David C. Baulcombe

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An RNA World

David C. Baulcombe

Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom; email: dcb40@cam.ac.uk

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Abstract

My research career started with an ambition to work out how genes are regulated in plants. I tried out various experimental systems—artichoke tissue culture in Edinburgh; soybean root nodules in Montreal; soybean hypocotyls in Athens, Georgia; and cereal aleurones in Cambridge—but eventually I discovered plant viruses. Viral satellite RNAs were my first interest, but I then explored transgenic and natural disease resistance and was led by curiosity into topics beyond virology, including RNA silencing, epigenetics, and more recently, genome evolution. On the way, I have learned about approaches to research, finding tractable systems, and taking academic research into the real world. I have always tried to consider the broader significance of our work, and my current projects address the definition of epigenetics, the arms race concept of disease resistance, and Darwin's abominable mystery.

Contents

AN INTRODUCTION TO BOTANICAL BEACHCOMBING

Very few scientists are visionaries who can identify and solve the big questions at the frontiers of knowledge. The rest of us are beachcombers. Like Newton, we are as children "playing on the seashore, and diverting [ourselves] in now and then finding a smoother pebble or a prettier shell than ordinary" (19, p. 407). Newton, of course, was aware of the nearby "great ocean of truth," but the rest of us only see it if we pick up the smoother pebble.

Thomas S. Kuhn in *The Structure of Scientific Revolutions* (66) referred to this beachcombing as "normal science," in which either we are attempting to describe the natural world using the extended perception afforded by technology or we are testing hypotheses. The normal science outcome is often consistent with existing hypotheses, but we all hope for the unexpected result—the smoother pebble that will reveal a paradigm shift.

My life as a beachcomber started when I decided to pursue a PhD. Jim Callow[1] was my undergraduate tutor in the Department of Botany at Leeds University, and he advised that for my PhD I should focus on what I thought was the most important question in biology. For me, it involved genetic regulation in eukaryotes. I thought that this topic was the key to the mysteries of biology and meeting real-world challenges in healthcare and agriculture.

Jacob and Monod had started to unravel genetic regulation in bacteria, but when I started my PhD in 1973, we knew virtually nothing about the equivalent processes in animals and plants. Britten and Davidson, however, were developing attractive models, and they were an inspiration. Their original gene-battery model did not pan out completely (20), but it involved the insightful concept of regulatory RNA. The layers of transcriptional and posttranscriptional mechanisms proposed by Britten, Davidson, and colleagues (53) are still a useful framework for thinking about genetic regulation in plants and animals.

My interest in RNA and posttranscriptional processes led me to the beautiful city of Edinburgh for my PhD. The University was, and still is, a leading center of molecular biology, and I had the good fortune to be accepted into John Ingle's laboratory. He was well known for seminal work with Joe Key on auxin (56), and he had a range of interesting projects, including one on flax epigenetic

[1]He later moved to Birmingham (**https://www.birmingham.ac.uk/staff/profiles/biosciences/callow-james.aspx**).

genotrophs (104)—lines that acquired heritable changes to their growth in response to either high or low fertilizer in the first generation.

Elsewhere in Edinburgh, Ken Murray was developing gene cloning (82), Ed Southern devised his famous gel blotting method (103), and John Bishop was using hybridization kinetics—the approach of Britten and Davidson—to characterize complex messenger RNA (mRNA) populations (48). I wanted to use these new and exciting methods in plants, so after the peculiarly truncated PhD training offered in UK universities, I looked for a suitable opportunity.

My first stint as a postdoc was with D.P.S. Verma in Montreal, where I hoped to gain molecular biology experience with leghaemoglobin and other root nodule genes. We made some progress (3, 13), but I had an opportunity to move to the University of Georgia with Joe Key. The Key lab's soybean system was attractive because it involved a defined stimulus—auxin. We knew already that there were clear auxin effects on RNA polymerase I (43), and if protein-coding genes were affected, this would be a good system in which to find them.

The University of Georgia was vibrant, and there were good faculty in plant science, biochemistry, and genetics. I especially valued the advice and support from Rich Meagher (77) in the days when some people thought that plant DNA was not cloneable in *Escherichia coli* (see below). We identified auxin-regulated mRNAs in the Key lab (11), but a lesson learned from my time in Georgia was to listen to your friends: They are the people to tell you the uncompromising truth! I had some intriguing data—still unpublished—showing a counterintuitive superinduction of these auxin-regulated mRNAs by an inhibitor of RNA synthesis. My postdoc colleague Fritz Schöffl told me that these were the most interesting experiments of my career and that I should follow them up. He may have been right, but I moved on to the Plant Breeding Institute (PBI) at Cambridge before we could establish the connection between auxins and gene expression (**Figure 1**).

At the PBI, I switched to gibberellins because, from the classic work of Joe Varner (24), we knew that these hormones stimulated α-amylase gene expression in the aleurone layer of germinated barley. We also identified other gibberellin-regulated mRNAs (7, 8, 10, 25, 67), but the wheat aleurone system was not ideal for what I wanted to do. One of the problems—inefficient cloning of wheat genomic DNA in the λ phage vectors—was overcome with the help of information in the *Maize Genetics Corporation Newsletter*. Maize geneticists had encountered a similar problem, and their remedy was to use *E. coli* K803 that could tolerate methylated DNA inserts (37). Rob Martienssen (who was a graduate student at the time), postdoc Colin Lazarus, and I raised a glass or two to *E. coli* strain K803. This methylated DNA problem thwarted many of the plant gene cloning pioneers.

Unfortunately, by using this strain, we made our task more difficult. The high-efficiency cloning with K803 included methylated and gene-poor heterochromatic DNA that was a much bigger component of the wheat genome than the unmethylated and gene-rich euchromatin. It was, consequently, a huge task to screen for clones of our gibberellin-regulated genes in the K803 libraries. We used very large square plates to grow and screen the libraries in phage λ vectors, what seemed like acres of Millipore filters, and huge amounts of radioactive probes.

In retrospect, I can now see that we could have screened on a smaller scale by using the host strains that would only tolerate the gene-rich regions of unmethylated DNA. Rob subsequently developed this idea into methylation filtration to facilitate genomic sequencing in maize (88). Perhaps the lesson from this experience is to ask whether a perceived problem really is a problem.

AVOIDING THE OBVIOUS

Until I joined the PBI, I was a genetics sceptic because I thought that the connection between a geneticist's gene and a piece of DNA could be made most easily using biochemistry and

Figure 1

The Plant Breeding Institute (PBI) at Cambridge had an unusual mix of traditional breeding, plant pathology, and plant physiology combined with cutting-edge research in molecular genetics. It was in a good position to bridge the gap between basic and applied science, but the breeding research was privatized and the basic research moved to the John Innes (JI) Centre. This photograph from 1987 shows Harold Woolhouse, the charismatic and inspirational Director of the combined institutes, describing this change to a joint meeting of PBI and JI scientists. Thirty years later, I was pleased to help establish the Cambridge Crop Science Centre by raising funds for a building and a Professorship. The Centre is based at the National Institute of Agricultural Botany (NIAB), and it is rebuilding the mix of basic and applied research that was lost with the privatization of the PBI. The photograph is used with permission from the John Innes Centre photograph archive.

molecular biology. But after a year or so at the PBI, I was a genetics convert. I was persuaded because advancing technology—transposon tagging and molecular mapping of genomes—meant that making the connection was now feasible. I also began to understand that I did not need a gene in a test tube to discover interesting biology: Careful deduction and thoughtful genetic analysis can be very informative.

