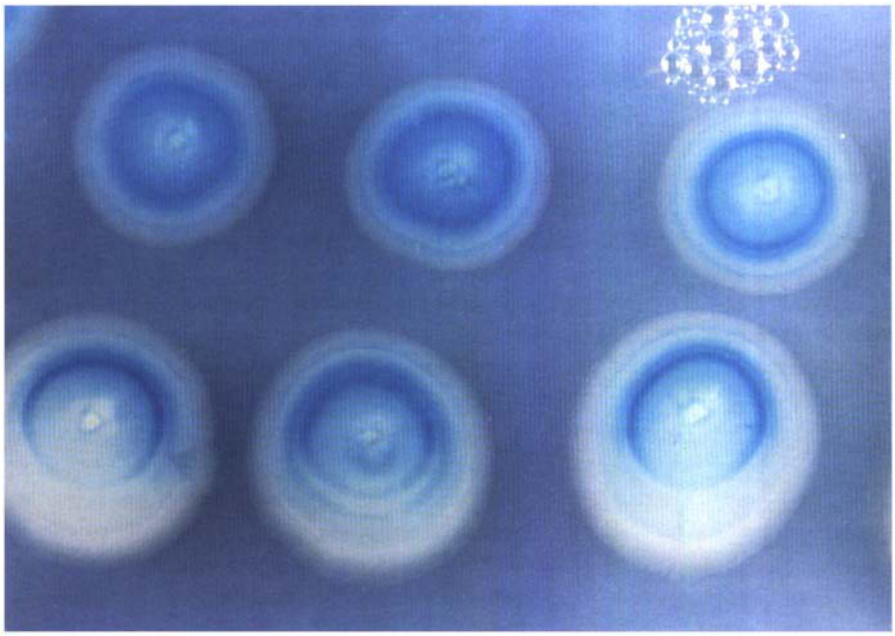

Fig. 3. Interaction of *E. coli* colonies carrying the Mu*dlac* element with a nutritional gradient in the substrate. These colonies were grown on a plate of β-galactosidase indicator agar containing glucose inoculated with many colonies. The edge of the plate was at the bottom, where substrates were less depleted due to the absence of colonies. In this zone, the colonies in the last row grew more extensively and displayed less enzyme activity. In the more depleted zone, however, β-galactosidase expression was confined to specific concentric ring zones of the colonies and thus was not determined solely by nutrient concentration in the substrate.

colony organization[6]; it is a phage Mu derivative capable of regulated replication and transposition and carries a decapitated *lac* operon, which can only be expressed if the Mu*dlac* inserts in the correct phase into an actively transcribed and translated open reading frame[17]. In some strains, the Mu*dlac* element was not located at a position where it could direct β-galactosidase synthesis. Nonetheless, colonies of these strains displayed concentric rings of enzyme synthesis (Fig. 3). The rings appeared because differentiated populations in which the Mu*dlac* element's transposition was derepressed formed at certain positions where the resulting replicative transposition created active *lacZ* fusions[18]. The correlation of Mu*dlac* replication with XGal staining was established by high-resolution colony hybridization with Mu-specific DNA probes, and the dependence of β-galactosidase expression on replicative transposition was confirmed using mutants defective in transposase. These results meant that the colony XGal-staining patterns were actually images of the locations where a particular class of DNA rearrangement was triggerred to occur at relatively high frequency. On Petri dishes with many colonies, nutritional gradients formed in the substrate, and the most intense *lacZ* expression was seen in the most depleted areas. However, even within colonies in the depleted zones, β-galactosidase activity was still limited to concentric rings, indicating that only certain organized subpopulations of bacteria could respond to the necessary chemical cues for Mu*dlac* derepression (Fig. 3)[12].

