sequesters actin monomers, thymosin-β4, which was not included in this study.

Profilin may also bind to VASP, the second stimulatory component examined by Carlier and colleagues. VASP interacts with the bacterial surface protein ActA, and may 'shuttle' profilin–actin subunits to the new barbed ends[13]. However, profilin mutants that do not bind VASP are able to stimulate motility[14]; and VASP without profilin has a stimulatory effect on its own, perhaps by directly interacting with actin filaments[15].

Finally, α-actinin, which crosslinks actin filaments, is known to be necessary for bacterial movement in living cells[16]. Carlier and co-workers found that it did not influence the speed of bacterial movement but did affect the morphology of the tails — without α-actinin, tails were splayed, not compact, because filaments were not crosslinked. This crosslinking function may be essential *in vivo*, where the forces necessary for movement should be greater than those in Carlier and colleagues' purified *in vitro* system.

Other components, not tested here, may also be necessary or stimulatory for bacterial motility in cells. The requirements for motility *in vivo* may be different or more demanding than those in the reconstitution system. For example, the need for profilin may be greater if thymosin-β4 acts to buffer the supply of actin monomers.

As well as providing a wonderfully simple model for actin-based motility, Carlier and colleagues' landmark study provides a new motility assay to analyse components and regulators of the actin cytoskeleton. It complements other methods for measuring actin polymerization and network formation, and greatly increases our ability to analyse actin assembly *in vitro*. ■

Laura M. Machesky is in the Division of Molecular Cell Biology, School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK.
e-mail: L.M.Machesky@bham.ac.uk
John A. Cooper is in the Department of Cell Biology, Washington University, St Louis, Missouri 63110, USA.
e-mail: jcooper@cellbio.wustl.edu

1. Loisel, T. P., Boujemaa, R., Pantaloni, D. & Carlier, M. F. *Nature* **401,** 613–616 (1999).
2. Dramsi, S. & Cossart, P. *Annu. Rev. Cell Dev. Biol.* **14,** 137–166 (1998).
3. Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J. & Pollard, T. D. *J. Cell Biol.* **127,** 107–115 (1994).
4. Welch, M. D., DePace, A. H., Verma, S., Iwamatsu, A. & Mitchison, T. J. *J. Cell Biol.* **138,** 375–384 (1997).
5. Welch, M. D., Rosenblatt, J., Skoble, J., Portnoy, D. A. & Mitchison, T. J. *Science* **281,** 105–108 (1998).
6. Mullins, R. D., Heuser, J. A. & Pollard, T. D. *Proc. Natl Acad. Sci. USA* **95,** 6181–6186 (1998).
7. Carlier, M.-F. & Pantaloni, D. *J. Mol. Biol.* **269,** 459–467 (1997).
8. Carlier, M.-F. *et al. J. Cell Biol.* **136,** 1307–1322 (1997).
9. Maciver, S. K., Pope, B. J., Whytock, S. & Weeds, A. G. *Eur. J. Biochem.* **256,** 388–397 (1998).
10. Blanchoin, L. & Pollard, T. D. *J. Biol. Chem.* **274,** 15538–15546 (1999).
11. Machesky, L. M. *et al. Proc. Natl Acad. Sci. USA* **96,** 3739–3744 (1999).
12. Pantaloni, D. & Carlier, M. F. *Cell* **75,** 1007–1014 (1993).
13. Chakraborty, T. *et al. EMBO J.* **14,** 1314–1321 (1995).
14. Egile, C. *et al. J. Cell Biol.* **146,** 1–14 (1999).
15. Laurent, V. *et al. J. Cell Biol.* **144,** 1245–1258 (1999).
16. Dold, F. G., Sanger, J. M. & Sanger, J. W. *Cell. Motil. Cytoskeleton* **28,** 97–107 (1994).

Plant defence

Long view from a high plateau

Jeff Dangl

Scientific endeavour tends towards a punctuated equilibrium — slow periods during which systems and tools are developed, followed by bursts of new knowledge. For the molecular understanding of plant–pathogen interactions there have been three recent explosions. The first was the isolation of plant disease-resistance (*R*) genes (Fig. 1); second was the ability to isolate mutants in 'model' plants such as *Arabidopsis thaliana* and tomato; and third was the (initially improbable) finding that bacterial pathogens of both plants and animals rely on a conserved delivery system to ferry the effectors of disease into their hosts. Experiments and achievements deriving from these three breakthroughs were reported at two meetings earlier this year*.

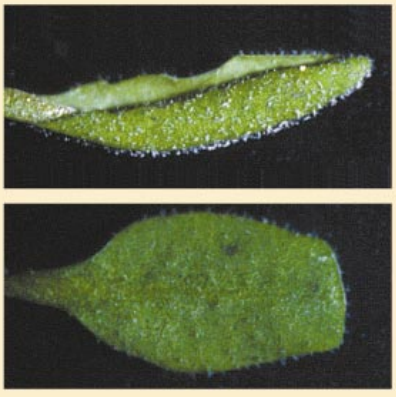
One current debate swirls around how the diversity of the *R* genes evolves and is maintained. This debate reminds me of (and draws from) discussions that followed the isolation of major histocompatibility complex genes in the early 1980s — full of structures and sequence comparisons, with enlightening forays into population genetics and molecular evolution.

