birds. However, a later arrival in Europe of migratory birds wintering south of the Sahel has been reported (*7*, *26*). For these species, the decision when to start spring migration may become maladaptive when the cue for migration is independent of the environmental change in the breeding area (*7*). Climate change may thus be a serious threat to species that migrate from tropical wintering grounds to temperate breeding areas. They may arrive at an inappropriate time to exploit the habitat and compete with larger numbers of individuals of resident species as more of them survive the winter. These arguments may partly explain the decline of these long-distance migratory species in Western Europe (*8*), although short-distance migrants may be more flexible. These findings support previous results demonstrating that shifts in global climate patterns can affect migratory birds (*27*).

These changes in plant phenology and bird migration show that climate warming may lead to a decoupling of species interactions, for example, between plants and their pollinators or between birds and their plant and insect food supplies (*2*). Changes not only in mean temperatures but also in temperature patterns may affect these interactions even more strongly because they may alter the synchronization between species (*28*). An example of such decoupling was recently reported. The Great Tit still breeds at the same time, but its food supply has been advanced because of earlier plant development in recent years (*29*). Different phenological responses (*7*, *30*) may alter the competitive ability of different species and thus their ecology and conservation, resulting in unpredictable impacts on community structure and ecosystem functioning.

The observed phenological changes have occurred with a warming only 50% or less of that expected for the 21st century (*1*). Many ecological (carbon sequestration, nutrient and water cycles, species competition, pests and diseases, bird migration and reproduction, and species-species interactions), agricultural (crop suitability, yield potential, length of growing season, risk of frost damage, epidemiology of pests and diseases, timing and amount of pesticide use, and food quality), and socioeconomic and sanitary (duration of the pollen season and distribution and population size of disease vectors) factors depend strongly on plant and animal phenology. Phenology is therefore increasingly relevant in the framework of global change studies (*31*).

As in many areas of environmental science, the key requirement is long-term data sets. Today, thousands of people—professionals and volunteers—record phenological changes all over the world, as do international and national phenological monitoring networks such as Global Learning to Benefit the Environment (GLOBE) or the European Phenology Network. Together with remote sensing, atmospheric, and ecological studies, these data will help to answer the many questions raised by the recently reported climate effects on phenology: What are the limits of the lengthening of the plant growth season and the consequent greening of our planet? Will the (less seasonal) tropical ecosystems be less affected than boreal, temperate, and Mediterranean ecosystems? How will different aquatic ecosystems respond? How will responses to temperature and other drivers of global change interact to affect phenology and the distribution of organisms? How will changes in synchronization between species affect population dynamics both in terrestrial and aquatic communities? Will appropriate phenological cues evolve at different trophic levels?