I should have picked up sooner on the power of genetics because I had heard a talk by Salvador Luria, one of the giants of twentieth-century science (112). Luria started by asking the audience whether we wanted a geneticist's or a molecular biologist's talk. A geneticist, he explained, may not have much data but has plenty of ideas. A molecular biologist, in contrast, would have lots of data.

I also learned from my PBI colleague Enrico Coen who worked on pin- and thrum-eyed primroses and the symmetry of *Antirrhinum* flowers. He said that it was not a hindrance to work with difficult species rather than the model organisms used by mainstream biology. With model species, it is easy to do the obvious experiments, but with the other plants, one is more likely to do the informative experiments.

In my subsequent attempts to avoid the obvious, I have used several species, including *Arabidopsis*, tobacco, potato, tomato, *Chlamydomonas*, and an Australian weed—*Nicotiana benthamiana*. One of my preferred journals likes to use common names rather than Latin binomials, and

the galley proofs of our papers would always come back with *N. benthamiana* changed to tobacco (*Nicotiana tabacum*). We corrected these errors, but then, in the final proof, the tobacco references were restored. Eventually the editor conceded defeat when I found out that the indigenous name for *N. benthamiana* is *tjuntiwari*.

POSSIBLE SCIENCE: TRACTABLE SYSTEMS

At the PBI there were several groups in Dick Flavell's Department of Molecular Genetics, and we worked in a completely open laboratory, sharing grant money, equipment, space, and ideas. It is an ethos that I have tried to recreate in my later career.

We also worked alongside very successful plant breeders including John Bingham, who had produced most of the wheat varieties grown in the UK. He liked to remind us that the real world was uncompromising: "An innovation that is almost the best is actually useless." One of the other breeders gave a retirement speech in which he said, "I want to talk about the impact of molecular biology on plant breeding," followed by a long silence. This skepticism should have prepared me for later campaigns with genetically modified (GM) crops.

But perhaps the most seismic shift for me at the PBI was my introduction to virology. It was a fairly low-key start prompted by the potato breeders who screened for virus in thousands of virus-inoculated plants. Their enzyme-linked immunosorbent assay (ELISA) protocol was simple, but they had a high frequency of false-positive or false-negative results. Dick Flavell and I thought that a nucleic acid hybridization assay would be more reliable, and I provided the breeders with complementary DNA (cDNA) clones and a protocol that they then started to use routinely. Spots of dried sap from the infected plants could be dried onto nitrocellulose membranes and hybridized with radiolabeled cDNA probes corresponding to the viral genome, and the results were a reliable indicator of the virus-susceptible plants in their breeding populations (9).

It was rewarding to be involved in a useful side project, but, more importantly, it introduced me to virology and plant pathology—topics that had largely passed me by until then. I was at a key point in my science career because, as described above, I was learning the hard way with wheat that progress in basic research needs a tractable experimental system. It needs a plant that is compact, has good genetics, is accessible to the tools of molecular biology, and is easy to bulk up for biochemistry. Wheat, at least in the mid-1980s, did not have all of those benefits, and I was looking around for something else. Following my involvement in diagnostics, I wondered whether viruses could be useful tools in my quest to understand genetic regulation in plants. There was ample precedent for this approach from animal virology.

At this point, I started talking to Bryan Harrison, an eminent virologist at the Scottish Crop Research Institute in Dundee, and we ended up having fruitful collaborations over many years. Bryan was enthusiastic about molecular biology, but having worked at the Rothamsted Research Institute with F.C. Bawden and N.W. Pirie, he was also a link with the early pioneers in virology (46). Bawden and Pirie discovered that tobacco mosaic virus contains RNA, and their findings contradicted the erroneous Nobel Prize–winning work of Wendell Stanley, who claimed that it was an infectious protein.

Between 1936 and 1940, Pirie was at Cambridge and his collaborator, Bawden, was at Rothamsted. Bryan speculated that purified viral RNA would have degraded and lost infectivity in transit and that institutional separation had prevented this pair from making the huge discovery that viral nucleic acids are infectious. Had Bawden and Pirie been at the same institution, they might have taken Stanley's place at Stockholm and anticipated the later discovery of Avery and Macleod that nucleic acids are the material of heredity.

I like this story because it illustrates how plants are good model systems for general biology. Others have also made this point (59), but the message needs to be shouted from the rooftops.

It is not that we need to be chauvinistic about plants. A more compelling reason is that plants shed light on fundamental aspects of biology because they can be easy to work with. Plant research might also help make agriculture more productive or sustainable. I return to this point in a later section titled Reaping the Benefits and Sustainable Intensification in Agriculture.

One of my first viral projects involved noncoding RNA that caused disease. We characterized noncoding viral satellite RNAs that triggered a spectacular yellow mosaic on tobacco plants or that caused tomato seedlings to keel over and die (34, 57). These findings were not compatible with the standard disease paradigm involving virus-encoded proteins, and we inferred that the viral RNA was somehow preventing the expression of a host gene. The idea was right, but we did not have the technology to prove it and, unfortunately, dropped the project. That was a very bad decision for reasons explained in the following section.

A VERY PRETTY SHELL

In parallel with these experiments on satellite RNA, I was also taking advantage of the *Agrobacterium* know-how brought to the PBI by Mike Bevan from Mary-Dell Chilton's St. Louis lab. Using Mike's vectors, we produced disease-resistant GM plants (12, 47), and although they were not grown in the field, they did support my successful application to join the newly established Sainsbury Laboratory in Norwich (**Figure 2**).

The Sainsbury Laboratory was set up by supermarket grandee David Sainsbury—later Minister for Science—who had been persuaded by his advisors that plant pathology is important and interesting. His aim was to establish a well-resourced facility in which the researchers were free to follow their scientific noses. There was no requirement to stock the shelves of his supermarket, but if there was a chance to do something useful with our research findings, we had a responsibility to follow up.

It was a fantastic opportunity, although positions in the Sainsbury Laboratory were on five-year contracts and I had to give up my tenure at the PBI in the Scientific Civil Service. I now had to reapply for my job after each contract period, but I was happy because the facility and resources

Figure 2

The Sainsbury Laboratory in Norwich was established in 1988 with Mike Daniels, Jonathan Jones, and myself as group leaders. We moved into a new custom-built laboratory, and working there was a wonderful experience. The left panel shows myself, Jonathan, and Mike (*left* to *right*) admiring the architect's maquette of the yet-to-be-built laboratory (photograph used with permission from the John Innes Centre photograph archive). I hope we justified the faith and investment of David Sainsbury, the benefactor of the Gatsby Charitable Foundation that supported the work of the laboratory. Jonathan is a demanding colleague requiring concentration and attention to detail even when we sailed my Norfolk Punt (*right*) (photograph used with permission of **https://myerscoughphotography.org/**)

were so good. If I could not justify my continued employment in that setting, then clearly I should go and do something else.

Some of our first experiments in the Sainsbury Laboratory explored the concept of parasite-derived resistance in which a gene is transferred from a parasite into the host—a type of genetic immunization. This approach worked well in *E. coli* (96), and I wanted to test it in GM plants. Our early results with tobacco were very clear: The plants were resistant specifically against the virus used for the transgene (72). To get such a definite result was exciting, but it was strange that the immunity was strongest in plants in which the viral transgene was not expressed. Eventually, however, I realized that the process causing the virus resistance also silenced the transgene. Others came to the same conclusion with their experimental systems (71).