With the Mu*dlac* replication/transposition patterns as a readily-scored marker, it has become possible to initiate a genetic analysis to identify components of the signal transduction network controlling metabolically induced DNA changes. Introduction of well-characterized mutations linked to antibiotic resistance by P1 transduction showed that the Clp protease[19] and the cyclic AMP-CRP complex (ref. 12; J. A. Shapiro, G. Maenhaut-Michel and P. Westfall, unpublished observations) are required for Mu*dlac* derepression. Transposon mutagenesis of Mu*dlac* strains has implicated other factors, such as the *gidA* product[20], in the control of when during colony development zones form where DNA rearrangements occur at high frequency form. Thus, another significance of bacterial colony patterns has been to help bring the problem of physiological influence on genetic change ('adaptive mutation') into the realm of cell biology[21-23]. Since colonies of non-motile bacteria preserve a physical image of each clonal lineage, they are ideal tools for detecting regularities in sectoring patterns which indicate control of genetic change[24].

Different modes of colony development

Through their use of bacteria as hosts for cloned DNA fragments, most biologists are familiar with the compact circularly symmetrical colonies produced by *E. coli* and many other bacteria on standard laboratory media containing high concentrations of agar and excess nutrients. The fact that these colonies display organization and complex morphogenesis means that interactive behavior is not limited to special stress conditions. However, the well-fed surface colony produced by non-migrating bacteria is only

one of several modes of bacterial colony development – and perhaps not the most interesting. Some bacteria form extended colonies on standard media by a sequence of events that includes active migration by cell groups over the agar surface, a process known generically as 'swarming'[25]. Other bacteria produce distinct colony morphologies depending on the environmental and nutritional conditions. Bacteria that are non-motile on hard surfaces can often swim chemotactically in semi-solid media, and many species adjust to nutritional deprivation by spreading dendritically. Analysis of these alternative colony morphogenesis styles has become a rich source for documenting additional instances of cellular differentiation and multicellular coordination in bacteria.

Swarming

Swarm colony morphogenesis occurs in many different bacterial taxa, including enterics like *Proteus* and *Serratia*, marine *Vibrios*, and gram-positive *Bacilli* and *Clostridia*[25]. All these bacteria form elongated, hyperflagellated swarmer cells which can migrate over agar surfaces. Swarmer cells almost never form in liquid medium, and at least some species use resistance to flagellar rotation as a sensory mechanism to determine that they are on a solid surface and trigger swarmer cell differentiation[26]. In many species, only groups of cells ('rafts') can migrate over empty agar. When an individual cell detaches from a raft, it is immobilized until incorporated into another passing raft. This observation suggests that raft mobility involves coordinated action, and videotapes reveal synchronized movement of flagella in rafts. In *Serratia* swarming, the bacteria produce wetting agents which are important factors in colony expansion[27]. The mechanics of how flagellar rotation translates into multicellular movement over a solid surface are unknown, but 'rafts' of swarmer cells were observed to be encased in exopolymer 'cocoons' when fixed for scanning electron microscopy[28]. Perhaps flagellar movements within a viscous gel provide propulsion over a 'lubricated' agar surface. In speculating about possible roles for exopolymers in bacterial group movement, it is important to recall that slime trails deposited by myxobacteria are also important in group movement and swarm colony development by these 'gliding' bacteria, which do not use flagella for motility[29]. Extracellular polymeric material can also play a role in the development of colonies by non-motile bacteria, such as *Pseudomonas putida*; polymerized materials were readily visible in scanning electron micrographs of colonies produced by this species[8].

Despite the apparent randomness of swarm cell movements, swarm colonies can be exquisitely geometrical and propagate structural features with great precision (Fig. 4). Morphogenesis of highly terraced *Proteus* colonies involves an intricate series of steps[11,28,30-32]. These include cyclical alternation over periods of several hours, between a 'swarming' phase involving active outwards migration and a 'consolidation' phase where the colony perimeter is stationary but cell divisions and rearrangements occur inside the colony. Within each phase, other rhythmic events can be observed, with periods ranging from fractions of a second (flagellar rotation) to many minutes (internal wave movements within the swarm phase). By observing spontaneous deformations of colony morphogenesis and interactions of swarm colonies with obstacles such as other colonies, it is clear that swarm cell migration and terrace formation are subject to quite precise orientation systems (Fig. 4). These systems appear to utilize bacterially created chemical fields as cues for migration, because trenches cut out of the agar substrate as chemical diffusion barriers 'shadow' areas of the growth medium where the expanding swarms do not enter, even after circumnavigating the trench barriers[1,11]. Additionally, sharp boundaries form between adjacent colonies inoculated at different times, indicating that systems are also at work providing internal coherence and identity to each swarm colony (Fig. 4).