References and Notes

1. Intergovernmental Panel on Climate Change, *Climate Change 2001: The Scientific Basis. Third Assessment Report of Working Group I*, J. T. Houghton *et al.*, Eds. (Cambridge Univ. Press, Cambridge, 2001).
2. H. Q. Crick, C. Dudley, D. E. Glue, D. L. Thomson, *Nature* **388**, 526 (1997).
3. A. Walkowszky, *Int. J. Biometeorol.* **41**, 155 (1998).
4. A. Menzel, P. Fabian, *Nature* **397**, 659 (1999).
5. N. L. Bradley, A. C. Leopold, J. Ross, W. Huffaker, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9701 (1999).
6. M. Abu-Asab *et al.*, *Biodivers. Conserv.* **10**, 597 (2001).
7. J. Peñuelas, I. Filella, P. Comas, *Global Change Biol.*, in press.
8. C. Both, M. E. Visser, *Nature* **411**, 296 (2001).
9. E. G. Beaubien, H. J. Freeland, *Int. J. Biometeorol.* **44**, 53 (2000).
10. F. M. Chmielewsky, T. Roetzer, *Agric. Forest Meteorol.* **108**, 101 (2001).
11. R. Ahas, *Int. J. Biometeorol.* **42**, 119 (1999).
12. M. D. Schwartz, B. E. Reiter, *Int. J. Climatol.* **20**, 929 (2000).
13. P. C. Reid, M. Edwards, H. G. Hunt, A. J. Warner, *Nature* **391**, 546 (1998).
14. R. B. Myneni *et al.*, *Nature* **386**, 698 (1997).
15. L. Zhou *et al.*, *J. Geophys. Res.* **106**, 20069 (2001).
16. C. D. Keeling, J. F. S. Chin, T. P. Whorf, *Nature* **382**, 146 (1996).
17. H. Spiecker *et al.*, Eds., *Growth Trends in European Forests: Studies from 12 Countries* (Springer, Berlin, 1996).
18. C. P. Osborne *et al.*, *Plant Cell Environ.* **23**, 701 (2000).
19. W. Larcher, *Physiological Plant Ecology* (Springer, Berlin, 1995).
20. M. V. Price, N. M. Waser, *Ecology* **79**, 1261 (1998).
21. Note, however, that species requiring a certain number of frost days for budbursting (*19*) may suffer an impact not linearly correlated with temperature.
22. R. A. Fleming, G. M. Tatchell, in *Insects in a Changing Environment*, R. Harrington, N. Stork, Eds. (Academic Press, London, 1995), pp. 505–508.
23. W. N. Ellis, J. H. Donner, J. H. Kuchlein, *Entomol. Ber. Amsterdam* **57**, 66 (1997).
24. D. B. Roy, T. H. Sparks, *Global Change Biol.* **6**, 407 (2000).
25. J. P. Gibbs, A. R. Breisch, *Conserv. Biol.* **15**, 1175 (2001).
26. C. F. Mason, *Bird Study* **42**, 182 (1995).
27. T. S. Sillet, R. T. Holmes, T. W. Sherry, *Science* **288**, 2040 (2000).
28. R. Harrington, I. Woidwod, T. Sparks, *Trends Ecol. Evol.* **14**, 146 (1999).
29. M. E. Visser *et. al.*, *Proc. R. Soc. London B* **265**, 1867 (1998).
30. A. H. Fitter *et al.*, *Funct. Ecol.* **9**, 55 (1995).
31. A conference, "The times they are a changing. Climate change, phenological responses and their consequences for biodiversity, agriculture, forestry and human health," will be held in Amsterdam in December 2001; see www.dow.wau.nl/msa/epn/conference.

PERSPECTIVES: PLANT BIOLOGY

A Baroque Residue in Red Wine

Herman Höfte

The walls of higher plants contain small amounts of a mysterious polysaccharide known as rhamnogalacturonan II (RGII). RGII is thought to be the most complex polysaccharide on Earth, and its presence and strong conservation in all higher plants suggest that it is important for the structure or growth of plant cell walls. The study by O'Neill *et al.* (*1*) on page 846 of this issue convincingly shows, 23 years after its discovery (*2*), that RGII is essential for plant growth and that minor changes in its structure cause growth defects.

