

antimony atoms among all the silicon atoms in the crystal is like searching for the proverbial needle in a haystack. To 'see' the antimony dopant atoms, the researchers adapted a powerful technique — scanning transmission electron microscopy<sup>5</sup> — to image almost all the antimony atoms in the silicon crystal, even individual antimony atoms.

The first critical step was to prepare thin crystals — only 9 or 11 atoms thick — lacking any surface or near-surface disorder that could scatter the incoming electron beam of the scanning transmission electron microscope and prevent it from being guided through the channels formed by the rows of atoms in the lattice. The cross-section (or probability) for electron scattering increases with the atomic number,  $Z$ , as  $Z^{1.7}$ , so an antimony atom, with  $Z=51$ , scatters electrons nine times more strongly than a host silicon atom with  $Z=14$ . By detecting the intensity of the scattered electrons, Voyles *et al.*<sup>1</sup> could readily distinguish rows in the atomic lattice that contained a single antimony atom from rows containing only silicon atoms (Fig. 1).

From these elegant measurements, Voyles *et al.* conclude that the electrically inactive atoms are grouped in clusters containing two antimony atoms. But their measurements also revealed that the basis on which impurity dopant atoms remain isolated or form small

clusters is random. This suggests that overcoming carrier saturation at very high dopant concentrations will be a major challenge.

Seeing is frequently the first step towards understanding. This first, unambiguous observation of single atoms bonded inside a bulk solid environment has wide-ranging implications for the analysis of single atoms and of clusters of two, three or four atoms — and for our efforts to understand the structure of impurities and alloy constituents in crystalline solids. Voyles *et al.*'s results<sup>1</sup> are important in understanding the distribution of impurities in silicon at an atomic level;

they will also be important in increasing our understanding of a wide range of complex materials. ■

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## Plant biology

# On guard

Pierre J. G. M. de Wit

Microorganisms that infect plants must suppress their hosts' defence mechanisms before they take up residence. But some plants use molecular guards to sense when they are being manipulated by pathogens.

As a rich source of sugars and amino acids, plants attract a variety of intruders, from viruses, bacteria and fungi to insects. To protect themselves, plants have in their armoury of passive defence mecha-

nisms such as strengthened cell walls and antimicrobial compounds, as well as active healing responses. Only a few microbes can breach these 'basal' defences, and are then fought by the plant's innate immune system.

## Agriculture

# Champagne surprise

Pinot noir is an important grape variety that is used for making wine, including champagne. It is derived from an ancient strain that has been cultivated for maybe 2,000 years. Cultivated grapevines (*Vitis vinifera*) are usually reproduced from cuttings, so all individuals are genetically identical. But sometimes mutations arise, and long ago this resulted in the generation of another champagne grape variety, Pinot Meunier (pictured here), from Pinot noir. Pinot Meunier plants are genetically indistinguishable from Pinot noir in most cells, but their outer layer, the 'L1' epidermal cell layer, is different — meaning that Pinot Meunier has a furry surface on its leaves whereas Pinot noir does not. Elsewhere in this issue (*Nature* **416**, 847–850; 2002), Paul K. Boss and Mark R. Thomas describe the precise mutation that causes this difference. Surprisingly, it is the grapevine equivalent of the 'dwarfing' mutations used to increase wheat yields during the green revolution.

Boss and Thomas started by producing grapevines that carried the mutation in all their cells, not just

their skin: using tissue culture, the authors regenerated whole plants from Pinot Meunier L1 cells. As well as having hairy leaves, these plants were semi-dwarfed — they were shorter and stockier than usual. This provided a clue about which gene might be mutated. Sure enough, it turned out to be the grapevine equivalent of the gene that, when mutated, causes dwarfing in wheat. Gene sequencing showed that the gene had a similar mutation in the L1-cell plants and in dwarfed wheat.

What does the gene do? It was first identified and cloned from the thale cress *Arabidopsis thaliana*, and named *GA INSENSITIVE* because of the effect on the plant of mutating it. It is a regulatory gene that normally keeps a brake on plant growth; the brake is released by gibberellic acid (GA). Thus the plant can regulate its growth by controlling the production and location of this hormone. Some mutations in the gene disrupt the encoded protein so that gibberellic acid no longer releases the brake on growth. This means that the brake is permanently on, and the plant is



smaller. Of course this only occurs if all cells are mutant; if the mutation is limited to the epidermis the only change seen is increased hairiness — gibberellic acid presumably also suppresses hair growth.