Chemotactic autoaggregation

Some of the most striking bacterial patterns have been found in expanding colonies of chemotactic bacteria in semi-solid media[33]. It has long been known that chemotactic bacteria formed expanding concentric ring patterns in soft agar. As they consumed nutrients in the central inoculated zone, sequential uptake of different nutrients in the medium led to the formation of several gradients of chemoattractants across the Petri plate, and the bacteria migrated outwards chasing these gradients. Eventually, the substrates were exhausted, and the swarm rings disappeared[34]. This behavior was interpreted statistically as reflecting the average movement of many independent cells[35]. But different types of patterns formed in chemotactic colonies growing on intermediates of the TCA cycle or in medium containing a stress agent, such as hydrogen peroxide or low levels of antibiotic (Fig. 5). Genetic or chemical blockage of the Tar chemoreceptor prevented pattern formation[33]. Amino acid analysis of the products secreted by the aggregating bacteria showed that the stressed bacteria began to secrete aspartate and glutamate, both powerful chemoattractants sensed by the Tar chemoreceptor[36]. Thus, these excreted amino acids served as autoaggregation signals. Once in an aggregate, a high proportion of the bacteria frequently lost their motility and so produced a dot consisting of nonmotile cells (E. O. Budrene, personal communication). Distinct patterns of

