

Sex and self-denial

Ed Newbigin and Richard D. Vierstra

Many plants are self-incompatible — that is, they have mechanisms to prevent fertilization by their own pollen. A familiar and uncommonly versatile protein, ubiquitin, is found to be a central player in one such system.

Ubiquitin is certainly living up to its name. This small protein, first named because of its seemingly ubiquitous presence in animal and plant cells, now seems to be an almost universal regulator molecule. Ubiquitin attaches to a multitude of other proteins, and its well-known role is as a tag that directs unwanted proteins to the machinery that eliminates them. But ubiquitin attachment is also turning out to influence events as diverse as gene transcription and the movement of proteins and other large molecules around a cell^{1,2}. In plants, ubiquitin is known to be involved in regulating responses to hormones and light, in the formation of seeds and flowers, and in the entrainment of circadian rhythms and defence against invading microbes. But there's more. As described in *The Plant Cell*, a group led by Daphne Goring — Stone *et al.*³ — now show that ubiquitin also helps to regulate mate selection in plants.

Much like animals, plants must choose mates from among many suitors. Being hermaphrodites presents them with an additional complication: how to prevent self-fertilization. Left unchecked, self-fertilization reduces genetic diversity and leads to 'inbreeding depression' that, at worst, can contribute to the extinction of a population or species. One mechanism to prevent inbreeding is self-incompatibility. Self-incompatible plants, such as *Brassica napus* (oilseed rape), the species studied by Stone *et al.*, have a high level of variation at a particular genetic location, the *S* locus. The different versions of the genes at this locus — alleles — enable brassicas to detect their own pollen and thus prevent self-fertilization.

The surface papilla cells of the female reproductive organ, the pistil, actively reject pollen grains (which contain the male gametes) when both have the same *S* allele, as happens when a plant is pollinated by self pollen. When pollen and papilla cells have different *S* alleles, the pollen is judged non-self, or compatible, and fertilization can occur. Rejection of self pollen is surprisingly precise: if a compatible pollen grain and a self pollen grain are simultaneously placed on the same papilla cell, only the self pollen is rejected. Rejection means that the papilla cell fails to undergo several localized changes that normally allow a compatible pollen grain to enter the reproductive system and deliver its gametes to the ovary, where the egg

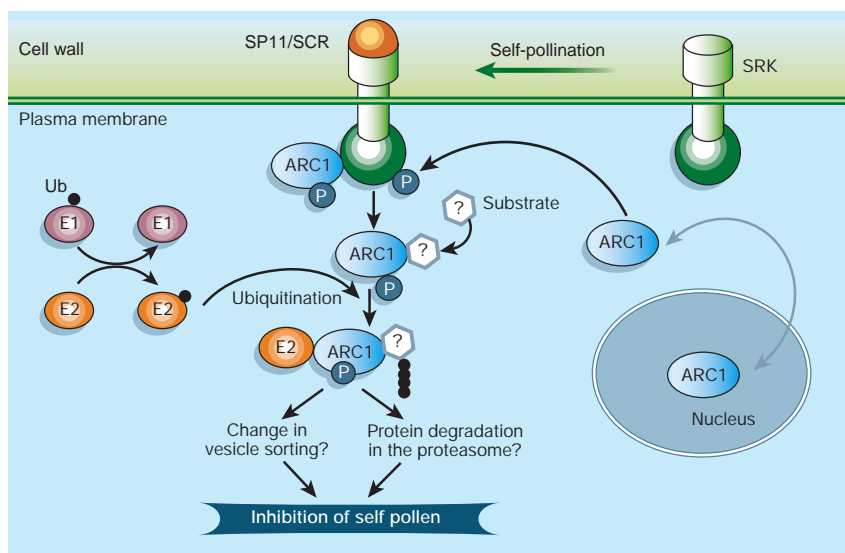


Figure 1 Rejection of self pollen by a brassica papilla cell. In the absence of a self pollen grain, the protein ARC1 shuttles between the nucleus and cytoplasm. In the presence of such pollen, SP11/SCR, released from the pollen coat, binds to and activates SRK, a receptor in the papilla cell's plasma membrane; SRK then phosphorylates (P) both itself and ARC1. Phosphorylated ARC1 in the cytoplasm causes ubiquitin (Ub) to become attached to unknown substrates by means of a conjugation cascade that requires the ubiquitin-conjugating enzyme E2. The experiments of Stone *et al.*³ show that ubiquitination is crucial for self-incompatibility, but how is not yet clear. Ubiquitination might lead to degradation of proteins in the 26S proteasome and their localized depletion, or it might produce a change in vesicle sorting that prevents proteins and other material from being delivered to the pollen contact site. Whatever the case, the upshot is that the papilla cell fails to undergo the changes that allow a compatible pollen grain to enter the plant's reproductive system and eventually perform fertilization.

cells are held⁴. Self-incompatibility in effect slams the door on self pollen.