Most R proteins contain leucine-rich repeats (LRRs), and there is overwhelming evidence that solvent-exposed surfaces of these repeats are subject to diversifying selection[1,2]. Selection acts on point mutations and on short tracts of DNA exchanged between chromosomes by recombination and, probably, gene conversion. Many *R* genes are found in linked clusters on the chromosomes. At the *R* cluster of each parental chromosome, those *R*-gene sequences (or 'haplotypes') that derived from a common ancestor (orthologues) seem to be more related than those that originated by duplications (paralogues; Richard Michelmore, Univ. California, Davis). If so, *R*-gene divergence is an ancient event[3].

A related and equally compelling argument is that architecture of the *R*-gene cluster determines the recombinational outcome and influences subsequent diversity (Jonathan Jones, Sainsbury Laboratory, Norwich). Two haplotypes at a region known as *RPP5* in *Arabidopsis* are scrambled by rearrangements, so it is impossible to define orthologues here. Recombination between these two haplotypes is suppressed. The extraordinary divergence in the *R*-gene cluster is backlit by considerable homology between the DNA sequences that flank *RPP5*. By contrast, the tomato *Cf-9* and *Cf-4* genes are orthologues embedded in unique, but fairly linear, haplotypes, and evidence for recombination between these two genes can, occasionally, be found.

When combined, these results (Michelmore; Jones) indicate that, at the *R* clusters examined, unequal recombination events can influence the evolution of a particular stretch of DNA, but that they contribute little to its diversification. Evolution of an *R*-gene cluster can also be influenced by chromatin dynamics, a view supported by molecular analyses of the flax *L* and *M* regions. When sequences from 11 of the 13 alleles at *L* were compared, diversity was found to be generated by deletion and expansion of LRRs. This region is also subject to diversifying selection (Peter Dodds, CSIRO, Canberra). So, similar events occur at simple and complex *R* genes.

Diversity aside, what is the function of the R proteins? The simplest idea is that they act as receptors for ligands encoded by the *avr* genes, but this has been difficult to



J. MCDOWELL & D. AVIV

Figure 1 **Downy mildew disease of many plants is caused by obligate oomycete parasites. *Peronospora parasitica* infects one *Arabidopsis thaliana* accession (top), but a second *Arabidopsis* inbred line is resistant (bottom) owing to the action of a single disease-resistance (*R*) gene.**

Attack and Defence: The Thirteenth John Innes Symposium 20–23 July, Norwich, UK; and *Ninth International Congress of Molecular Plant–Microbe Interactions* 25–30 July, Amsterdam, The Netherlands.

demonstrate. An LRR domain encoded by the rice *Pi-ta* gene has been shown to bind its cognate Avr protein, though, and this interaction can be terminated by exchanging a single amino acid on the carboxy-terminal side of the final LRR (Barbara Valent, DuPont, Wilmington). Interactions between R and Avr might require a third (or even a fourth) partner, and, although sequence variability in the LRRs determines specificity, a broad structural signature might be derived from an R–Avr complex containing other proteins. For example, a yeast three-hybrid screen was used to isolate partners for the AvrPto–Pto pair (Greg Martin, Cornell Univ.). Pto is a rather exceptional *R*-gene product — it has no LRRs and, although it binds AvrPto, it also requires a more catholic LRR-containing protein called Prf in order to function.

How can we identify more genes required for *R* function? Genetic screens for loss of resistance pick out only a few genes owing to redundancy or lethality. For instance, the barley Rar1 protein is required for cell death after recognition of a pathogen (Paul Schulze-Lefert, Sainsbury Laboratory), and its homologue in the nematode *Caenorhabditis elegans* is also involved in germ-cell death. Hence, what Schulze-Lefert terms "transgenomics" can identify conserved functions across kingdoms. Yet despite the difficulties, the spectrum of *Arabidopsis* mutants with effects on disease resistance grows continuously[4]. In conditional *avr*-expression screens of several hundred thousand progeny, for example, we have identified rare mutations of new genes required for *RPM1* function (Pablo Tornero and myself).

There is also redundancy in the steps that occur after the R protein has recognized its ligand. When parsley cells are exposed to a fungal protein that initiates defence responses, five mitogen-activated protein (MAP) kinases are activated (Dierk Scheel, Inst. Plant Biochem., Halle). All tested isoforms seem to activate defence genes in parallel. And, using the powerful 'fast-forward genetics' method, a calcium-dependent protein kinase has been identified upstream of other, probably redundant, MAP kinases that transduce the function of the *Cf-9* and *Cf-4 R* genes (Tina Romeis, Sainsbury Laboratory).