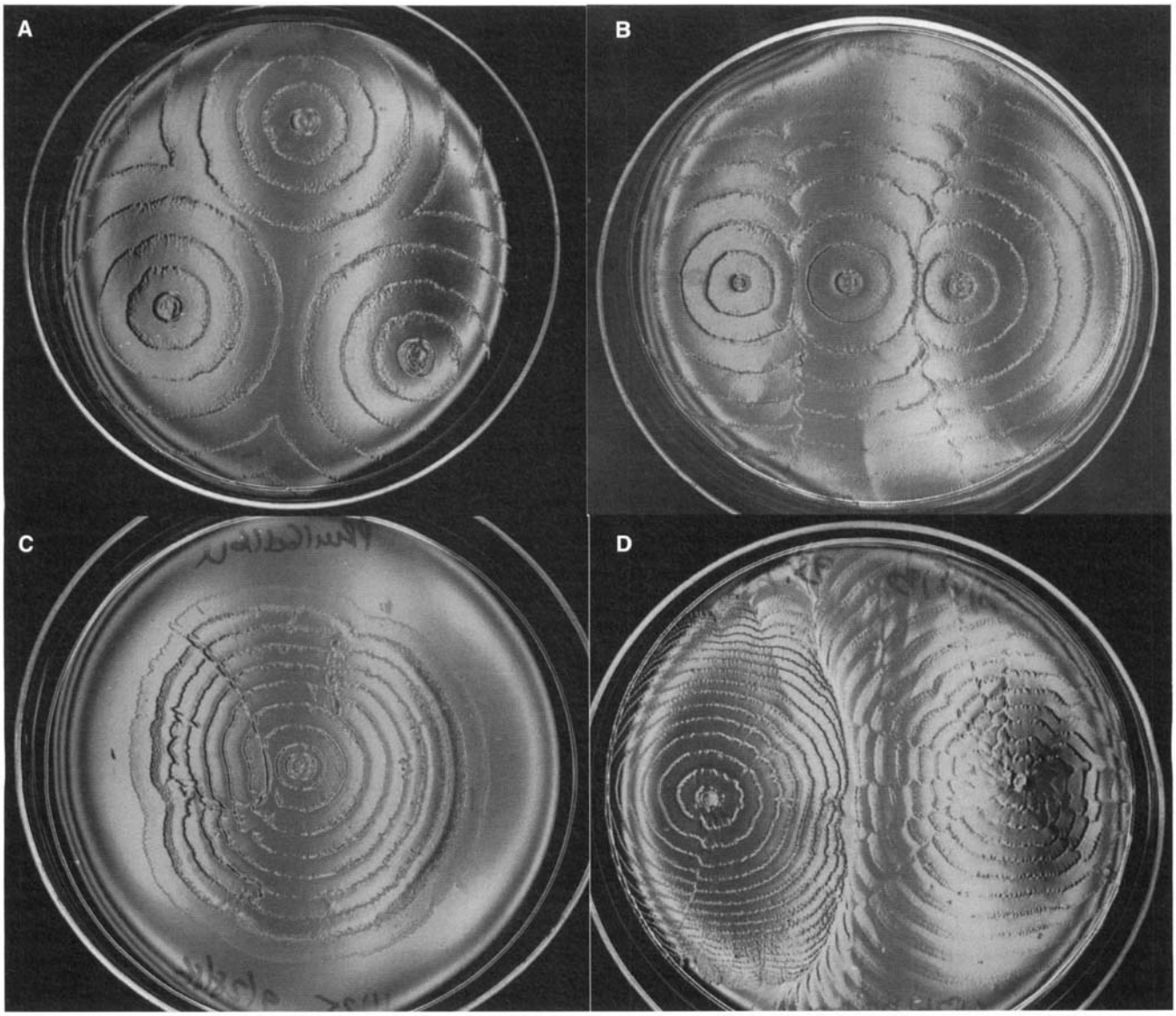

Fig. 4. *Proteus mirabilis* colonies. The colonies in the top row were produced by strain PRM 48,000, a clinical isolate. The colonies at upper left (A) were all inoculated at the same time (note how the terraces coalesced) while the colonies at upper right (B) were inoculated at different times (and their terraces did not coalesce). The colonies in the bottom row (C,D) were produced by strain PRM 16, a mutant of a clinical isolate with more closely spaced terraces. These plates displayed spontaneous irregularities that appeared in the first few terraces and then propagated during subsequent terraces.

these dots formed in different soft agar media. The geometry of each pattern appeared to depend on several key variables: cell density, magnitude of the chemotactic response, concentration of the chemoattractants and the rate of loss of motility in aggregates. Under certain conditions, aggregates remained intact for long periods of time and sometimes behaved as integrated units. For example, they could migrate, split and fuse, and so it is possible that additional forms of intercellular communication were operating within and between the aggregates (E. O. Budrene, personal communication). Clearly, these patterns demonstrated situations in which migrating *E. coli* cells did not move independently but were coordinated by means of secreted signals processed by the chemotaxis system. From the perspective of biological utility, it should be noted that bacteria in multicellular aggregates have a much better chance than isolated cells of surviving exposure to toxic substances[1,37].

Colonies under nutritional deprivation

A major significance of bacterial colony patterns resides in their contribution to the solution of basic problems of self-

