PLANT BIOLOGY

Defence at dawn

A remarkable example has been discovered of a plant tuning its immune defence against a pathogen. The tuning consists of maximal expression of the relevant genes at the time of day when attack is most likely. SEE LETTER P.110

C. ROBERTSON MCCLUNG

Plants cannot avoid pathogens by fleeing, so they have evolved mechanisms to resist attack. Those mechanisms include forms of immunity, and on page 110 of this issue Wang *et al.*[1] reveal an unexpected connection between plant immunity and the circadian clock.

Plant immunity is controlled by a complex and incompletely understood signalling network. The first level of defence consists of the recognition of molecules called pathogen-associated molecular patterns (PAMPs) and the subsequent initiation of PAMP-triggered immunity[2]. Because many pathogens can suppress this response, often by means of proteins generically termed effectors, plants have evolved disease-resistance (*R*) genes that recognize effectors or the action of effectors on their host-cell targets. The consequent effector-triggered immunity involves the reprogramming of gene transcription, which culminates in physiological changes that include the hypersensitive (cell death) response[2].

Plant pathogens can be divided into necrotrophs, which kill the host and feed on the dead tissues, and biotrophs, which infect and feed off a living host without killing it. *R*-gene-mediated defence is the primary response to biotrophs, including the oomycete *Hyaloperonospora arabidopsidis*, which causes downy mildew disease in the thale cress *Arabidopsis* (Fig. 1). In their paper, Wang *et al.*[1] describe novel components of *R*-gene-mediated resistance against downy mildew in *Arabidopsis*, and show that these components are controlled by a transcription factor called CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1), which is an essential component of the circadian clock.

Wang *et al.*[1] employed a systems approach. They compared changes in *Arabidopsis* gene expression in response to *H. arabidopsidis* infection over time in wild-type seedlings (which successfully resist this pathogen owing to the presence of the *RPP4* disease-resistance gene) with gene-expression changes in an *rpp4* mutant (which has lost RPP4 function and is sensitive to infection). Their examination of events shortly after infection revealed several genes that responded to *H. arabidopsidis* challenge in an RPP4-dependent manner but that had not previously been identified as immune regulators or as components of the response to *R*-gene activation.

The authors then tested mutants in which each of these novel plant genes was non-functional, to define a subset that is important for successful disease resistance. These genes could be divided into two clusters — one associated mainly with the hypersensitive response and the other with physico-chemical responses to infection. Intriguingly, the promoter sequences of both groups of genes showed over-representation of binding sites for CCA1, implicating the circadian clock in the regulation of immunity.

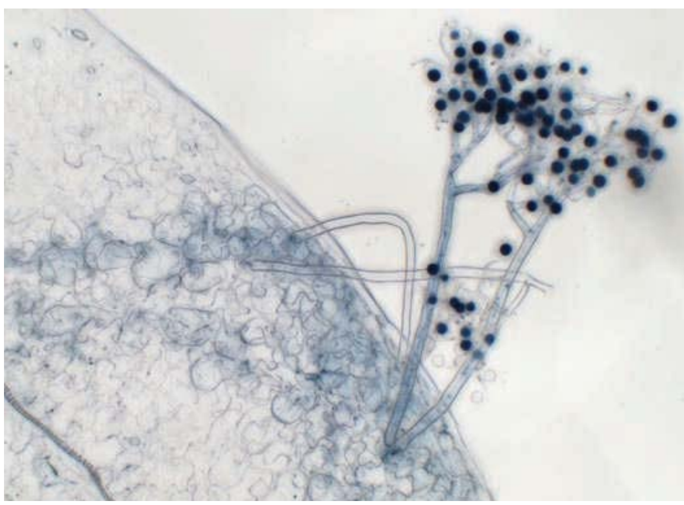

Figure 1 | *Hyaloperonospora arabidopsidis* in spore-production mode. The spores (dark particles) are borne on a structure known as the sporangiophore, which here is about 0.7 millimetres in height.