The virus in these experiments, potato virus X (PVX), has an RNA genome, and we consequently referred to this process as RNA silencing. Using a combination of molecular biology and genetics, we and others described aspects of its mechanism in a fair amount of detail (14, 27, 28, 50–52, 54, 97, 101, 114). We used genetic screens to identify several factors involved in RNA silencing, but the biggest contribution of my lab was when Andrew Hamilton found the small RNAs (sRNAs) that are the specificity determinant of the silencing mechanism (44, 45). These findings converged satisfyingly with results from various other plant, animal, and fungal groups, and we were clearly all on the same RNA bandwagon looking at homologous processes (117). I am not quite sure whether the collective discovery of these RNA-based mechanisms is a Kuhnian paradigm shift, but if not, it has to be at least a nudge in the gene expression field and certainly a prettier shell or smoother pebble.

If we had persisted with our analysis of viral satellite RNAs, we might have made progress ten years earlier because the tobacco yellow mosaic is due to sRNAs: They are produced from the satellite RNA, and they target the mRNA of a chlorophyll biosynthetic enzyme (98, 102). Looking back, I am not sure why we did not look harder. However, even if we had, I am not sure that it would have made much of a difference. The findings might have been dismissed as being a niche discovery in specialist virological journals. By contrast, in 1999, when Andrew Hamilton and I published his data on sRNAs (44), RNA silencing had momentum due to the convergence of findings from several plant, animal, and fungal systems. It was the right time.

REAPING THE BENEFITS AND SUSTAINABLE INTENSIFICATION IN AGRICULTURE

In principle, we could extend RNA-mediated virus resistance in GM plants to protect any crop against any virus. We could design transgenes based on problem viruses in agriculture and use *Agrobacterium* or particle bombardment to transform the affected crops. A similar approach would also be effective with cellular pathogens because there is a transfer of silencer RNA from a host into an infecting bacterium, fungus, or oomycete (5). Problem-solving in the real world, however, requires more than good ideas. We also have to persuade people (and be sure) that our solution does not introduce complications that are worse than the original problem, and we need to understand the environmental, economic, and social implications of the new technology.

My introduction to this broad perspective was in 2008 when, with a Royal Society working group, we reported on the role of bioscience and technology in crops. Our report, *Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture* (92) (**Figure 3**), was well received, and its main messages were that research should be focused on major crops, including those in horticulture; ecosystem-based approaches should be given more emphasis; and there should be support for high-risk, high-return projects.

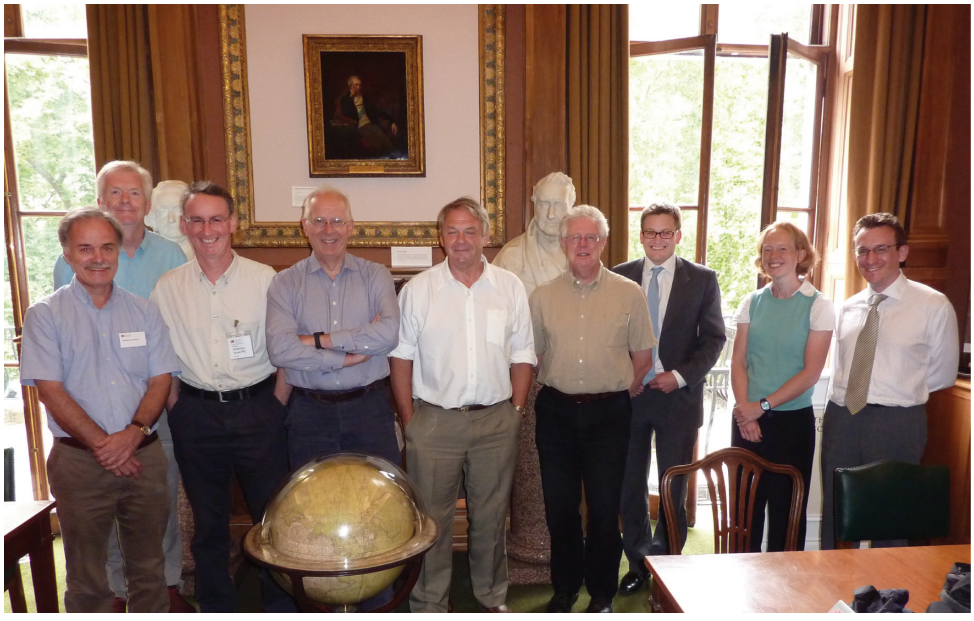

Figure 3

Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture is a Royal Society report produced by a small group, including Jules Pretty, Bill Davies, Jonathan Jones, Mike Gale, Jim Dunwell, Ian Crute, Camilla Toulmin, and me as chair. Jules introduced the concept of sustainable intensification. It is a highly useful and appropriate term, but unfortunately, widely misinterpreted. This photograph, used with permission from Bill Davies, shows (from *left* to *right*) Dunwell, Davies, Jones, myself, Gale, and Crute with members of the Royal Society policy team Jack Stilgoe, Sarah Mee, and Nick Green. We were at the Royal Society headquarters in London in July 2009 having just signed off on the final version of the report. Two days later Mike Gale died suddenly after a game of golf. It was a premature loss of a fine colleague, excellent scientist, and good friend.

Specifically, in the context of ecosystem-based approaches, we discussed agronomy and the management of crops and soils. The high-risk targets for technology included enhanced photosynthesis, reduced fertilizer use, perennialization of annual crops, and strategies for pest and disease resistance. We also suggested that the UK government should establish an independent food security advisory body. This body would work openly with stakeholders to help the government put future technological options into a broad social and economic context and appraise their benefits and uncertainties alongside alternatives. It would feed into and stimulate similar international efforts by CGIAR and the United Nations.

These recommendations remain relevant, although science and technology have moved on. The number of potential GM applications has increased greatly (84), we have gene editing, and there are useful studies on the environmental impacts of crop protection chemicals and nonbiological technology for food production. Unfortunately, despite this progress, global food systems are failing many people. There are mal- and undernourished people in both developed and less-developed regions, and agriculture, including crops, continues to deplete ecosystem services. These challenges are coming into very sharp focus in the aftermath of the pandemic and as a consequence of the war in Ukraine.

To reverse the decline, there must be more effort made toward understanding the social and economic contexts of technology in our food supply systems, as recommended in our original

report. Those of us in biotechnology should be more aware of the broader context in which our innovation could be used, and there is scope for productive dialogue with the green environmental movement.

Some of the real-world challenges for the food supply are set out in a recent discussion paper from Benton & Harwatt (18), who compare two extreme scenarios for future agriculture: sustainable intensification and land sparing versus agroecology and land sharing. On reading this research, I realized that we should have been clearer about the meaning of sustainable intensification in our original report. The intensification term, in particular, is often misinterpreted as being inevitably linked to market disruption and poor nutrition due to overproduction and cheap calorie-rich food.

Benton & Harwatt's (18) critique of sustainable intensification, for example, invokes a paradox of productivity (Jevons paradox) in which innovation in agriculture leading to price reduction would have rebound effects. It would increase demand, distort markets, and undermine the potential for land sparing or other benefits to the food system. Unfortunately, the critique ignores the pressing need for new technology in a sustainable food supply system. In the case of disease resistance, for example, the technology would allow less use of farm machinery for spraying (and thereby less soil compaction and use of fossil fuels), less application of chemicals that would affect beneficial organisms, and less land used per output of crop. We will not provide sufficient food sustainably without these and other innovations.

It is true that there may well be price reduction as a consequence (which is not necessarily a bad outcome), but we can avoid the paradox of productivity if we address directly the potential for market forces to undermine any potential benefits. The Royal Society of London, for example, has called for outcome-based regulation to reduce the rebound effects of new genetic and other technology in agriculture (**https://royalsociety.org/blog/2021/03/gmo-regulation/**). My preferred approach would avoid the blunt tool of regulation because it is not sufficiently flexible for complex agricultural systems, and I would use regulation only where safety is a real issue. In other situations, a better approach would involve subsidy or other incentives for good practice by growers. Various nudge strategies applied to growers or consumers could also be used to help markets support sustainably intense food production.