There was one more surprise from Boss and Thomas's study: in the L1-cell plants, tendrils were replaced by flowering stems. Normally, a new shoot produces several bunches of flowers (and thus grapes) opposite the first few leaves, and tendrils opposite leaves that form later. The tendrils anchor the vine as it grows in search of light. But in the semi-dwarfed mutant, flowering stems continued to form in place of tendrils. Presumably, the explanation is again that the plants cannot respond correctly to gibberellic acid. Normally,

this hormone may ensure that the later-arising structures become tendrils rather than flowering stems. Gibberellic acid was known to influence flowering in other plant species, but this function is apparently new. Interestingly, tendrils in some plants such as peas have a different origin — they are modified leaflets rather than flowering stems. New knowledge about hormone-response genes may allow us to fine-tune both the vegetative architecture of grapevines, and how many bunches of grapes they produce.

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100 YEARS AGO

Our present state of civilisation has of necessity resulted in an annual increase in the amount of capital borrowed by man from the store of energy accumulated by our earth in bygone times, and the diversion of this capital to uses for which the world's annual income of solar energy was formerly deemed adequate. An instance of this tendency is afforded by the experiments of Dr. Selim Lemström, of Helsingfors, on the uses of electricity in stimulating the growth of cereals, vegetables and other plants... The investigation seems to have been suggested in the first instance by an attempt to connect the luxuriant growth of plants in high latitudes with the influence of electric currents associated with the Aurora Borealis. The experiments showed that for plants growing on arable land of medium quality an increase of 45 per cent. in the crops is obtainable; but the better the field is ploughed and cared for the greater will be the increase. On poor soil the effect is trifling. Certain plants, such as peas, cabbages and turnips, only lend themselves to electrical treatment after being watered. It is, however, injurious to most, if not all, plants to submit them to the influence of electricity in hot sunshine... A further suggestion is that we have here an explanation of the needle-shaped leaves of coniferous plants which are well adapted to facilitate the passage of electricity, or in common parlance, "attract electricity."

From *Nature* 24 April 1902.

50 YEARS AGO

Crystalline penicillin (sodium salt) was added at the rate of 12.6 gm. per ton to a standard turkey-rearing mash... The experiments briefly summarized show that the addition of small amounts of penicillin to the diet of growing turkeys not only produced a significant increase in growth-rate but also that it appeared to have a profound effect in stimulating metabolism, tiding chicks over a critical period of susceptibility to chilling and other non-specific causes of mortality during the first few weeks of life. Rate of growth and mortality during rearing are factors of primary importance to the turkey industry and largely determine the profitability of the undertaking... Its administration to birds destined for the table within the shortest possible time would appear to be strongly indicated.

From *Nature* 26 April 1952.

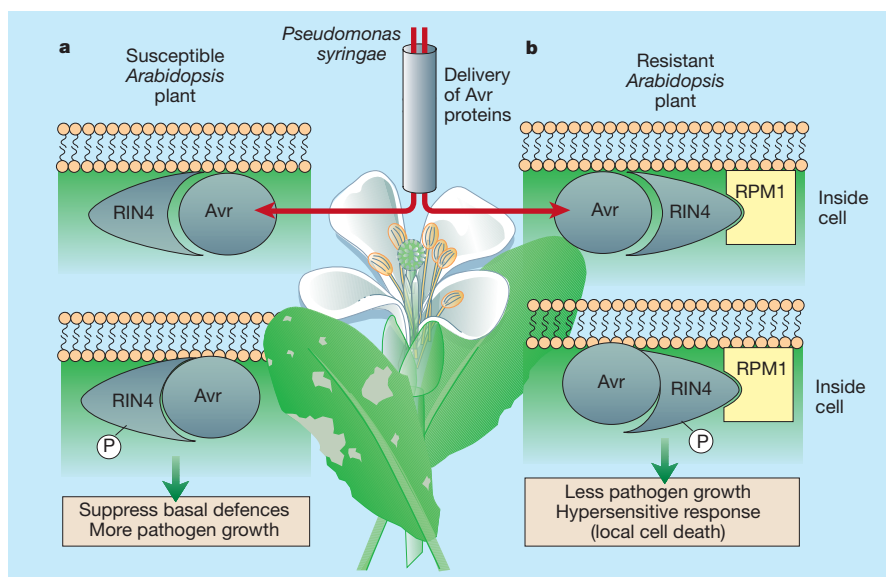


Figure 1 At the crossroads of disease resistance and susceptibility. Mackey *et al.*<sup>3</sup> studied the *Arabidopsis thaliana* resistance (R) protein RPM1, and the *Pseudomonas syringae* avirulence (Avr) proteins AvrRpm1 and AvrB. (*P. syringae* infects *A. thaliana* leaves.) The authors found that the two bacterial proteins can bind to the plant protein RIN4, tag it with phosphate groups (circled 'P') and upregulate its concentration and its activity as a negative regulator of plant basal defence mechanisms. a, In susceptible plants, this downgrading of plant defences results in the spread of the bacterium. b, Resistant plants make use of the RPM1 protein to sense these manipulations of RIN4 by the bacterial Avr proteins, activating the hypersensitive response which prevents bacterial spread. Figure modified from ref. 3.