The technique of fast-forward genetics was developed by David Baulcombe and collaborators (Sainsbury Laboratory), and is called virus-induced gene silencing (VIGS)[5]. This method allows the expression of any gene to be silenced, and it is being used to identify the genes required for a variety of *R*-gene functions. In principle, VIGS can ascribe function to genes with lethal loss-of-function effects, and it can be used to silence several (possibly redundant) members of one gene family if they are more than 80% homologous. With the development of the tobacco rattle virus this method should soon be applicable in *Arabidopsis*.

Another question is how bacterial pathogens deliver the effectors of disease to plants and trigger the defence responses in the first place. The highly conserved type-III delivery system is encoded by the *hrp*/*hrc* cluster, expression of which is induced by contact with the host (Christian Boucher, CNRS-INRA). One border of the *hrp*/*hrc* pathogenicity island is flanked by a variable domain that can encode different effector proteins (Alan Collmer, Cornell Univ.). When expressed inside plant cells, these can trigger *R*-gene-dependent responses — that is, they behave like Avr proteins. They can also contribute to virulence in hosts that lack the appropriate *R* gene. Plant pathogens maximize their potential virulence by 'collecting' type-III effectors and deploying them blindly into potential host cells. If none of these effectors is recognized by a host *R*-gene function then they may contribute to virulence.

A newly discovered pathogenicity island encodes at least five type-III effectors, four of which contribute to virulence (John Mansfield, Wye College)[6]. These are linked in a sea of insertion sequences and transposons, suggesting that many genetic events have created a pile-up of potential virulence functions. We have found that the host response can, in fact, initiate the excision of a pathogenicity island from the pathogen's chromosome. We are currently exploring whether this plasmid can be horizontally transferred to a new bacterial host. So the weaponry of bacterial pathogens can respond rapidly to the host's defences.

These lines of enquiry will slow as the next wave of challenges form, challenges that are increasingly cell-biological and biochemical in nature. The effects of genomics are beginning to be felt, and, along with the further refinement of genetic screens, will provide fodder for the next evolutionary steps in this arena. The development of new tools and systems will pique anticipation of the next punctuation in the equilibrium. Like evolution, we will stumble down several blind alleys — but the way forward will inevitably be found. ■

Jeff Dangl is in the Department of Biology and Curriculum in Genetics, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA.

e-mail: dangl@email.unc.edu

1. Parniske, M. *et al. Cell* **91,** 821–832 (1997).
2. Michelmore, R. W. & Meyers, B. C. *Genome Res.* **8,** 1113–1130 (1998).
3. Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M. & Bergelson, J. *Nature* **400,** 667–671 (1999).
4. McNellis, T. W. *et al. Plant J.* **14,** 247–258 (1998).
5. Angell, S. M. & Baulcombe, D. C. *EMBO J.* **16,** 3675–3684 (1997).
6. Jackson, R. W. *et al. Proc. Natl Acad. Sci. USA* **96,** 10875–10880 (1999).

Daedalus

Instant diamond

A diamond is a single crystal, a vast regular lattice of carbon atoms. But graphite can also be obtained in single-crystal form, both as a natural mineral and by slow annealing of polycrystalline graphite. Daedalus now proposes to flip one crystal form into the other.

The transition from graphite to diamond is fast and martensitic, taking only a few nanoseconds. A sudden pressure-pulse, compressing a perfect graphite crystal above the transition pressure while heating it above the transition temperature, should convert it to diamond. Daedalus wants to do this by explosive forming.

The explosive would be laid against a carefully shaped piece of single-crystal graphite, and detonated. The shock-wave traversing the graphite would collapse its lattice into the denser diamond one. The geometry of the process needs careful study. Each carbon atom must be projected into the graphite structure ahead of it, in just the direction to take up its proper place in the growing diamond lattice. Daedalus reckons that the explosion front needs to be normal to a main bonding direction in the graphite planes, and at a dihedral angle to them of about 22°. The transition should then be handed on through the graphite lattice as a self-replicating molecular mechanism.

The explosive formation of bulk diamond from single-crystal graphite will send a violent shock through the jewellery trade. But Daedalus goes further. Graphite reacts with many substances — alkali metals, halogens and ions of various kinds — which get between the layers of carbon atoms. The explosive transformation of such lamellar graphite compounds would give doped diamond.

Some natural diamonds are doped with nitrogen or other elements; they are often coloured. But solid-state and semiconductor physicists should leap at the chance to dope diamond with controlled amounts of other well-chosen impurities. Pure diamond is almost a perfect insulator. By contrast, doped diamond could be a splendid new semiconductor. Not only might it work at unprecedented temperatures and huge voltages. It could be the basis of many new logical, piezoelectric, thermo- and photo-voltaic devices. In particular, the transparency of diamond should make it ideal for LEDs and solid-state lasers. These cheap, mass-produced products would allow even ordinary folk to flaunt real diamond jewellery with a truly brilliant sparkle. **David Jones**