As things stand, it cannot be right that because we have not yet come up with specific solutions to Jevons paradox, we are avoiding intensification technologies, including GM and gene-edited crops that could reduce the environmental failures in the global food system. The unmet challenge is for social scientists and policymakers to devise strategies that promote constructive use of these new technologies. To meet this challenge is not optional. Much of current practice in crops and agriculture is not sustainable, and we must, if we are to avoid catastrophic loss of biodiversity and other ecosystem services, use science and technology to help achieve equitable and sustainable production in agriculture.

ON EPIGENETICS

My first introduction to epigenetics was in the flax genotroph projects of my PhD supervisor (104), but this topic otherwise passed me by until we became interested in transgene silencing. In parallel with various viral projects, including ours (29, 36, 71, 72), there were intriguing findings about the coordinate suppression (or cosuppression) of a transgene and the corresponding endogenous gene in *Petunia* and tomato (30, 83, 105). Some groups interpreted the effect as being epigenetic (62), but in our systems, we knew that the target of silencing was an RNA virus, and we were thinking of more direct mechanisms. Our lab motto, "keep it simple," led us to sRNA and, together with other groups and with input from nonplant systems, the now well-known

Figure 4

Alan Herr (*right*) was one of my most talented postdocs. His discovery of PolIV and its involvement in epigenetic RNA silencing was for sure a smoother pebble, but it was not lying on the beach. Alan had to dig down in the sand to find it. This photograph of him with me was at a conference in the French Alps.

posttranscriptional mechanisms involving Dicer(-like) and Slicer (Argonaute) emerged (4). There is nothing fundamentally epigenetic about this process.

But the *Petunia* groups were not wrong: There is undeniably an epigenetic dimension to RNA silencing in plants (76), fission yeast (110), and other organisms (68). This aspect of silencing was illustrated clearly in one of the nicest experiments to come from my lab, done by Louise Jones. She showed that a modified RNA virus could target the silencing and DNA methylation of a transgene promoter. The silencing and DNA methylation persisted for several generations although the virus was not transmitted through the seed (61).

Alan Herr was able to reinforce the link between RNA silencing and epigenetics when he characterized one of our RNA silencing mutants and discovered PolIV, an RNA polymerase II homolog required for sRNA biogenesis and DNA methylation of transposon and repeated DNA (51). Craig Pikaard homed in on the same discovery almost simultaneously (86). Alan died of cancer last year, and I would like to pay tribute to him as a very fine person, wonderful colleague, and excellent scientist (**Figure 4**).

These and other findings from several groups indicated that variations on the basic post-transcriptional silencing mechanism have effects at the DNA and chromatin levels (76). Our viral promoter silencing system represents one of these variations, and the process clearly fit the definition of epigenetics: There was no change to the DNA sequence of the transgene promoter and the effect was heritable. On reflection over several years, however, I think it is too simplistic to put genetic and epigenetic effects in separate categories. It is more appropriate to recognize that epigenetic effects are dependent, at several levels, on genetic features. In the virus-induced promoter silencing, for example, the establishment of the silent state was dependent on a genetic factor—the modified virus-carrying sequence with similarity to the promoter target. Additionally,

different promoter regions vary in the extent to which they can be targeted by this process, dependent to some extent on their GC content—a genetic feature (87).

The general relationship between genetic features and epigenetic effects overlaps with Mark Ptashne's perspective on epigenetics that he illustrated with a thought experiment based on an autoregulatory transcription factor gene (89, 90). He envisioned that, following activation of this gene by an external stimulus, its continued activity would be autonomous: It would be independent of the continuing stimulus because the transcription factor would drive its own expression. This system would not have the typical epigenetic features associated with DNA methylation or histone modification, but it nevertheless has the separate establishment and maintenance phases that are a defining feature of epigenetics.

With plant systems, the different epigenetic phases may be separated in space and time so that they are sometimes easier to study than in other organisms. Separation in space is allowed because sRNA with a role in establishment can move symplastically, most likely in a double-stranded form (33, 79, 106, 109). Separation in time is illustrated, for example, by Louise Jones's virus experiments in which establishment of the promoter DNA methylation occurred in the infected plant, whereas maintenance continued in the absence of the virus in later generations (61). In mammals, by contrast, the epigenome is reset in each generation and transgenerational separation is rare (49).

My lab, at Cambridge University since 2007, continues to be interested in the genetics of epigenetics, and we are exploiting the potential of plant systems to identify features of and cofactors involved in the separate establishment and maintenance phases (41, 42, 69, 75, 78, 111). Although the biology in our systems is specific to plants, the concepts and some of the mechanisms are common to animals and other organisms (49). These examples reinforce my earlier point that plants can be good model systems for basic mechanisms in general biology. We can corrupt the famous Monod aphorism to say, "What is true for peas may also be true for people." At least it is a better alliteration than "*E. coli* and elephants."

BEYOND THE DISEASE-RESISTANCE ARMS RACE

A common description of disease resistance often uses an evolutionary arms race metaphor: Hosts have greater fitness if they have strong immunity and pathogens have a selective advantage if they can evade or suppress these defense systems (60). This framework influences thinking about host–pathogen interactions, and it shapes many approaches to control disease in plants and animals. Our recent findings, however, lead us to a more nuanced perspective that looks beyond the arms race.

I have been interested in immunity and disease resistance research ever since my group started using viruses as tractable experimental systems. One strand of our interest involved dominant disease resistance genes, and we focused initially on *Rx* in potato. Rx-mediated resistance completely suppressed PVX in the initially infected cell, and I predicted that the encoded protein would be a repressor of virus replication. The PVX resistance, however, was induced by the viral coat protein that is not required for viral replication (16, 65), and after heroic potato gene mapping by Abdel Bendahmane, we identified Rx as a nucleotide-binding site leucine-rich repeat (NLR) protein (15). We had converged on the effector-triggered immunity (ETI) field pioneered by our Sainsbury Laboratory neighbor—Jonathan Jones—and others. Effector-triggered immunity is one of the main components of the plant innate immune system responsible for many examples of pathogen race-specific disease resistance (85, 116).

The other strand of our disease resistance research originated with the transgenic projects that led to our interest in RNA silencing. We were engineering artificial parasite-derived resistance in

these projects, but in some of our transgenic lines, the plants were totally resistant (72, 81). The resistance was specific for the virus corresponding to the transgene, but it seemed unlikely that an artificial mechanism would be so effective unless it was based on a natural process.

To explore the possibility of natural RNA silencing, we were guided by Bryan Harrison to papers from 1928 describing how plants could recover from virus infection and resist secondary infection (113). We thought that this recovery could represent natural RNA silencing, and consistent with that hypothesis, the recovery was dependent on RNA sequence similarity between the primary and secondary viruses (93, 94).

The most compelling support for silencing as a natural antiviral defense, however, was from the discovery of viral sRNAs (44) and of virus-encoded suppressors of RNA silencing (26). The production of antiviral sRNA in infected plants would represent a primary defense system, and the suppressors of RNA silencing would be the counter-defense. Vicki Vance and Jim Carrington first came up with evidence that a virus, tobacco etch virus, encodes a suppressor of silencing (2, 64), and my group confirmed that many, if not all, viruses encode proteins with similar functions, although the structures and mechanisms were different with each class of viruses (108). Cellular pathogens are also affected by RNA silencing. Many of the pathogens' virulence genes may be targeted by *trans*-kingdom silencing from the host (22, 55), and like viruses, these pathogens encode virulence factors that are suppressors of RNA silencing (91, 115).