The innate immune response is well described genetically by what is known as the gene-for-gene model<sup>1</sup>, because it requires a pathogen protein encoded by an 'avirulence' (*Avr*) gene to be recognized by a plant protein encoded by a resistance (*R*) gene. This activates an array of defence mechanisms, including the hypersensitive response<sup>2</sup>, in which a few plant cells at the site of infection die, thereby limiting the spread of disease.

Although many Avr and R proteins have been identified, we have still much to learn about what they do. For example, do Avr proteins bind directly to R proteins? And what are the main functions of Avr proteins? They are surely not there just to enable the plant to detect the intruder. Writing in *Cell*, Mackey *et al.*<sup>3</sup> have proposed some answers. The authors studied the R protein RPM1 from the thale cress *Arabidopsis thaliana* and two Avr proteins, AvrB and AvrRpm1, from the bacterium *Pseudomonas syringae*. They show that RPM1 is a molecular guard that prevents the bacterial proteins from taking advantage of another plant protein, RIN4, that downgrades plant basal defences. The results provide concrete support for the 'guard hypothesis' of plant defence (reviewed in refs 2,4).

Some predictions about the molecular connections between Avr and R proteins have emerged from studies of their structures and amino-acid sequences. For the most part, pathogen Avr proteins are structurally unrelated to each other. But most plant R proteins do have similar structures and contain sequences with a high proportion of leucine amino acids.

These 'leucine-rich repeats' (LRRs) are thought to be involved in protein-protein

interactions<sup>5</sup>, and so are expected to allow R proteins to lock directly onto Avr proteins, although (despite much searching) so far this has been shown only for the rice Pi-ta protein and the AvrPita protein from the rice-blast fungus *Magnaporthe grisea*<sup>6</sup>. LRRs may also specify interactions with other plant proteins (although the specificity of R proteins is not determined solely by their LRRs<sup>7</sup>). Some R proteins have LRRs in their extracellular regions; here, the LRRs are assumed to be involved in recognizing pathogens outside plant cells<sup>8</sup>. By contrast, the *A. thaliana* RPM1 protein resides wholly within plant cells, associated with the plasma membrane, where it was thought to interact directly with Avr proteins like *P. syringae* AvrRpm1 and AvrB<sup>9</sup>. These Avr proteins are injected into leaf cells by the tubular bacterial type III secretion machinery<sup>10</sup>.

But it seems that things are not so simple. Mackey *et al.*<sup>3</sup> find that the *A. thaliana* RPM1 and *P. syringae* AvrRpm1 and AvrB proteins interact not directly but indirectly, through the newly discovered plant protein RIN4 (for 'RPM1-interacting protein-4'). The authors first identified RIN4 because of its interaction with AvrB, and showed that it also interacts with RPM1 and AvrRpm1. They then discovered that RIN4 suppresses the expression of plant genes needed for basal defence; moreover, both AvrRpm1 and AvrB induce the covalent addition of phosphate groups to RIN4, possibly enhancing its ability to negatively regulate plant defence. Conversely, however, RIN4 is essential for RPM1 to activate the hypersensitive response to the two *P. syringae* proteins, thereby inhibiting bacterial growth — this response does not occur in

plants with low levels of RIN4. Reductions in RIN4 levels also cause reductions in RPM1 levels and (for as yet unknown reasons) resistance to both *P. syringae* and, intriguingly, the unrelated fungus-like *Peronospora parasitica*.

All of which suggests that RIN4 sits at a crossroads between susceptibility and disease resistance, and that RPM1 guards *A. thaliana* against pathogens that use AvrRpm1 and AvrB to manipulate RIN4 activity<sup>3</sup> (Fig. 1). So, when susceptible plants are infected by *P. syringae*, the Avr proteins interact with RIN4, induce its phosphorylation, and increase its concentration, thereby inhibiting basal defences and leading to susceptibility. But in plants that are resistant to *P. syringae*, these manipulations are somehow sensed by RPM1, which launches a local cell-death programme that leads to resistance.

So Mackey *et al.*<sup>3</sup> have shown how one R protein and two Avr proteins work at the molecular and cellular levels, causing either disease or the hypersensitive response according to the balance of power between the proteins. In so doing, the authors have answered the questions (at least for this set of proteins) of how an R protein can sense the presence of its cognate Avr proteins — through their manipulation of RIN4 — and what the Avr proteins do. It is known that some fungal and bacterial Avr proteins function in susceptible plants as 'virulence factors', thought to be required for maximum virulence of the pathogen<sup>11</sup>. Mackey *et al.*<sup>3</sup> have revealed that Avr proteins can do this by increasing the activity of a plant defence inhibitor.