W. WANG & X. DONG

The circadian clock is a timekeeping mechanism that enables the anticipation of environmental conditions or biological events that occur at predictable times of day[3]. For example, cold stress typically occurs at night, and the plant circadian clock modulates cold responses accordingly[4]. Similarly, *H. arabidopsidis* sporulates at night and disseminates its spores at dawn, perhaps because enhanced humidity optimizes germination and the colonization of new hosts[5].

Wang *et al.*[1] found that some *Arabidopsis* mutants in which clock function was impaired showed increased susceptibility to *H. arabidopsidis* infection. They propose that, in wild-type plants, disease resistance is maximal at dawn, when the likelihood of encountering *H. arabidopsidis* spores is greatest, and reduced at dusk. They suggest that the plant circadian clock mediates a pulse of defence-gene expression near dawn, when *CCA1* expression is high, in anticipation of possible *H. arabidopsidis* challenge at that time. This anticipatory expression of defence genes, at a time when the potential for pathogen challenge is perhaps higher than during the rest of the day, could allow the need for maximal disease resistance to be balanced against the growth decrement associated with sustained expression of defence genes[6]. The central role of *CCA1* is emphasized by the reduced resistance to *H. arabidopsidis* at dawn in *cca1* loss-of-function mutants; these same mutants showed no reduction in resistance, compared with wild-type plants, in response to *H. arabidopsidis* challenge at dusk, when *CCA1* is not expressed.

It is intriguing that CCA1 serves as an integrator between the immune response and the circadian clock to elicit enhanced resistance, whereas a closely related transcription factor, LHY, does not — *lhy* mutants show no decrement in disease resistance, yet display a similar shortening of circadian period to that seen in *cca1* mutants[3]. That may indicate that the primary defect in disease resistance in *cca1* mutants is due to the action of CCA1 on an output pathway that is modulated by the circadian clock, rather than on the oscillator mechanism itself. It is also intriguing that CCA1, but again not LHY, has a crucial role in the integration of nitrogen assimilation and the circadian clock[7].

The work of Wang *et al.*[1] has implications for understanding other plant pathogens. One example is another oomycete, *Phytophthora infestans*, which causes late blight in potatoes and was responsible for the potato famines of the 1840s[8]. A second example, of more recent notoriety, is the biotrophic Ug99 strain of wheat stem rust (*Puccinia graminis*, a basidiomycetous fungus), which was first identified in Uganda in 1999 but now threatens wheat-growing regions in Asia[9]. Ug99 overcomes the resistance afforded by most of the *R* genes in current cultivars. New insights into *R*-gene-mediated disease resistance may contribute to the development of wheat that is resistant to this devastating pathogen.

The results[1] may also apply beyond the plant world. For instance, many genes involved in innate immunity in fruitflies exhibit cyclic expression controlled by the central oscillator component CLOCK[10]. Wild-type flies show circadian variation in resistance to bacterial infection, peaking in the middle of the night[11]. Similarly, evidence is accumulating in support of circadian modulation of human immune responses[12].

Finally, returning to *H. arabidopsidis*, it is not known whether the apparently rhythmic sporulation[5] is a direct response to the light–dark cycle or instead represents a circadian rhythm driven by an internal clock. Nor is it known whether rhythmic sporulation is a characteristic of oomycetes in general. Rhythmic asexual spore production (conidiation) in the ascomycetous fungus *Neurospora crassa* has provided one of the most fruitful systems for investigating circadian rhythms[13], and evidence from genome sequencing of many fungal species indicates that some clock proteins were present in the common ancestor of the fungi[14]. Oomycetes are only distantly related to fungi[15], but the identification of a circadian component in plant resistance to oomycete infection justifies investigation of potential circadian rhythmicity in oomycetous and fungal pathogens as well[16]. ■

C. Robertson McClung *is in the Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755, USA.*
e-mail: c.robertson.mcclung@dartmouth.edu

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ASTROPHYSICS

Big black hole found in tiny galaxy

Conventional wisdom tells us that supermassive black holes are found exclusively in massive galaxies undergoing little star formation. But one such object has now been discovered in a star-forming dwarf galaxy. **SEE LETTER P.66**