In my laboratory, the RNA silencing and Rx/NLR/ETI projects started off as completely separate lines of research, but they converged because the viral suppressors of silencing may be protein triggers of ETI (31, 95, 107) and because there are endogenous sRNAs—microRNAs—targeting NLR mRNAs in tomato and other species (32, 99, 118). Extended analyses of this NLR silencing revealed that NLRs are implicated in race-nonspecific (23, 58) as well as in the race-specific disease resistance mechanisms investigated by many others (60). Our analysis also revealed that NLR silencing is less effective in virus-infected plants (23). This effect is most likely because the suppressors of silencing referred to above block endogenous NLR silencing just as they reduce the biochemically similar pathway of antiviral RNA silencing (26). I am intrigued by this intersection of RNA silencing and innate immunity because it points beyond the basic arms race idea of disease and disease resistance (6, 73) in which immunity benefits only the host and virulence gives advantage specifically to the pathogen. We need a more nuanced perspective.

From the host side, the need to go beyond the arms race is illustrated by the fitness cost of immunity (63). There is a rough correlation of NLR gene numbers with endogenous sRNAs targeting the NLR mRNAs (40), and the NLR silencing could reduce the fitness cost of disease resistance. The NLR silencing would, however, be reversed in infected plants due to the action of the silencing suppressors. Blocking the NLR silencing by the pathogen-encoded suppressors would activate the innate immune system in infected plants. Overall, the NLR silencing may reduce the level of immunity, but in the longer term, a host may be better protected against disease if its immune systems are not so strong that they select for resistance-breaking pathogens (21).

The need to look beyond the arms race model is also illustrated by the involvement of RNA silencing and suppressors of RNA silencing in the relationship between pathogens and the host. The suppressors of silencing promote virulence of the pathogen in some contexts because they would block the action of antiviral or *trans*-kingdom sRNAs. In other contexts or perhaps at certain stages in the infection cycle they do the opposite. They would block sRNA-mediated silencing of the NLR mRNAs and thereby induce the host's immune system. To reconcile this paradox, we are exploring the possibility, particularly with biotrophs, that a pathogen may be more fit and spread more easily if any disease-mediated damage to the host is moderated by the immune system. In

this light, the ability of pathogens to suppress NLR silencing and activate immunity could increase their fitness. The enhanced NLR immunity in infected plants would allow the pathogen to survive and spread to other plants. Clearly there is no simple relationship between fitness and the strength of the immune system in the host or virulence in the pathogen (1, 35), and I am attracted to the idea that evolution selects hosts and pathogens that accommodate rather than outrun each other. It is not a race but a dance in which the partners glide gracefully and try not to tread on each other's feet!

This issue has more than academic importance because it is relevant to disease resistance in people, farm animals, and crops. The conventional approach, based on the arms race metaphor, is to use antimicrobial chemicals, vaccines, or disease resistance genes with the strongest protection (21). The repeated lesson, however, is that this approach selects for pathogens that overcome or evade the protection system. Perhaps by better understanding the interaction of RNA silencing, NLR, and other innate immune systems in plants, we will be able to dance with disease and develop more durable strategies for crop protection than we have at present.

DARWIN'S ABOMINABLE MYSTERY, SYMBIOSIS, AND DISEASE RESISTANCE

My career started with what I hoped would be a big question related to the regulation of gene expression, but like many aging biologists, my interest has shifted toward evolution. I am particularly puzzled by Darwin's abominable mystery: the unexplained rapid radiation of flowering plant species starting about 60 million years ago and continuing from the Paleogene into the Quaternary Period. Vertebrate clades did not have a similar radiation, and there are around 300,000 species of angiosperms but approximately tenfold fewer vertebrates (17).

Darwin's explanation of this mystery, suggested by his correspondent Saporta, involves coadaptation of plants and their insect pollinators as a driver of evolutionary change (39). But wind-pollinated grasses are as diverse as other families of flowering plant, and there must be more to this story. Various alternative hypotheses invoke plant carbon economy, resistance to climatic stresses, nutrient economy, and biotic interactions (17). Of these, the coadaptive biotic interactions of plants with other rapidly evolving organisms are likely to be potent drivers of evolutionary change. The dance with disease would be important, but nonpathogenic symbionts, including mutualists, epiphytes, and components of the soil microbiome, could drive rapid coadaptive change in the associated plant genomes. Vertebrates with adaptive immune systems would be buffered against biotic interactions as drivers of genomic change.

Transposons are likely also part of the solution to the abominable mystery because they facilitate rapid plant genome evolution (38). They promote evolvability because they can be exapted as new protein-coding genes and they can affect gene expression either directly if they insert into promoters or indirectly if they are the genetic determinant of epigenetic marks. Probably the most important contribution of transposons to evolvability, however, is through their potential to cause structural genomic changes, including chromosomal rearrangement and gene duplication or loss. Such changes would be particularly important in a period of postpolyploidization diploidization (70) that ultimately explains why plant genomes have evidence of repeated cycles of whole-genome duplication and are able to generate new genotypes in rapid evolutionary time.

My group's interest in transposons and genomic change involves transposon-derived sRNAs, especially in hybrid plants. Transposons are the most highly variable components of genomes, and they are abundant sources of sRNA (80). Our working hypothesis is that in hybrids, the sRNAs from one parent would find new targets in the other genome and vice versa (100). Using crosses of tomato with a wild relative, we have data that are consistent with the hypothesis. Some

of the hybrid-induced genomic changes have paramutation-like characteristics (42), and others may involve endogenous pararetroviruses (74). It has been a challenge to keep the momentum of these projects during the COVID-19 pandemic, but my group zoomed up to the task admirably. I am looking forward to further analysis of these systems and using the data to refine the basic hypothesis.

IN ANOTHER LIFE?

In early life I wanted to be a saxophonist (**Figure 5**), but anyone who has heard me play is grateful that I went into science. Instead of a band, I have had a research group for more than forty years, and, almost entirely, it has been a deeply rewarding experience. We had, and have, smart people with good social skills so that vigorous scientific (and general) discussion was and is a pleasure (**Figure 6**). I heard from many of them recently for my seventieth birthday, and it would be good to have news from others if they read this article. I am sorry that I have not mentioned them all by name. In all of my institutions, I have benefitted from working alongside other researchers, but I would like to thank Jonathan Jones and Rico Coen especially for their inspiration and friendship.

I am grateful to my funders, including the Biotechnology and Biological Sciences Research Council, Royal Society, the European Research Council, and, especially, the Gatsby Charitable

Figure 5

Never to be Charlie Parker. The less said the better. . .

Figure 6

A dream team. This is my group from 2018 sharing a summer picnic and punting expedition with the Ian Henderson and Sebastian Eves-van den Akker groups with whom we share lab space. Ever since my time at the PBI I have enjoyed sharing lab space with other groups. This is one of a long line of dream teams. Photograph used with permission of Xiao Wang.

Foundation. They provided generous funding for more than 20 years in a manner that allowed my group to find, perhaps, a few smoother pebbles and prettier shells. I hope I will be able to continue beachcombing for a while because reports of my death are greatly exaggerated (**Figure 7**).