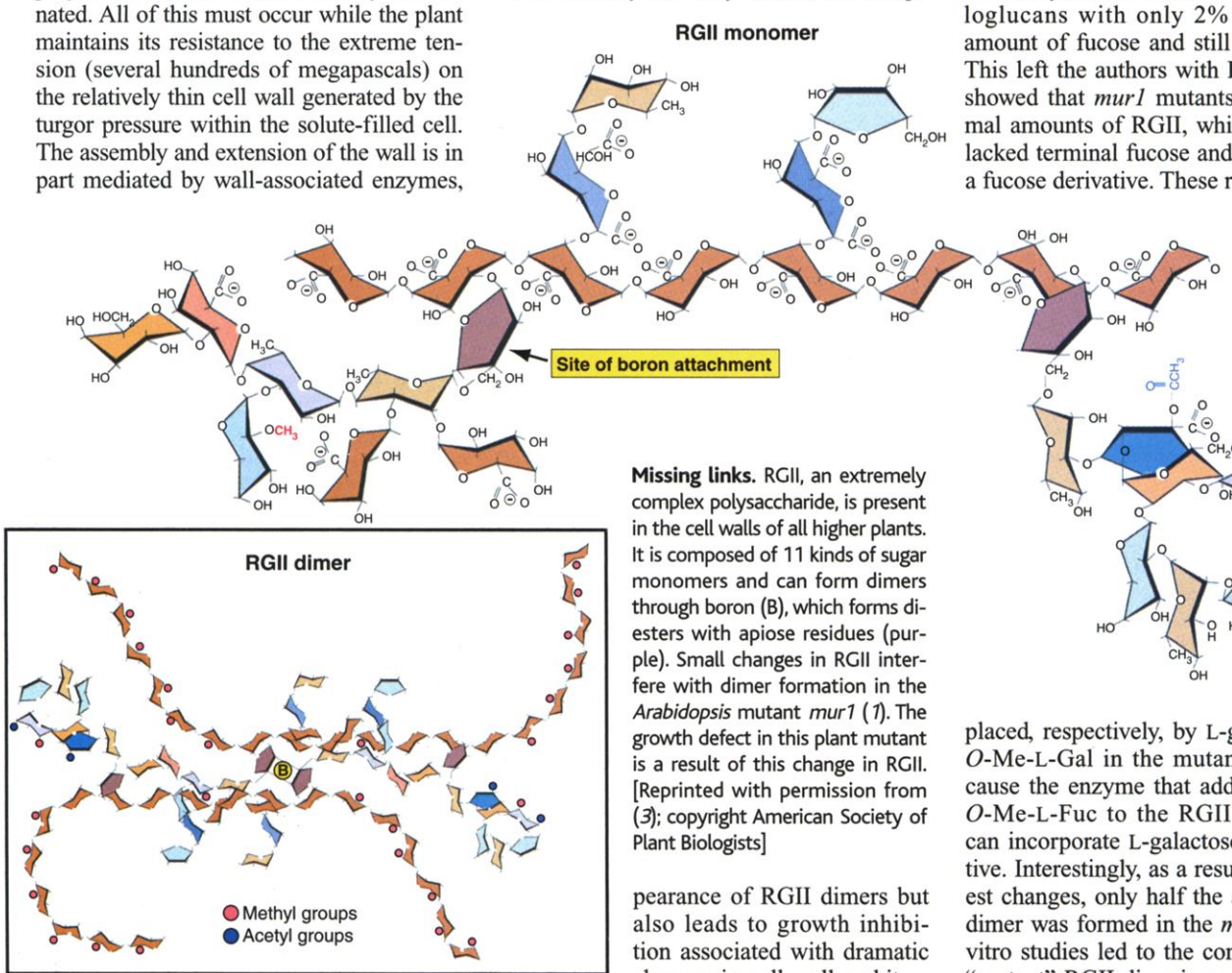
More than 300 years ago, Robert Hooke pointed his primitive microscope at a slice of cork and discovered the cellular basis of organisms. Sadly, since then, plant cell walls, which formed the compartments he actually observed, have never been considered particularly entertaining structures. Indeed, the word wall itself evokes something dull and rigid, built only to enclose, support, divide, and protect. However, a closer look reveals just how erroneous this view is. Walls of growing plant cells are extremely sophisticated composite materials made of dynamic networks of polysaccharides, protein, and phenolic compounds. Cellulose microfibrils with a tensile strength comparable to that of steel provide the plant with a load-bearing framework. These microfibrils are rigid wires made of crystalline arrays of β-1,4-linked chains of glucose residues, which are extruded from little hexameric spinnerets in the plant cell plasma membrane and surround the growing cell like the hoops around a barrel. Because cellulose microfibrils constrain turgor-driven cell expansion in one preferential direction, they control the shape of plant cells and ultimately that of the plants themselves. Hemicelluloses, such as xyloglucans, are tethered by hydrogen bonds to cellulose and form cross-links that may control the separation of the cellulose microfibril hoops. The cellulose-hemicellulose network is embedded in a matrix of complex galacturonic acid–rich pectic

The author is in the Laboratoire de Biologie Cellulaire, INRA, 78210 Versailles Cedex, France. E-mail: hofte@versailles.inra.fr

polysaccharides (including RGII) that form a hydrated gel inside the wall and provide a dynamic operating environment for cell wall processes.