JENNY E. GREENE

On page 66 of this issue, Reines and collaborators[1] report a most unlikely discovery: a big black hole in the centre of a very small galaxy. The low-mass (or 'dwarf') galaxy Henize 2-10, the focus of their research, is currently undergoing a major growth spurt. It seems to have formed an appreciable fraction of its stars in the past 10 million years[2], which is unusually fast for present-day galaxies. Now it appears that Henize 2-10 is growing more than just stars. The authors detect a source of light that they attribute to gas falling into a supermassive black hole at the heart of the galaxy. This is the first potential detection of a black hole in a rapidly star-forming dwarf galaxy such as Henize 2-10. If confirmed, this observation gives us the hitherto impossible opportunity to study at first hand the growth of black holes in a forming galaxy.

Over the past three decades, astronomers have used Henize 2-10 and galaxies like it as laboratories for what might have happened in the first days of galaxy formation[3]. They can study how gas is converted into stars, and how and where those stars are formed, as the galaxy grows. It turns out that most young stars form in compact clusters within the growing galaxy.

Despite many years of study, however, no central supermassive black hole had been discovered in Henize 2-10. Light from the young, newly forming stars had overwhelmed the unique light signatures of the black hole and masked its presence. But using new and archival high-resolution data, Reines *et al.*[1] found a source of both radio and X-ray emission far from any site of new star formation. Although massive star birth and death is accompanied by sources of radio and X-ray emission, the relative strengths of the radio and X-ray sources, combined with their distance from newly forming stars, strongly suggests a more exotic origin — a radiating black hole.

Reines and collaborators were aided by better data, but ultimately it was good intuition and imagination that led to their discovery. Conventional wisdom tells us that supermassive black holes with masses millions to billions times that of our Sun are found exclusively in massive galaxies, not in dwarf galaxies such as Henize 2-10. And usually, supermassive black holes are found in the parts of galaxies with regular elliptical shape and very little ongoing star formation, not in lumpy star-forming galaxies. The new discovery, while unexpected, may represent an important opportunity to investigate the unknown origins of supermassive black holes. We do not yet have telescopes powerful enough to witness the interactions between the first black holes and their parent galaxies. Because Henize 2-10 is currently forming a large fraction of its stars, much like the host galaxies of the first black holes, we may finally have our chance to observe a growing black hole within a forming galaxy.

The case is not yet watertight for a black hole in the centre of Henize 2-10. We cannot rule out the chance alignment of two less exotic objects associated with star birth and death in such a complicated and dusty region. Furthermore, some expected signatures of a black hole, such as emission from highly excited neon atoms, are not observed. But without knowing more precisely the mass and total power of the putative black hole, it is hard to guess how bright the emission should be. Reines *et al.* estimate a range of black-hole masses between 100,000 and 10 million times the mass of the Sun, which places it at the low-mass end of supermassive black holes. Accurate estimates of the black-hole mass must await the next generation of optical telescopes. In the meantime, Reines and collaborators are obtaining a more precise measure of the total power output of the black hole.

Although the discovery of a massive black hole in a dwarf star-forming galaxy is unexpected, there are many known examples of black holes in small galaxies. Indeed, recent work[4–8] has uncovered many examples of small galaxies containing black holes with masses approximately 10,000 times that of our Sun. Unfortunately, obtaining an accurate count of the black-hole population becomes increasingly difficult for low-mass black holes in small galaxies. Emission from these black holes is always faint, and because they are so small we cannot directly observe their gravitational pull on surrounding stars.

Reines and collaborators[1] have identified an entirely new parent population of galaxies where many unknown supermassive black holes could be lurking. There are many things Henize 2-10 may teach us. First, black-hole growth might inhibit star formation in fragile, first-generation galaxies[9]. To test this hypothesis, we can look for signs that energy from the black hole is heating or removing gas in Henize 2-10. Second, the location of the putative black hole away from any star cluster is intriguing, because most proposed mechanisms for making new black holes involve massive stars[10]. And perhaps