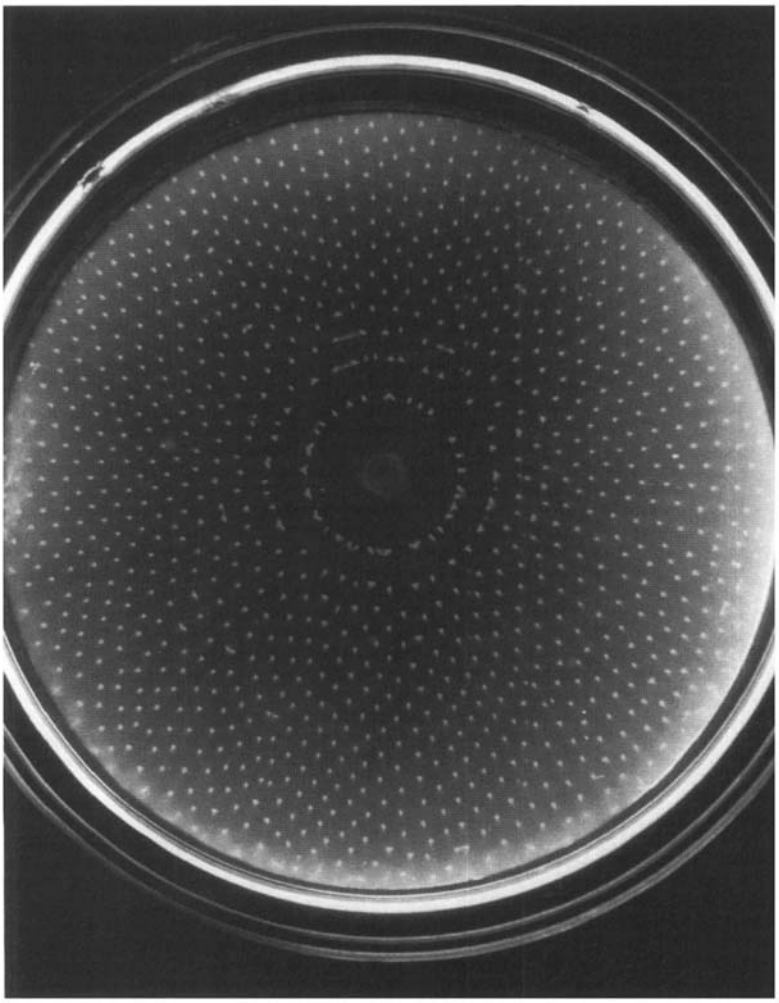

Fig. 5. A punctate pattern produced by chemotactic *E. coli* cells after migrating outwards in soft agar containing succinate as carbon source (courtesy of Dr Elena Budrene, Harvard University).

organization and morphogenesis which cross traditional disciplinary boundaries. Colonies constitute some of the most easily studied illustrations of the micro/macro problem in pattern formation: how indescribably complex series of events at the microscopic (cellular) level can lead to geometrically regular and predictable morphologies at the macroscopic (whole colony) level. Some physical scientists interested in general problems of pattern formation have begun to utilize bacterial colonies as their experimental material. Rather than looking at colonies on standard media where there is a nutritional excess, they have focused on patterns produced under suboptimal conditions[38,39]. It has been very instructive to display colony outlines in a two-dimensional graph, varying the nutrient concentration along one axis and agar concentration along the other, to produce morphology diagrams analogous to the plots used to display physical phase transitions[39,40]. At certain positions, the colonies assume morphologies identified in inorganic systems. As substrate concentrations fall and agar concentration increases, colonies of many species become more branched and dendritic, frequently showing fractal patterns predicted by diffusion-limited aggregation (DLA) models. This suggests that nutrient diffusion is the primary morphogenetic determinant when resources are scarce and bacterial motility is inhibited. However, there are exceptions to this general rule. For example, *B. subtilis* colonies can become more compact and symmetrical at the lowest nutrient concentrations. To make computer simulations of these more compact patterns, an equation for repulsive chemotactic signalling between the bacteria must be added to the mathematical model[41]. While mathematically inelegant because it is *ad hoc* rather than derived from first principles, addition of such extra equations is biologically realistic because it suggests the operation of particular intercellular communication systems under specific physiological conditions.

Microscopic examination of *B. subtilis* in branched colonies reveals that morphogenesis involves the behavior of coordinated multicellular groups, not isolated cells[41]. The branches are each encased in a viscous

