ESSAY

Microbial awakenings

A theory of how microbes 'wake up' from dormancy could help to solve scientific mysteries and improve disease control, says **Slava S. Epstein**.

Dormancy is a useful tool. Many plants and animals survive the winter in this way; spore-forming microbes use it to survive for perhaps millions of years. But what of non-sporulating species, which make up the great majority of microbial diversity?

Since at least the 1920s, these microbes have been strongly suspected to have a dormant state. But the picture isn't clear. Dormancy is exceedingly difficult to demonstrate directly, unequivocally prove or even to study in non-spore-forming microbial species. But why is it so hard to crack?

The answer may be simple: perhaps these microorganisms have a different mechanism of revival than those we are used to. Rather than awakening to environmental cues (as plants, animals and spores do), these microbes may wake stochastically — randomly.

I envision a situation in which microbial populations consist of a mix of active and dormant cells. Faced with an adverse environmental change, more cells are induced into dormancy, and survive the challenge. Individual cells would then periodically exit dormancy as a result of infrequent and essentially random events, such as a change in the expression of a master regulatory gene. I call such awakened cells 'scouts'. If the adverse conditions persist, the scout dies. If a scout forms under growth-permissive conditions, it starts a new population. In some species, scouts might even use growth-inducing signalling compounds to wake up the rest of the dormant population.

Waking the dead

Such an 'abnormal' pattern of revival, which differs from that of other microbes and contrasts with scientific expectations, would be easy to overlook. Researchers might see some evidence of revival events but, given the random nature of such events, be unable to reproduce the effect in further experiments, and so throw away legitimately positive results. Alternatively, researchers attempting to revive dormant microbes might instead find themselves accidentally and futilely attempting to wake up dead cells rather than dormant ones, thus providing a false negative.

The scout theory helps to explain apparently random disease recurrence, as happens for example with tuberculosis. Suppose that a small population of dormant cells escapes both the host's immune system and an antibiotic treatment (today's antibiotics target mainly actively growing cells). The possibility of disease recurrence would be determined by the balance between scout formation and the ability of the immune system to eliminate the scouts' progeny. When the pathogen succeeds, the relapse would seem to the observer as a random event.

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If this is how microbes truly work, it has important implications. If the molecular mechanism of scout formation were known, researchers could potentially devise a way to make it less frequent, possibly to the point of rendering a population permanently dormant and thus harmless. Alternatively, they could artificially wake up all dormant cells into scouts, and dose them with drugs while they are active and vulnerable.

The scout model also redefines the nature of 'persisters', a small proportion of cells that are curiously tolerant of high concentrations of antibiotics and yet genetically identical to the vulnerable cells (by contrast with genetic mutants that might be resistant to a given antibiotic). Previous theories have proposed that microbial populations harbour specific, specialized dormant cells that survive the antibiotic challenge and grow once the challenge has been removed. It was unclear, however, how such persister cells could be both sufficiently dormant to not divide in an otherwise growing population the moment before the challenge, and then, under identical conditions after the challenge, be sufficiently active to form a colony.

My model postulates that the pool of dormant cells that survives the dose of antibiotic is substantially larger than the pool of persisters currently thought to survive. These dormant cells are effectively invisible to researchers because they do not grow, and so have been overlooked or dismissed as dead. If this pool of dormant cells continues to produce a small number of random scouts — before, during and after the antibiotic challenge — then the results are logical. Persisters are thus simply scouts generated by the large — and so far ignored — pool of true survivors.

Survival scouts

Dormancy and random scout formation could also help explain the 'great plate-count anomaly': the remarkable disparity between the high number of cells in a sample, and the small number of colonies these cells form *in vitro*. This was first observed in the late 1800s, making it arguably the oldest unresolved microbiological mystery.

Environmental samples harbour tens of thousands of species, most of which contain just a handful of cells per population. Low abundance suggests inactivity, and dormancy. Statistically, only a small minority of such rare and dormant populations would be expected to contain scouts at any given time. The majority of populations would therefore not grow, regardless of the conditions. In principle, scouts should appear in any population given a long enough period of incubation, but in practice Petri dishes are often thrown away by that time, or are overgrown by other, fast-growing species.

This suggests several ways to recover the 'missing' microbial diversity. Single cells could be cultivated, allowing them enough time for scout production unimpeded by faster growing species. Alternatively, researchers could attempt to identify signalling compounds used by scouts, if they exist, and use these molecules to induce growth of dormant kin.

The scout model makes other predictions. For example, in a prolonged incubation, a given microbial species would be expected to produce colonies not at a specific time but continuously over the entire duration of the growth experiment. These and other predictions can be checked through very simple experiments, several of which are under way in our laboratory. ■

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