Most of the components of the pectic matrix are notable for their heterogeneity, but intriguingly, RGII is highly conserved. During the growth of plant cells, which can extend hundreds and even thousands of times their original length, the synthesis, deposition, assembly, and remodeling of wall polysaccharides must be carefully coordinated. All of this must occur while the plant maintains its resistance to the extreme tension (several hundreds of megapascals) on the relatively thin cell wall generated by the turgor pressure within the solute-filled cell. The assembly and extension of the wall is in part mediated by wall-associated enzymes, which cleave, rearrange, or cross-link polysaccharides. Plants have evolved unique strategies to fine-tune the activity of these extracellular enzymes indirectly, for example, by modifying the extracellular pH or the redox state of the plant cell or by generating localized oxygen radicals. Pectic polysaccharides are also thought to be important in this control, because they can influence the porosity of the cell wall and hence the mobility of the wall enzymes.

RGII is a remarkable molecule (see the figure). It is composed of 11 kinds of sugar monomers, and it is thought that at least 21 enzymes are dedicated to the construction of all the linkages between the sugar residues. This molecule must adopt a well-defined three-dimensional conformation, as suggested by its marked resistance to enzymatic degradation. As a result, large amounts of RGII can be found in fermented products such as wine. Why do all higher plants invest so much effort in producing this baroque structure in their walls? The first hint came from the observation that RGII exists as a dimer that is cross-linked by a borate diester (see the figure). Boron is an essential micronutrient of plants, and its deficiency not only causes the disappearance of RGII dimers but also leads to growth inhibition associated with dramatic changes in cell wall architecture. These findings suggest, but do not prove, that the absence of RGII dimers causes growth defects. To demonstrate a causal relationship, plant mutants with specifically altered RGII would be needed.



Missing links. RGII, an extremely complex polysaccharide, is present in the cell walls of all higher plants. It is composed of 11 kinds of sugar monomers and can form dimers through boron (B), which forms diesters with apiose residues (purple). Small changes in RGII interfere with dimer formation in the *Arabidopsis* mutant *mur1* (*1*). The growth defect in this plant mutant is a result of this change in RGII. [Reprinted with permission from (*3*); copyright American Society of Plant Biologists]

In the absence of such specific mutants, O'Neill *et al.* (*1*) took advantage of the known pleiotropic mutant *mur1* in the model plant *Arabidopsis thaliana*. The altered gene in this mutant codes for the enzyme GDP-D-mannose-4,6-dehydratase, which is required for the synthesis of GDP-L-fucose. This activated sugar is the substrate used for the fucosylation of RGII. The mutant plants are dwarfed and have more fragile cell walls. Watering the mutant with fucose restores normal growth. The problem is that fucose is not only found in RGII. Two other types of polymer are also fucosylated: xyloglucans and the glycan side chains of many proteins. But which altered polysaccharides are responsible for the growth defect in *mur1*? To resolve this whodunit, the authors commandeered observations reported for two other mutants. Indeed, mutant *cgl1* entirely lacks fucose in its glycoproteins and surprisingly does not show an observable phenotype. Similarly, *mur2* mutants synthesize xyloglucans with only 2% of the normal amount of fucose and still grow normally. This left the authors with RGII. They next showed that *mur1* mutants contained normal amounts of RGII, which, as expected, lacked terminal fucose and 2-*O*-Me-L-Fuc, a fucose derivative. These residues were replaced, respectively, by L-galactose and 2-*O*-Me-L-Gal in the mutant, probably because the enzyme that adds fucose and 2-*O*-Me-L-Fuc to the RGII backbone also can incorporate L-galactose and its derivative. Interestingly, as a result of these modest changes, only half the amount of RGII dimer was formed in the *mur1* mutants. In vitro studies led to the conclusion that the "mutant" RGII dimerizes more slowly and has a reduced stability at low pH. It also requires higher concentrations of boron to dimerize. This also turned out to be true in vivo, because watering the plants with boron restored the formation of RGII dimers. The most exciting observation of this study is that this treatment also restored normal growth to *mur1* plants, thus demonstrating unequivocally that RGII dimer formation is essential for normal plant growth.