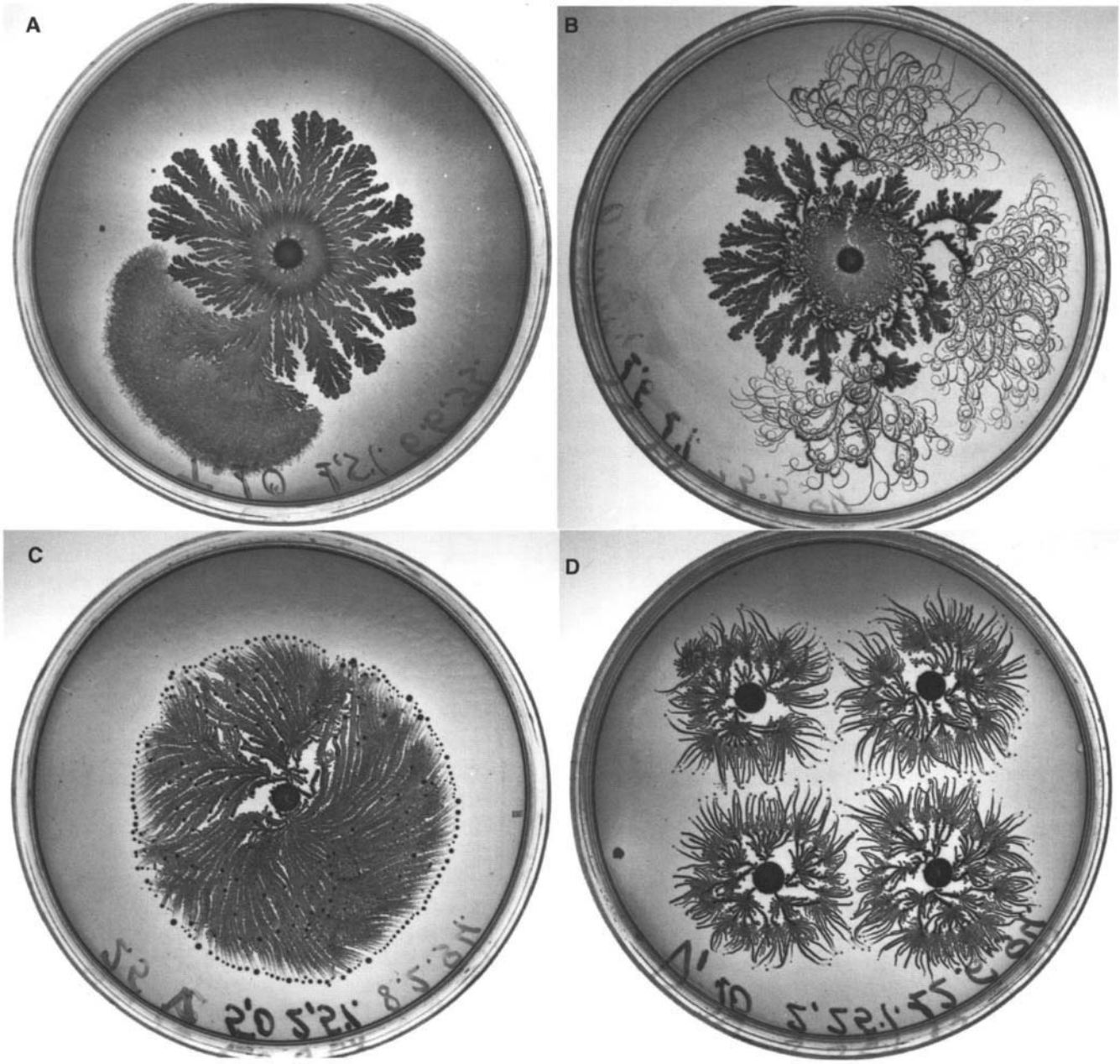

Fig. 6. Patterns produce by *Bacillus subtilis* on agar containing limiting peptone substrate (courtesy of Dr Eshel Ben-Jacob, Tel Aviv University). In the top row (A,B), note the sectors which emerged from the colony perimeter displaying novel growth patterns. In the bottom row (C,D), the dark spots at the perimeter were rotating, migrating minicolonies or 'vortices', which left behind trails of non-migrating cells.

'envelope', probably composed of materials analogous to those which make up the extracellular polysaccharide gels which surround *Proteus* swarmers. Within these envelopes, the bacteria are often highly motile. The importance of motility is confirmed by the observation that *B. subtilis* mutants lacking flagella produce a distinct, more limited morphology diagram than do flagellated strains[40]. Morphologically distinct sectors often form at the periphery of colonies (Fig. 6). By selecting variant bacteria from these sectors, it is possible to obtain pure cultures that display remarkable new patterns[39,42]. Some of these patterns arise from the migration of rotating mini-colonies ('vortices'), which leave behind trails of non-moving cells (Fig. 6)[42]. This kind of whole-colony migratory behavior has been known for some time in species named *Bacillus circulans* and *B. rotans*, and dramatic films of these group

migrations are available[43]. In some of the sequences filmed under polarized light conditions, it can be observed that the colonies are themselves polarizing, which suggests a microcrystalline arrangement of the cells.

