Knocking out nodules

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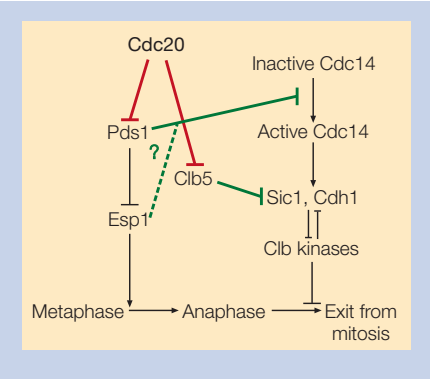

Figure 1 **Two-step regulation of exit from mitosis by Cdc20, as proposed by Shirayama *et al.*[4]. After completion of metaphase (the stage of mitosis when chromosomes condense and a mitotic spindle forms), Cdc20, together with the anaphase-promoting complex, ubiquitinates Pds1. Degradation of Pds1 frees its partner Esp1, which then initiates the partitioning of the genetic material between daughter cells. Degradation of Pds1 also allows the release of Cdc14 from the nucleolus. Whether Esp1 mediates the Pds1-dependent inhibition of Cdc14 is not clear (dotted line). Cdc20 also degrades the Cdc14 antagonist Clb5. The Clb5–Cdc28 kinase phosphorylates Sic1 and Cdh1 more rapidly than Cdc14 dephosphorylates them. So, Cdc20 enables Cdc14 to dephosphorylate its targets Sic1 and Cdh1, which, in turn, allows these proteins to become active and destroy mitotic CDKs, resulting in exit from mitosis.**

with its activator Cdh1, induces degradation of the mitotic cyclins Clb1, Clb2, Clb3 and Clb4, and, as a result, inactivates the mitotic CDKs. Binding of Sic1 to the kinase complex also downregulates mitotic CDKs and triggers exit from mitosis. So Cdc14 works by dephosphorylating Cdh1 and Sic1, facilitating both Clb degradation and Sic1 accumulation[1]. But how is Cdc14 inactivated when it is not needed during G1, S phase and metaphase? It is then sequestered in the nucleolus by Cfi1/Net1 (ref. 1). Only during anaphase, when a signal-transduction pathway termed the mitotic-exit pathway[7,8] releases it from the nucleolus, can Cdc14 reach and dephosphorylate its targets.

To determine how Cdc20 regulates exit from mitosis, Shirayama *et al.*[4] did a genetic selection based on the premise that exit from mitosis requires degradation of a certain factor by Cdc20, and that the absence of this factor will allow cells lacking *CDC20* and *PDS1* to exit mitosis, divide and form colonies. Using this mutant hunt the authors identified a gene called *CLB5*, which encodes a cyclin required for progression through S phase. The Clb5 cyclin, when complexed with the protein kinase Cdc28, is important for promoting S phase[7].

Having confirmed that Clb5 is indeed degraded by the APC–Cdc20 complex, Shirayama and colleagues worked out how the degradation of Clb5 and Pds1 regulates exit from mitosis. They examined the levels of mitotic CDKs and localization of Cdc14 in *cdc20 clb5* and *cdc20 pds1* double mutants, and in *cdc20 clb5 pds1* triple mutants. As expected, neither *cdc20 pds1* nor *cdc20 clb5* double mutants could inactivate mitotic CDKs (and, therefore, exit mitosis), whereas the *cdc20 clb5 pds1* triple mutant could. Interestingly, though, the two double mutants differed with respect to Cdc14 localization. Whereas Cdc14 remained in the nucleolus in *cdc20 pds1* mutants, the protein was released in *cdc20 clb5* mutants. The authors concluded that Cdc20 is required at two steps during the initiation of mitotic exit. In the first, Cdc20-mediated degradation of Pds1 releases Cdc14 from the nucleolus. And in the second, Cdc20 degrades Clb5, allowing Cdc14 to dephosphorylate Cdh1 and Sic1 after it has been released from the nucleolus.

How does degradation of Pds1 facilitate liberation of Cdc14 from the nucleolus? And how does destruction of Clb5 enable Cdc14 to dephosphorylate critical cell-cycle targets? Pds1 is known to inhibit the onset of anaphase by inhibiting another protein called Esp1 (Fig. 1)[8]. So Shirayama *et al.* determined whether Pds1 prevented Cdc14 release by inhibiting Esp1. They found that *esp1* mutants showed, at best, a modest delay in the inactivation of mitotic CDKs. This suggests that Pds1 inhibits Cdc14 release by means other than inhibition of Esp1. But other reports point to the involvement of Esp1 in inactivating mitotic CDKs[9]. Irrespective of whether Pds1 inhibits Cdc14 release by blocking Esp1 or some other, unidentified protein, it will be interesting to see if, and how, Pds1 interferes with the mitotic-exit pathway[1].