This study should give a boost to those studying the molecular interactions between plants and microbes. It is likely that the direct interaction shown for the rice and rice-blast fungus proteins<sup>6</sup> is the exception, not the rule; mechanisms like that described by Mackey *et al.*<sup>3</sup> may be more common. Many labs are now hunting for virulence-related targets of Avr proteins, akin to RIN4, in other model gene-for-gene systems. It will be interesting to see whether different pathogens use the same targets. For instance, if the *P. parasitica* Avr proteins (which have not yet been identified) also interact with RIN4, that might explain why plants that are susceptible to *P. syringae* are also susceptible to *P. parasitica*. Another question is whether different Avr proteins from the same pathogen are recognized by the same plant protein, as are AvrB and AvrRpm1. I anticipate that most pathogens have a set of Avr proteins that work together to afford full virulence.

Finally, as mentioned above, some Avr proteins — such as those from the tomato pathogen *Cladosporium fulvum*<sup>8</sup> — are detected by LRR motifs on the outside of plant cells, rather than inside. Might a similar guard mechanism protect against pathogens like these, too? Support for this idea comes from the finding that the hypersensitive response of tomato to one *C. fulvum*

Avr protein (Avr2)<sup>12</sup> requires not only the cognate R protein (Cf-2) but also a further tomato protein (Rcr3), found outside cells, which might be a virulence-related target<sup>13</sup>. Perhaps the R protein protects this target from the pathogen protein. Molecular guards may be widely used to prevent plant proteins from being subverted for pathogenic purposes<sup>4</sup>. ■

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## Relativity

# Testing times in space

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We take for granted that physical 'constants', such as the speed of light, are fixed values. But they might not be, and experiments in space may allow us to investigate this possibility.

A basic assumption of Einstein's theory of relativity is that the fundamental physical laws and parameters do not depend on the position, orientation or uniform velocity of the laboratory in which they are measured — a property generally known as Lorentz invariance. Relativity has been tested, implicitly and explicitly, in countless experiments; as yet, no failure of the theory has been observed. But most explicit tests have been confined to laboratories on Earth. In *Physical Review Letters*, a theoretical analysis by Bluhm *et al.*<sup>1</sup> shows that experiments in space — some already planned for the International Space Station — could offer better sensitivity, as well as extending the range of tests that could be performed.

Some of the most exacting tests of relativity have involved atomic clocks. These 'tick' by electrons moving between energy levels emitting a photon with a certain frequency. The tests compare the tick rates of two different atomic clocks as a function of their orientation and velocity through space. The idea is that, if the two clocks are based on different types of energy-level transitions, any failure of Lorentz invariance would show up as a relative shift in the two frequencies of the clocks, because the physical 'constants' governing the ticks of the clocks would not actually be constant, but would change with the clocks' orientation and velocity.

In Earth-based experiments, the orientation and velocity of the clocks are determined by the Earth's rotation and revolution about the Sun, and by the motion of the Solar System relative to the Universe as a whole. Typically, the differential clock frequencies are measured as a function of time. Given the Earth coordinates of the clocks, together with the time and date, the time-dependent orientation of the clock can be determined. Then,

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any violation of relativity can be correlated with some aspect of orientation or velocity.

An example of a clock comparison experiment is to test whether the speed of light,  $c$ , is a universal constant — is there a limiting speed for matter,  $c_m$ , that is different from the speed of light? For instance, my colleagues and I have sought<sup>2</sup> a difference in the values of these numbers by comparing the behaviour of two atomic nuclei, <sup>199</sup>Hg and <sup>201</sup>Hg. In an applied magnetic field, the magnetic-moment vector of a nucleus precesses about the field direction at a particular frequency (this process is also the basis of magnetic resonance imaging). The <sup>199</sup>Hg nucleus is spherical and so its orientation in space (usually defined relative to distant, 'fixed' stars) does not affect the precession frequency. But the <sup>201</sup>Hg nucleus is egg-shaped — its lack of spherical symmetry means that the angle between its velocity vector and its magnetic moment becomes important; and if  $c_m$  is not identically equal to  $c$ , a shift in the precession frequency of <sup>201</sup>Hg compared to that of <sup>199</sup>Hg appears. In this Earth-bound experiment, no difference between  $c_m$  and  $c$  was detected, implying that if such a difference exists, then  $1 - c_m/c < 10^{22}$  — a rather astoundingly accurate limit.

This experiment is prototypical of many of the experiments described by Bluhm *et al.*<sup>1</sup>, and we can question whether there would be an advantage to performing it in space. Among the advantages that Bluhm *et al.* specifically address is the ability to change the orientation and velocity of space-borne clocks to arbitrary directions; orientation changes could also be made more rapidly than the once-per-day change for an Earth-based experiment (which would avoid problems due to slow drift in the clock frequencies). In fact, in the specific