Observations of multicellular structure and coordinated movements leave little doubt that intercellular signalling is occurring in these nutritionally stressed *Bacillus* colonies. One intriguing aspect of the patterns is how sectors with new growth characteristics arise. Frequently, many such sectors emerge more or less synchronously from different sites around the colony perimeter (Fig. 6)[42]. These apparently coordinated yet independent events of genetic change may be similar to the coordinated activations of Mu*dlac* elements in *E. coli* colonies, described above. If so, pattern formation in nutritionally challenged *B. subtilis* colonies may provide another example of regulatory links between cell physiology, cell-cell interactions and the biochemistry of genomic reorganization.

Conclusion

An emerging new paradigm in prokaryotic biology

The picture that one gets from looking at the various examples of bacterial colonies cited here (and at other cases not discussed, such as morphogenesis of Myxobacterial fruiting bodies[29]) is decidedly interactive and multicellular. Spatial organization, differential gene expression, intercellular communication and pattern formation are all phenomena unambiguously documented in several bacterial taxa, including the archetypical *E. coli* and *B. subtilis*. Consideration of coordinated cell movements by *E. coli, Proteus, Bacillus* and the myxobacteria justifies using the term 'group behavior' for prokaryotes, a perspective reinforced by viewing movies and videotapes of colony development[10,11,43,44].

Attention to multicellular phenomena like colony patterns and group motility are at the heart of a new paradigm in prokaryotic biology which emphasizes intercellular communication and cellular decision-making based (to a considerable extent) on information received from other cells. The research agenda of this new paradigm will be ample. Continued identification of intercellular signals and the intracellular signal transduction networks which respond to them is critical. In the laboratory, a key avenue of research will be genetic analysis of cellular functions involved in multicellular development, which has already produced significant results with *Myxococcus xanthus*[29] and *Proteus mirabilis*[45]. The interpretation of genetic experiments will not be simple, however. For example, transposon mutagenesis revealed that a *selB* mutation altered *E. coli* colony morphology in a very specific way, changing the colony center from a dome to a crater. The *selB* locus encodes a translation factor for the incorporation of seleno-cysteine into formate dehydrogenase[46]. Thus, to explain this result, we will ultimately have to understand the interplay between cellular physiology and colony morphology. In the environment, an important new topic will be the documentation of further examples where organized, differentiated populations allow bacteria to proliferate and survive better in natural settings. Perhaps the most fascinating subject will be unravelling the evolutionary aspects of bacterial multicellularity. One basic question is, how have multicellular regulatory networks evolved during the radiation of so many physiologically diverse bacterial taxa? A complementary question arises out of observations such as concentric ring patterns of Mu*dlac* replication/transposition: how does bacterial multicellularity influence fundamental processes of genetic change?

Bacterial systems are at the forefront of issues like pattern formation by complex self-organizing systems and biological feedback onto the mechanisms of genome reorganization. The realization is growing that bacteria are also suitable for studying problems of intercellular communication and multicellular development. These topics are emerging as focal points of contemporary research. Science is undergoing its greatest intellectual revolution since the time of Descartes. The new emphasis is on connections, complexity and the behavior of systems rather than the inherent properties of the smallest possible units. Living systems are the key to this new approach because well-established biological concepts like homeostatic feedback regulation, sensory information processing, behavioral responsiveness and hierarchical integration are applicable to all complex systems. Given the great virtues of bacteria as experimental material, especially the unparalleled nucleotide-by-nucleotide resolution of bacterial genetics, it is hard to escape the conclusion that these small but highly sophisticated cells will once again be the organisms of choice for clarifying basic problems at the leading edge of science.

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