To explain how Clb5 inhibits exit from mitosis, Shirayama *et al.* propose that the Clb5–Cdc28 complex phosphorylates Cdh1 and Sic1 at a rate that exceeds Cdc14's dephosphorylation activity towards these proteins. Clb5 seems to be the only Clb cyclin that can antagonize Cdc14 effectively. For instance, when the authors eliminated other Clb cyclins, such as Clb1, Clb3 or Clb4, the *cdc20 pds1* double mutants could no longer exit mitosis. By working out why only Clb5 seems to inhibit Cdc14, we may learn not only how exit from mitosis is regulated, but also how Clb kinases differ in their ability to select and phosphorylate different substrates.

We now have a good understanding of how Cdc20 regulates exit from mitosis. The next challenge will be to determine how Cdc20's substrates — Pds1 and Clb5 — regulate the activity of Cdc14. Our ultimate goal will be to understand how cells ensure that exit from mitosis is not initiated before the chromosomes have segregated. A prime candidate for coupling these two events is Pds1, which inhibits both anaphase and release of Cdc14 from the nucleolus. But cells that lack *PDS1* still exit mitosis only after the chromosomes have separated completely[4]. So the pathways that regulate sister-chromatid separation in the absence of *PDS1*, and that couple this event to exit from mitosis, still need to be identified. ■

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Plant genetics

Knocking out nodules

Herman P. Spaink

Plant genetics has had a great impact over the past 150 years. In the late nineteenth century Gregor Mendel discovered the basic principles of inheritance using the pea (a leguminous plant). Then, in the 1940s, Barbara McClintock's pioneering work on maize uncovered the existence of mobile DNA elements called transposons. In the 1970s, DNA transfer from *Agrobacterium tumefaciens*[1] allowed foreign genes to be efficiently introduced into the plant genome. And the development of simple, rapid methods to obtain transgenic offspring from *Arabidopsis thaliana* plants has allowed large collections of mutants to be generated. But genetics has been difficult in other species of plant, for which there are no such rapid methods.

On page 191 of this issue, Schauser *et al.*[2] show that this lack of methodology will no longer be the main barrier for genetic studies of leguminous plants. The authors have shown that a transposon called *Ac*, which is derived from maize, can be successfully used to disrupt genes in the leguminous plant *Lotus japonicus*. This is good news for those

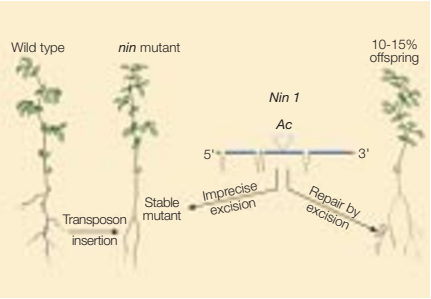

Figure 1 **Strategy used by Schauser *et al.*[2] to characterize the 'nodule inception', *nin*, mutant. By inserting a mobile DNA element (transposon) called *Ac* into wild-type *Lotus japonicus*, the authors generated mutant plants with *Ac* inserted into the *nin* gene. This transposon can excise itself from the DNA. If it does so imprecisely, a mutation remains in the *nin* gene. But if it excises precisely, the gene can be repaired and the plants return to a wild-type phenotype. This is a powerful technique for generating mutations in plants.**

studying root nodulation in the legume family. Root nodules are the result of a symbiotic (mutually beneficial) interaction between plants and various soil bacteria, collectively called rhizobia[1]. Within these nodules, the bacteria fix atmospheric nitrogen into ammonia. This means that leguminous plants do not depend on fixed nitrogen in the soil — usually a major growth limitation.

Two decades of research into the molecular basis of this symbiosis have shown that it is due to an exchange of signal molecules. These molecules are recognized specifically by the host plants and their bacterial partners[3], and they include the bacterially produced nodulation factors, which can trigger the roots of leguminous plants to form a root nodule[4–6]. The bacterial genes involved in the symbiosis have been studied in detail[7], stimulating research into the genomes of rhizobia in general (for example, the sequence of the entire *Sinorhizobium meliloti* genome is close to completion[8]).