EXCELLENCE IN SCIENCE

THE ROYAL SOCIETY

Notices 2010

The Royal Society regrets to announce the deaths of the following Fellows in 2010:

Sir David Charles Baulcombe FRS
19 March 1942 - 22 December 2010 [elected 2001]
Regius Professor of Botany and Royal Society Research Professor, Department of Plant Sciences, University of Cambridge
Medal: Royal (2006)
Council Service: 2007-2009

Figure 7

An exaggerated report. I was alerted to this mistake by a friend who sent an email asking whether I was dead! Unfortunately, the Royal Society corrected the mistake before I could read my obituary.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Alizon S, Hurford A, Mideo N, Van Baalen M. 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. *J. Evol. Biol.* 22(2):245–59
2. Anandalakshmi R, Pruss GJ, Ge X, Marathe R, Smith TH, Vance VB. 1998. A viral suppressor of gene silencing in plants. *PNAS* 95(22):13079–84
3. Auger S, Baulcombe DC, Verma DPS. 1979. Sequence complexities of the poly(A)-containing RNA in uninfected soybean root and the nodule tissue developed due to infection by *Rhizobium*. *Biochim. Biophys. Acta Nucleic Acids Protein Synth.* 563:496–507
4. Baulcombe D. 2004. RNA silencing in plants. *Nature* 431:356–63
5. Baulcombe DC. 2015. VIGS, HIGS and FIGS: small RNA silencing in the interactions of viruses or filamentous organisms with their plant hosts. *Curr. Opin. Plant Biol.* 26:141–46
6. Baulcombe DC. 2022. The role of viruses in identifying and analyzing RNA silencing. *Annu. Rev. Virol.* 9:353–73
7. Baulcombe DC, Barker RF, Jarvis MG. 1987. A gibberellin responsive wheat gene has homology to yeast carboxypeptidase Y. *J. Biol. Chem.* 262:13726–35
8. Baulcombe DC, Buffard D. 1983. Gibberellic acid regulated expression of α-amylase and six other genes in wheat aleurone layers. *Planta* 157:493–501
9. Baulcombe DC, Flavell RB, Boulton RE, Jellis GJ. 1984. The sensitivity and specificity of a rapid nucleic acid hybridization method for the detection of potato virus X in crude sap samples. *Plant Pathol.* 33(3):361–70
10. Baulcombe DC, Huttly AK, Martienssen RA, Barker RF, Jarvis MG. 1987. A novel wheat α-amylase gene (α-*Amy3*). *Mol. Gen. Genet.* 209(1):33–40
11. Baulcombe DC, Key JL. 1980. Polyadenylated RNA sequences which are reduced in concentration following auxin treatment of soybean hypocotyls. *J. Biol. Chem.* 255:8907–13
12. Baulcombe DC, Saunders GR, Bevan MW, Mayo MA, Harrison BD. 1986. Expression of biologically active viral satellite RNA from the nuclear genome of transformed plants. *Nature* 321(6068):446–49
13. Baulcombe DC, Verma DPS. 1978. Preparation of a complementary DNA for leghaemoglobin and direct demonstration that leghaemoglobin is encoded by the soybean genome. *Nucleic Acids Res.* 5:4141–53
14. Bäurle I, Smith L, Baulcombe DC, Dean C. 2007. Widespread role for the flowering-time regulators FCA and FPA in RNA-mediated chromatin silencing. *Science* 318(5847):109–12
15. Bendahmane A, Kanyuka K, Baulcombe DC. 1999. The *Rx* gene from potato controls separate virus resistance and cell death responses. *Plant Cell* 11(5):781–91
16. Bendahmane A, Köhm BA, Dedi C, Baulcombe DC. 1995. The coat protein of potato virus X is a strain-specific elicitor of *Rx*1-mediated virus resistance in potato. *Plant J.* 8(6):933–41
17. Benton MJ, Wilf P, Sauquet H. 2022. The Angiosperm Terrestrial Revolution and the origins of modern biodiversity. *New Phytol.* 233(5):2017–35
18. Benton TG, Harwatt H. 2022. *Sustainable agriculture and food systems*. Res. Pap., Chatham House, London, UK. **https://www.chathamhouse.org/2022/05/sustainable-agriculture-and-food-systems**
19. Brewster D. 1855. *Memoirs of the Life, Writings, and Discoveries of Sir Isaac Newton*. Edinburgh, UK: Thomas Constable and Co. **https://quod.lib.umich.edu/g/genpub/aat0604.0002.001?rgn=main;view=fulltext;q1=unitarian**
20. Britten RJ, Davidson EH. 1969. Gene regulation for higher cells: a theory. *Science* 165(3891):349–57
21. Brown JKM. 2015. Durable resistance of crops to disease: a Darwinian perspective. *Annu. Rev. Phytopathol.* 53:513–39

22. Cai Q, He B, Wang S, Fletcher S, Niu D, et al. 2021. Message in a bubble: shuttling small RNAs and proteins between cells and interacting organisms using extracellular vesicles. *Annu. Rev. Plant Biol.* 72:497–524
23. Canto-Pastor A, Santos BAMC, Valli AA, Summers W, Schornack S, Baulcombe DC. 2019. Enhanced resistance to bacterial and oomycete pathogens by short tandem target mimic RNAs in tomato. *PNAS* 116(7):2755–60
24. Chrispeels MJ, Varner JE. 1967. Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley and aleurone layers. *Plant Physiol.* 42(3):398–406
25. Cornford CA, Black M, Chapman JM, Baulcombe DC. 1986. Expression of α-amylase and other GA-regulated genes in aleurone tissue of developing wheat grains. *Planta* 169:420–28
26. Csorba T, Kontra L, Burgyán J. 2015. Viral silencing suppressors: tools forged to fine-tune host-pathogen coexistence. *Virology* 479–480:85–103
27. Dalmay T, Hamilton AJ, Rudd S, Angell S, Baulcombe DC. 2000. An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. *Cell* 101(5):543–53
28. Dalmay TD, Horsefield R, Braunstein TH, Baulcombe DC. 2001. *SDE3* encodes an RNA helicase required for post-transcriptional gene silencing in *Arabidopsis*. *EMBO J.* 20(8):2069–78
29. De Haan P, Gielen JJL, Prins M, Wijkamp IG, van Schepen A, et al. 1992. Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants. *Bio/Technology* 10:1133–37
30. de Lange P, de Boer G-J, Mol JNM, Kooter JM. 1993. Conditional inhibition of β-glucuronidase expression by antisense gene fragments in petunia protoplasts. *Plant Mol. Biol.* 23:45–55
31. de Ronde D, Pasquier A, Ying S, Butterbach P, Lohuis D, Kormelink R. 2014. Analysis of *Tomato spotted wilt virus* NSs protein indicates the importance of the N-terminal domain for avirulence and RNA silencing suppression. *Mol. Plant Pathol.* 15(2):185–95
32. de Vries S, Kloesges T, Rose LE. 2015. Evolutionarily dynamic, but robust, targeting of resistance genes by the miR482/2118 gene family in the Solanaceae. *Genome Biol. Evol.* 7(12):3307–21
33. Devers EA, Brosnan CA, Sarazin A, Albertini D, Amsler AC, et al. 2020. Movement and differential consumption of short interfering RNA duplexes underlie mobile RNA interference. *Nat. Plants* 6(7):789–99
34. Devic M, Jaegle M, Baulcombe DC. 1989. Symptom production on tobacco and tomato is determined by two distinct domains of the satellite RNA of cucumber mosaic virus (strain Y). *J. Gen. Virol.* 70:2765–74
35. Doumayrou J, Avellan A, Froissart R, Michalakis Y. 2013. An experimental test of the transmission-virulence trade-off hypothesis in a plant virus. *Evolution* 67(2):477–86
36. English JJ, Mueller E, Baulcombe DC. 1996. Suppression of virus accumulation in transgenic plants exhibiting silencing of nuclear genes. *Plant Cell* 8:179–88
37. Fedoroff N. 1983. Notes on cloning maize DNA. *Maize Genet. Corp. Newsl.* 57:154–55
38. Fedoroff NV. 2012. Transposable elements, epigenetics, and genome evolution. *Science* 338:758–67
39. Friedman WE. 2009. The meaning of Darwin's "abominable mystery." *Am. J. Bot.* 96(1):5–21
40. González VM, Müller SY, Baulcombe DC, Puigdoménech P. 2015. Evolution of NBS-LRR gene copies among dicot plants and its regulation by members of the miR482/2118 superfamily of miRNAs. *Mol. Plant* 8:329–31
41. Gouil Q, Baulcombe DC. 2016. DNA methylation signatures of the plant chromomethyltransferases. *PLOS Genet.* 12(12):e1006526
42. Gouil Q, Baulcombe DC. 2018. Paramutation-like features of multiple natural epialleles in tomato. *BMC Genom.* 19:203
43. Guilfoyle TJ, Lin CY, Chen YM, Nagao RT, Key JL. 1975. Enhancement of soybean RNA polymerase I by auxin. *PNAS* 72(1):69–72
44. Hamilton AJ, Baulcombe DC. 1999. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286(5441):950–52
45. Hamilton AJ, Voinnet O, Chappell L, Baulcombe DC. 2002. Two classes of short interfering RNA in RNA silencing. *EMBO J.* 21(17):4671–79
46. Harrison BD. 1994. Frederick Charles Bawden: plant pathologist and pioneer in plant virus research. *Annu. Rev. Phytopathol.* 32:39–47