As usual, these observations raise more questions than they answer. Why is RGII dimerization so important? RGII is covalently attached to a backbone composed of a linear chain of galacturonic acids, and dimerization promotes the formation of a

cross-linked pectin network. This network controls the porosity of the cell wall, as shown by the increased pore size of cell walls of boron-starved cell cultures. It will be interesting to see whether the altered pore size also affects the accessibility of polysaccharide-modifying enzymes to their substrates, which could be a mechanism by which reduced boron cross-linking affects growth of *mur1* plants. Does RGII cross-linking control normal plant growth? A reduction in pore size has been observed upon transition from the growth phase to the stationary phase in cell cultures. It is not known, however, whether RGII is involved in this change. What is the three-dimensional structure of this molecule? Insights come from molecular modeling, and attempts to crystallize this polysaccharide are under way. Maybe these approaches will explain its unusual stability. Finally, it will also be interesting to know whether this complex molecule carries out tasks other than those related to its ability to form dimers. Next time you drink a glass of red wine, rich in RGII, why not reflect on these intriguing questions.

References and Notes

1. M. O'Neill, S. Eberhard, P. Albersheim, A. G. Darvill, *Science* **294**, 846 (2001).
2. A. G. Darvill *et al.*, *Plant Physiol.* **62**, 418 (1978).
3. N. Carpita, M. McCann, in *Biochemistry and Molecular Biology of Plants*, B. Buchanan, W. Gruissem, R. Jones, Eds. (American Society of Plant Biologists, Rockville, MD, 2000), chap. 2, p. 73.
4. I thank K. Roberts, W. Tycho Willats, M. Pauly, T. Hayashi, H. North, and P. Lerouge for helpful comments.

PERSPECTIVES: MOLECULAR BIOLOGY

Glimpses of a Tiny RNA World

Gary Ruvkun

Over the years, a steady stream of structural and regulatory RNAs have been identified. Three papers published in this issue on pages 853, 858, and 862 from the Tuschl, Bartel, and Ambros labs continue the tradition, but now prospecting for tiny RNAs of ~22 nucleotides (nt) (*1–3*). The chain of reasoning that simultaneously attracted these groups to 22 nt is convoluted but interesting.

The first 22-nt RNAs, *lin-4* and *let-7*, were identified by genetic analysis of *Caenorhabditis elegans* developmental timing (*4*, *5*). The expression of the *lin-4* RNA during the first larval stage and the *let-7* RNA during the fourth larval stage triggers the down-regulation of target mRNAs via 3′-untranslated region (UTR) elements that are complementary to each regulatory RNA to specify the temporal progression of cell fates (*6*, *7*). The *let-7* RNA, as well as its temporal regulation, are conserved across much of animal phylogeny (*8*). These 22-nt RNAs are called small temporal RNAs or stRNAs

Tiny RNAs also emerged from the biochemical analysis of RNA interference (RNAi) by experimentally induced double-stranded RNA (dsRNA): 21- to 25-nt small intermediate RNAs (siRNAs) are processed from dsRNA and act as templates for their own amplification and the degradation of target mRNAs during RNAi (*9*, *10*). *lin-4* and *let-7* are predicted to be processed from partially double-stranded precursors as well (*4*, *5*, *8*). The common size of ~22 nt for stRNAs and siRNAs suggested that they are generated and perhaps act by a common mechanism. In fact, the same Dicer ribonuclease (RNase) that is required to process dsRNA to siRNAs also processes the stRNAs from their precursors (*11*, *12*). The developmental defects caused by Dicer mutations in plants and animals may be due to defects in processing of other endogenous tiny regulatory RNAs (*11–13*).