From the plant side, far less is known of the genes (and corresponding functions) involved in the symbiosis. This is mainly due to the technical bottlenecks in plant genetics. Various plants have been identified with an impaired capacity to be nodulated or infected by rhizobia[9], but these mutants were derived by chemical mutagenesis. So the resulting defects (such as point mutations) are still difficult to identify.

The transposition approach used by Schauser *et al.*[2] now makes it very easy to identify mutated genes, because they are tagged by the known nucleotide sequence of the transposon. The strategy yielded a mutant that could not form root nodules, so was called a *nin* (for 'nodule inception') mutant (Fig. 1). Interestingly, the mutant does not seem to be affected in any other aspect of plant development — such as root, shoot, leaf, flower or seed development — as long as nitrogen nutrients are supplied externally. This suggests that the mutated gene is dedicated to root-nodule formation.

The authors went on to show that the genetic defect in the *nin* mutants is indeed caused by integration of the transposon. To do this, they used the fact that the *Ac* transposon can excise spontaneously from the affected gene at a certain frequency. They found that when the transposon was precisely excised, mutant plants reverted to the wild-type phenotype (Fig. 1). Then, because the transposon acted as a tag for the mutated gene, Schauser *et al*. could easily obtain the nucleotide sequence of the complete gene using established procedures. This sequence showed that the Nin protein is probably a transcription factor, which may have a membrane-spanning domain. The *Nin* gene seems to be related to the *mid* ('minus dominance') genes from the alga *Chlamydomonas*. These genes regulate sexual reproduction in response to short supplies of nitrogen. Because root nodulation is also regulated by nitrogen limitation, this relationship might point to a conserved function for this type of regulator in evolution.

The identification of this hitherto-unknown regulator of nodule inception is a landmark discovery that will influence future studies in the legume field. This kind of approach will be strengthened by linking it to detailed analyses of leguminous-plant genomes. Two international consortia are currently obtaining nucleotide-sequence data from *L. japonicus* and another model legume, *Medicago truncatula*. Such large-scale projects are important owing to the role of leguminous plants in studies on the interaction of plants with symbiotic mycorrhizae[8] (which are involved in the supply of phosphorus), or in research on the various plant pathogens that are specific for the leguminous family. It will be exciting to follow research in this area, and to see the vast amount of biochemical and physiological data obtained as leguminous plants are linked to plant genetics. ■

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Daedalus

Asteroidal defence

One day, a stray asteroid or comet may smash into the Earth, when we shall go the way of the dinosaurs. Pessimists advocate a close watch on asteroidal neighbours, and the building of a vast nuclear missile to disrupt the incomer before it hits the Earth. Daedalus has a neater scheme.

He wants to put an asteroidal 'anti-missile' in a high Earth orbit. If a potential invader were sighted, we would arrange for the guard asteroid to collide with it. An asteroid already in orbit would patrol near-Earth space very densely. Even a slight nudge, if well calculated, could guarantee a successful interception, shattering both asteroids.

But how to capture a guard asteroid in the first place? Many asteroids, comets and planetesimals contain hydrogen, water, ammonia or methane ices, which can deliver useful thrust by direct evaporation. Indeed, it has been suggested that an asteroid could be steered by flying a big concave mirror alongside it, to focus sunlight on the icy surface. The resulting plume of hot gas would deflect its orbit. Daedalus goes even further. Most asteroids, he claims, are not loose rubble piles of the sort discussed by E. Asphaug on page 127. They have a compressed centre, in which the ices are converted to energetic hydroxonium, ammonium or methylammonium 'alloys'. Release the over-pressure, and these compounds will decompose to hot pressurized gas. A space probe that landed on such an asteroid and drilled down to its energetic core would release a gas-jet that would turn the asteroid into a natural rocket. With crafty enough steering, it could be detached from its solar orbit and put into one round the Earth. The drill-hole would then be plugged, restoring the stabilizing pressure. When the asteroid was called on to make the supreme sacrifice for its new primary, the hole would be unplugged again to steer it to its final, fatal encounter.

This elegant scheme can be put into effect long in advance of any threat. With the guard asteroid safely in orbit, the Earth is well defended. Of course, if the deadly incomer turns out to another ice-cored asteroid, it might still be feasible to send a spacecraft to intercept it on the old plan. But instead of blowing it up, it could be steered into Earth orbit as an additional defender. David Jones