47. Harrison BD, Mayo MA, Baulcombe DC. 1987. Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. *Nature* 328(6133):799–802
48. Hastie ND, Bishop JO. 1976. The expression of three abundance classes of messenger RNA in mouse tissues. *Cell* 9:761–74
49. Heard E, Martienssen RA. 2014. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* 157(1):95–109
50. Hernandez-Pinzon I, Yelina NE, Schwach F, Studholme DJ, Baulcombe DC, Dalmay T. 2007. SDE5, the putative homologue of a human mRNA export factor, is required for transgene silencing and accumulation of *trans*-acting endogenous siRNA. *Plant J.* 50(1):140–48
51. Herr AJ, Jensen MB, Dalmay T, Baulcombe DC. 2005. RNA polymerase IV directs silencing of endogenous DNA. *Science* 308(5718):118–20
52. Herr AJ, Molnàr A, Jones A, Baulcombe DC. 2006. Defective RNA processing enhances RNA silencing and influences flowering of *Arabidopsis*. *PNAS* 103(41):14994–5001
53. Hough BR, Smith MJ, Britten RJ, Davidson EH. 1975. Sequence complexity of heterogeneous nuclear RNA in sea urchin embryos. *Cell* 5(3):291–99
54. Huang LF, Jones AME, Searle I, Patel K, Vogler H, et al. 2009. An atypical RNA polymerase involved in RNA silencing shares small subunits with RNA polymerase II. *Nat. Struct. Mol. Biol.* 16(1):91–93
55. Hudzik C, Hou Y, Ma W, Axtell MJ. 2020. Exchange of small regulatory RNAs between plants and their pests. *Plant Physiol.* 182(1):51–62
56. Ingle J, Key JL, Holm RE. 1965. Demonstration and characterization of a DNA-like RNA in excised plant tissue. *J. Mol. Biol.* 11(4):730–46
57. Jaegle M, Devic M, Longstaff M, Baulcombe DC. 1990. Cucumber mosaic virus satellite RNA (Y strain): analysis of sequences which affect yellow mosaic symptoms on tobacco. *J. Gen. Virol.* 71:1905–12
58. Jiang N, Meng J, Cui J, Sun G, Luan Y. 2018. Function identification of miR482b, a negative regulator during tomato resistance to *Phytophthora infestans*. *Horticult. Res.* 5:9
59. Jones AM, Chory J, Dangl JL, Estelle M, Jacobsen SE, et al. 2008. The impact of *Arabidopsis* on human health: diversifying our portfolio. *Cell* 133(6):939–43
60. Jones JD, Dangl JL. 2006. The plant immune system. *Nature* 444(7117):323–29
61. Jones L, Ratcliff F, Baulcombe DC. 2001. RNA-directed transcriptional gene silencing in plants can be inherited independently of the RNA trigger and requires Met1 for maintenance. *Curr. Biol.* 11(10):747–57
62. Jorgensen RA. 1995. Cosuppression, flower color patterns and metastable gene expression states. *Science* 268:686–91
63. Karasov TL, Chae E, Herman JJ, Bergelson J. 2017. Mechanisms to mitigate the trade-off between growth and defense. *Plant Cell* 29(4):666–80
64. Kasschau KD, Carrington JC. 1998. A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. *Cell* 95(4):461–70
65. Kohm BA, Goulden MG, Gilbert JE, Kavanagh TA, Baulcombe DC. 1993. A potato virus x resistance gene mediates an induced, nonspecific resistance in protoplasts. *Plant Cell* 5(8):913–20
66. Kuhn TS. 1962. *The Structure of Scientific Revolutions*. Chicago, IL: Univ. Chicago Press
67. Lazarus CM, Baulcombe DC, Martienssen RA. 1985. α-Amylase genes of wheat are two multi-gene families which are differentially expressed. *Plant Mol. Biol.* 5:13–24
68. Lepère G, Bétermier M, Meyer E, Duharcourt S. 2008. Maternal noncoding transcripts antagonize the targeting of DNA elimination by scanRNAs in *Paramecium tetraurelia*. *Genes Dev.* 22(11):1501–12
69. Lewsey MG, Hardcastle TJ, Melnyk CW, Molnar A, Valli A, et al. 2016. Mobile small RNAs regulate genome-wide DNA methylation. *PNAS* 113(6):E801–10
70. Li Z, McKibben MTW, Finch GS, Blischak PD, Sutherland BL, Barker MS. 2021. Patterns and processes of diploidization in land plants. *Annu. Rev. Plant Biol.* 72:387–410
71. Lindbo JA, Silva-Rosales L, Proebsting WM, Dougherty WG. 1993. Induction of a highly specific antiviral state in transgenic plants: implications for regulation of gene expression and virus resistance. *Plant Cell* 5(12):1749–59