The three teams use a range of biochemical techniques to clone 21- to 25-nt RNAs (*1–3*) from three different organisms, and thus reveal the richness of the tiny RNA world. They detect almost 100 new tiny RNAs—microRNAs or miRNAs. The Tuschl group identified 14 new miRNAs from the *Drosophila* embryo and 19 miRNAs from HeLa cells (*1*). The expression of all the new miRNAs was verified. The Bartel group identified 55 new miRNAs from mixed-stage *C. elegans* and verified the expression of 20 out of 22 miRNAs tested (*2*). Lee and Ambros cloned and verified the expression of 15 *C. elegans* miRNAs, 10 of which were also identified by the Bartel group (*3*). While the entry point of these studies was biochemical, complete genome sequences were key in the analyses. All three groups used the genome sequences of a variety of organisms to determine that these miRNAs are not breakdown products of mRNAs or structural RNAs, to infer precursors, to determine the genetic locations of the new genes, and to determine whether the miRNAs are conserved in evolution.

All of these miRNAs are predicted to be processed from multiply bulged and partially duplex precursors, like the stRNA precursors. Therefore, they are likely to be processed by Dicer, as demonstrated for two of the new miRNAs (*3*). More of the miRNAs are processed from the 3′ region of the precursor stem loop than from the 5′ region, from which stRNAs are processed. One precursor produces miRNAs from both stems (*2*). Thus, as in siRNA processing from dsRNA, Dicer probably processes both strands of these precursors, but in many cases only one strand may be stable. Some of the miRNAs are expressed only as longer precursors at some developmental stages (*2*, *3*), suggesting possible regulation of processing rather than transcription.

Members of the RDE-1/Argonaute superfamily of proteins may also function in the maturation of miRNAs. The *C. elegans* Argonaute orthologs are required for the maturation and function of *let-7* and *lin-4* (*11*), and *C. elegans* RDE-1 and *Arabidopsis* Ago1 are necessary for RNAi (*14*, *15*). These proteins may form a complex with Dicer, as has been shown for *Drosophila* Argonaute2 (*16*). Genome sequences suggest that there are 24 *C. elegans* RDE-1/Argonaute genes, 7 in *Arabidopsis*, 4 in *Drosophila*, and 4 in humans. The distinct RDE-1/Argonautes may be specialized for processing subsets of miRNA genes. The developmental defects caused by mutations in *Drosophila* or *Arabidopsis* Argonaute genes may be due to defective processing of particular miRNAs (*17*, *18*).

Like *let-7*, a number of the miRNA genes are conserved in evolution. About 12% of the miRNAs are conserved between nematodes, flies, and mammals, but more than 90% of the *C. elegans* miRNAs are conserved in the 90% complete *Caenorhabditis briggsae* sequence (*2*). To detect these conserved segments in genome sequence comparisons, only one or two mismatches could be tolerated. But one of the new miRNAs, *mir-84*, is 5 nt diverged from *let-7*, temporally regulated like *let-7*, and conserved in flies and humans (*2*). Such a paralog could only be detected in the rarified sequence space of the miRNA sequence collection.

One of the more subtle results comes from what the papers did not find: There is almost no evidence of siRNAs diagnostic of RNA interference in normally growing animals (*2*). Thus, Dicer and its cofactors are normally used for miRNA production, and are only recruited for RNAi upon viral or other dsRNA induction.

Some of the miRNAs, like *lin-4* and *let-7*, are temporally regulated. A number of the *Drosophila* and *C. elegans* miRNAs are only expressed in germ line or early embryos, hotbeds of translational control. In addition, the analysis of miRNA expression in cell lines and tissues suggests cell type–specific expression (*1*, *3*). The regulated ex-

The author is in the Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA.