inherent response to stress or starvation; or the increased expression of pump proteins (reviewed in ref. 6).

Drenkard and Ausubel¹ tackled the problem of the antibiotic resistance of biofilms by studying *P. aeruginosa*. They first cultured a *P. aeruginosa* strain on agar plates containing one of several medically useful antibiotics, such as tobramycin, gentamycin or kanamycin. Antibiotic-resistant *P. aeruginosa* colonies developed, many of which were small and had rough edges (and so were dubbed 'rough small-colony variants'). Compared with the original strain, these variants also formed larger biofilms, and more rapidly. These observations imply that biofilm formation and antibiotic resistance are switched on together.

A similar situation may occur in cystic-fibrosis patients, as the authors showed by studying the colonies that arise after culturing sputum samples from patients' lungs. All the *P. aeruginosa* samples from patients who were being treated with antibiotics were small-colony variants, and many were highly resistant even to antibiotics not being used for treatment. (Resistance to a wide range of antimicrobial drugs is another characteristic of biofilms.) So the antibiotic-resistant, small-colony variants seem to be important in cystic-fibrosis patients, as well as *in vitro*. The authors found few or no small-colony variants in untreated patients.

How might the switch to antibiotic resistance and biofilm architecture be controlled? The small-colony variants derived from the original *P. aeruginosa* strain reverted to the original characteristics (phenotype) when cultured without antibiotics¹. This suggests that temporary phenotypic changes are involved, rather than permanent genetic alterations. More specifically, these changes could be due to 'phase variation' — a mainly random process whereby bacteria use several molecular mechanisms to express phenotypes that may be advantageous in a particular environment. The process is not typically controlled by the environment, and involves randomly producing many different phenotypic variants, the goal being one that can survive and proliferate.

But there are cases of environmental signals specifically controlling the rate of phenotypic switching⁷, and one well-studied example involves the expression by *Escherichia coli* of surface structures called Pap pili, which enable the bacterium to attach to its host. At 37 °C (body temperature) the apparently random addition of methyl groups to regulatory sequences flanking the *pap* genes dictates whether the expression of Pap pili is on or off. But changes in temperature are one environmental stimulus that affect this on/off switch, and the expression of Pap pili is completely shut down at 26 °C or below⁸ (presumably when *E. coli* has left its host

and no longer needs these structures).

Similarly, Drenkard and Ausubel's results¹ suggest that environmental cues — such as the conditions in the lungs of cystic-fibrosis patients — may control the degree of antibiotic resistance of *P. aeruginosa*. They identified a *P. aeruginosa* regulatory protein that, when overexpressed or mutated, altered the rate at which rough small-colony variants reverted to the antibiotic-sensitive phenotype. The authors propose that this protein is not directly involved in the modifications required for phenotype switching, but may regulate the factors that are required.

So Drenkard and Ausubel have described a general mechanism — environment-driven phenotypic switching — that explains how antibiotic-resistant biofilm variants of *P. aeruginosa* arise. They also propose that these particular variants account for the antibiotic resistance of *P. aeruginosa* in cystic-fibrosis patients. Previous work supports this contention: it seems that not all biofilm bacteria are highly antibiotic resistant. Indeed, most biofilm cells die after treatment with antibiotics, but a small population survives, and is eliminated only when high antibiotic concentrations are used^{9,10}. Moreover, it has been proposed that some biofilm cells develop a phenotype that renders them resistant to antimicrobial drugs^{11,12}, and that this developmental programme is triggered by environmental signals. The highly resistant cells are called persisters^{12,13}. Perhaps the small-colony variants identified by Drenkard and Ausubel¹ are likewise persisters.

We still do not know what characteristics enable these cells to survive in the face of antibiotics. But it might be possible to develop treatments that target persisters — for example, by modifying the regulatory protein of *P. aeruginosa* — thereby rendering remaining biofilm cells susceptible to conventional antibiotics. ■

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Daedalus

Very slow vacuum

Fast-moving particles or atoms leave tracks in many materials. Geologists assess the ages of specimens by studying the tracks of particles or recoil nuclei; microscopists use molecules or ions like light, firing them at surfaces or through samples to gain information. Technologists can make many films: polyalkane, polyester, polyamide, their laminates, and so on. DREADCO physical chemists are now firing atoms, molecules and ions through thin films from one side only. Most of the resulting damage-tracks will just be fine holes. But Daedalus reckons that, every so often, a molecular group will be pushed away but not detached. Even if it returns to its place it will easily give way again, in that same direction. He is looking for a molecular one-way valve, the equivalent of the rectifier in electronics.

Initial progress must depend on luck. Daedalus cannot guess what molecule or ion, fired at what energy at what film, will give the best one-way valve for what gas molecules. But the resulting DREADCO 'Vacfilm' will have many uses, especially for the molecules in air. Seal something inside it; if its pumping direction is outwards, it will slowly extract all internal air. The Maxwell-Boltzmann distribution gives some molecules twice the average energy or even more; these are the ones that will pass the little valves. But the process may take many weeks. Daedalus reckons that Vacfilm, like an inefficient Carnot engine, will be driven by small daily changes of temperature. Conversely, however, a mere atmosphere of adverse pressure is nothing to a molecular valve. In due course vacuum will be achieved and maintained; even small leaks will be pumped out.

The obvious use is food wrap. Vacuum packing is already used for products such as bacon. Many products would last almost indefinitely if protected from oxygen, now imperfectly excluded by aluminized film. A self-pumping food film would be widely welcomed. But Daedalus also recalls the refrigerator. It uses almost all its power pumping away heat, which just flows back through bad insulation. The best foamed insulation should be filled with vacuum, yet air always slowly leaks in. A block of foam inside sealed Vacfilm should be ideal. It would keep the heat out indefinitely, and any small air-leaks would soon be pumped away. The constant temperature-swings of the refrigerator thermostat would keep the Vacfilm working.

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