72. Longstaff M, Brigneti G, Boccard F, Chapman SN, Baulcombe DC. 1993. Extreme resistance to potato virus X infection in plants expressing a modified component of the putative viral replicase. *EMBO J.* 12:379–86
73. Lopez-Gomollon S, Baulcombe DC. 2022. Roles of RNA silencing in viral and non-viral plant immunity and in the crosstalk between disease resistance systems. *Nat. Rev. Mol. Cell Biol.* 23:645–62
74. Lopez-Gomollon S, Müller SY, Baulcombe DC. 2022. Interspecific hybridization in tomato influences endogenous viral sRNAs and alters gene expression. *Genome Biol.* 23:120
75. Martinho C, Wang Z, Ghigi A, Buddle S, Barbour F, et al. 2022. CHROMOMETHYL-TRANSFERASE3/KRYPTONITE maintain the *sulfurea* paramutation in *Solanum lycopersicum*. *PNAS* 119:e2112240119
76. Matzke MA, Mosher RA. 2014. RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. *Nat. Rev. Genet.* 15(6):394–408
77. Meagher RB, Shepherd RJ, Boyer HW. 1977. The structure of cauliflower mosaic virus: I. A restriction endonuclease map of cauliflower mosaic virus DNA. *Virology* 80(2):362–75
78. Melnyk CW, Molnar A, Bassett A, Baulcombe DC. 2011. Mobile 24 nt small RNAs direct transcriptional gene silencing in the root meristems of *Arabidopsis thaliana*. *Curr. Biol.* 21(19):1678–83
79. Molnar A, Melnyk CW, Bassett A, Hardcastle TJ, Dunn R, Baulcombe DC. 2010. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. *Science* 328(5980):872–75
80. Mosher RA, Schwach F, Studhollme D, Baulcombe DC. 2008. PolIVb influences RNA-directed DNA-methylation independently of its role in siRNA biogenesis. *PNAS* 105(8):3145–50
81. Mueller E, Gilbert J, Davenport G, Brigneti G, Baulcombe DC. 1995. Homology-dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing. *Plant J.* 7(6):1001–13
82. Murray K, Murray NE. 1975. Phage lambda receptor chromosomes for DNA fragments made with restriction endonuclease III of *Haemophilus influenzae* and restriction endonuclease I of *Escherichia coli*. *J. Mol. Biol.* 98(3):551–64
83. Napoli C, Lemieux C, Jorgensen RA. 1990. Introduction of a chimeric chalcone synthase gene into Petunia results in reversible co-suppression of homologous genes *in trans*. *Plant Cell* 2:279–89
84. Natl. Acad. Sci. Eng. Med. 2016. *Genetically Engineered Crops: Experiences and Prospects*. Washington, DC: Natl. Acad. Press
85. Ngou BPM, Ahn H-K, Ding P, Jones JDG. 2021. Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature* 592(7852):110–15
86. Onodera Y, Haag JR, Ream T, Nunes PC, Pontes O, Pikaard CS. 2005. Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell* 120(5):613–22
87. Otagaki S, Kawai M, Masuta C, Kanazawa A. 2011. Size and positional effects of promoter RNA segments on virus-induced RNA-directed DNA methylation and transcriptional gene silencing. *Epigenetics* 6(6):681–91
88. Palmer LE, Rabinowicz PD, O'Shaughnessy AL, Balija VS, Nascimento LU, et al. 2003. Maize genome sequencing by methylation filtration. *Science* 302(5653):2115–17
89. Ptashne M. 2007. On the use of the word "epigenetic." *Curr. Biol.* 17(7):R233–36
90. Ptashne M. 2013. Epigenetics: core misconcept. *PNAS* 110(18):7101–3
91. Qiao Y, Liu L, Xiong Q, Flores C, Wong J, et al. 2013. Oomycete pathogens encode RNA silencing suppressors. *Nat. Genet.* 45:330–33
92. R. Soc. 2009. *Reaping the benefits: science and the sustainable intensification of global agriculture.* Rep., R. Soc., London, UK. **https://royalsociety.org/topics-policy/publications/2009/reaping-benefits/**
93. Ratcliff F, Harrison BD, Baulcombe DC. 1997. A similarity between viral defense and gene silencing in plants. *Science* 276:1558–60
94. Ratcliff FG, MacFarlane SA, Baulcombe DC. 1999. Gene silencing without DNA: RNA-mediated cross-protection between viruses. *Plant Cell* 11(7):1207–15
95. Ren T, Qu F, Morris TJ. 2000. *HRT* gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. *Plant Cell* 12(10):1917–26
96. Sanford JC, Johnston SA. 1985. The concept of parasite-derived resistance—deriving resistance genes from the parasite's own genome. *J. Theor. Biol.* 113(2):395–405

97. Searle IR, Pontes O, Melnyk CW, Smith LM, Baulcombe DC. 2010. JMJ14, a JmjC domain protein, is required for RNA silencing and cell-to-cell movement of an RNA silencing signal in *Arabidopsis*. *Genes Dev*. 24(10):986–91
98. Shimura H, Pantaleo V, Ishihara T, Myojo N, Inaba J, et al. 2011. A viral satellite RNA induces yellow symptoms on tobacco by targeting a gene involved in chlorophyll biosynthesis using the RNA silencing machinery. *PLOS Pathog*. 7(5):e1002021
99. Shivaprasad PV, Chen H-M, Patel K, Bond DM, Santos BACM, Baulcombe DC. 2012. A microRNA superfamily regulates nucleotide binding site–leucine-rich repeats and other mRNAs. *Plant Cell* 24(3):859–74
100. Shivaprasad PV, Dunn RM, Santos BACM, Bassett A, Baulcombe DC. 2012. Extraordinary transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing RNAs. *EMBO J*. 31(2):257–66
101. Smith LM, Pontes O, Searle I, Yelina N, Yousafzai FK, et al. 2007. An SNF2 protein associated with nuclear RNA silencing and the spread of a silencing signal between cells in *Arabidopsis*. *Plant Cell* 19(5):1507–21
102. Smith NA, Eamens AL, Wang M-B. 2011. Viral small interfering RNAs target host genes to mediate disease symptoms in plants. *PLOS Pathog*. 7(5):e1002022
103. Southern EM. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–17
104. Timmis JN, Ingle J. 1974. The nature of the variable DNA associated with environmental induction in flax. *Heredity* 33(3):339–46
105. Van Blokland R, Van der Geest N, Mol JNM, Kooter JM. 1994. Transgene-mediated suppression of chalcone synthase expression in *Petunia hybrida* results from an increase in RNA turnover. *Plant J*. 6:861–77
106. Voinnet O, Baulcombe DC. 1997. Systemic signalling in gene silencing. *Nature* 389:553
107. Voinnet O, Lederer C, Baulcombe DC. 2000. A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana*. *Cell* 103:157–67
108. Voinnet O, Pinto YM, Baulcombe DC. 1999. Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. *PNAS* 96(24):14147–52
109. Voinnet O, Vain P, Angell S, Baulcombe DC. 1998. Systemic spread of sequence-specific transgene RNA degradation in plants is initiated by localized introduction of ectopic promoterless DNA. *Cell* 95(2):177–87
110. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SIS, Martienssen RA. 2002. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 297(5588):1833–37
111. Wang Z, Baulcombe DC. 2020. Transposon age and non-CG methylation. *Nat. Commun.* 11(1):1221
112. Watson JD. 1991. Salvador E. Luria (1912–1991). *Nature* 350(6314):113
113. Wingard SA. 1928. Hosts and symptoms of ring spot, a virus disease of plants. *J. Agric. Res.* 37:127–53
114. Yelina NE, Smith LM, Jones AME, Patel K, Kelly KA, Baulcombe DC. 2010. Putative *Arabidopsis* THO/TREX mRNA export complex is involved in transgene and endogenous siRNA biosynthesis. *PNAS* 107(31):13948–53
115. Yin C, Ramachandran SR, Zhai Y, Bu C, Pappu HR, Hulbert SH. 2019. A novel fungal effector from *Puccinia graminis* suppressing RNA silencing and plant defense responses. *New Phytol*. 222(3):1561–72
116. Yuan M, Jiang Z, Bi G, Nomura K, Liu M, et al. 2021. Pattern-recognition receptors are required for NLR-mediated plant immunity. *Nature* 592:105–9
117. Zamore PD. 2006. RNA interference: big applause for silencing in Stockholm. *Cell* 127(6):1083–86
118. Zhang Y, Xia R, Kuang H, Meyers BC. 2016. The diversification of plant *NBS-LRR* defense genes directs the evolution of microRNAs that target them. *Mol. Biol. Evol.* 33(10):2692–705