Self-recognition in brassicas depends on an interaction between two separate and highly polymorphic proteins encoded within the *S* locus^{5,6}. One product, called SRK, is found on the papilla cell's surface and is a membrane-bound receptor kinase — an enzyme that responds to external signal molecules by phosphorylating, and so activating, internal proteins. The other product, known variously as SP11 or SCR, is a small, soluble protein from the coat of the pollen grain. It is released whenever a pollen grain alights on a papilla cell, but it binds to SRK only when the two proteins are products of the same *S* allele. Binding of SP11/SCR activates SRK and initiates a kinase-signalling cascade that results in isolation of the incestuous suitor. Up to now, however, the identity of the molecules phosphorylated by SRK and what they do have largely been a mystery.

A few years ago, Goring's group identified⁷ a protein — ARC1 — in brassica papilla cells that could bind to and be phosphorylated by SRK. Plants with lower concentrations of the protein in their pistils had a diminished capacity to reject self pollen⁸. All this pointed to an involvement of ARC1 in self-incompatibility. But how? Scrutiny of the ARC1 sequence revealed a clue. In addition to motifs for localization and protein-protein interactions, there is a region related to the U-box, a domain involved in ubiquitin conjugation. The U-box, like its distant cousin the RING domain, is present in a variety of ubiquitin ligases, or E3s, which are enzymes that perform the final step in the ubiquitination reaction. In these E3s, the U-box/RING domain docks with another enzyme called E2 that carries an activated ubiquitin moiety, while other domains within the E3 select a suitable target. For ARC1, the U-box is near a sequence motif known as an armadillo

repeat that presumably functions in target selection.

In the new work, Stone *et al.*³ show that ARC1 is an E3 and that ubiquitination is crucial for self-incompatibility. They found that ARC1 can direct ubiquitination *in vitro* and that more proteins are ubiquitinated after a self-pollination than after a compatible pollination. An increase in ubiquitin conjugates was not seen in plants with lower concentrations of ARC1. Adding ubiquitin to a protein targets it for destruction by the cell's garbage-disposal unit, the 26S proteasome. Because the application of a proteasome inhibitor to the pistil also impairs the rejection of self pollen, the targets of ARC1 ubiquitination must be degraded by this multi-enzyme complex.

The substrate(s) of ARC1 ubiquitination, and how SRK affects ARC1 activity, remain unknown. Unfortunately, ARC1 is unlike any other known U-box protein, so guilt by association cannot be used to predict its targets. The large increase in ubiquitinated proteins observed after a self-pollination suggests that a marked shift in target selection occurs in the papilla cell. But how does this shift cause the changes seen during pollen rejection?

Figure 1 shows some possibilities. ARC1 might target proteins in the papilla cell that normally promote pollen germination and direct them to the proteasome for breakdown. Degradation must somehow be restricted to near where the papilla cell and self pollen grain touch, so another possibility is related to ubiquitin's role in vesicle sorting: ubiquitination of specific transport proteins helps to direct the flow of vesicles to appropriate cellular destinations¹. Consistent with this notion was the finding by Stone *et al.* that when ARC1 is phosphorylated by SRK it is no longer mostly in the nucleus but enters the cytoplasm, where it seems to associate with the endoplasmic reticulum and secretory system. Focused secretory activity at the contact site and localized loosening of the papilla cell wall are events seen soon after a compatible pollination⁴. ARC1 ubiquitination could prevent the delivery of proteins and other molecules that are essential for germination or wall loosening to the contact site of self pollen.

Stone and colleagues' work constitutes a step forward in our understanding of brassica self-incompatibility. But is there a bigger picture as well? Other plant families have evolved self-incompatibility mechanisms that are radically different to that found in brassicas. Self-incompatible species in the Rosaceae family, which includes apple and some other fruit trees, use a different *S*-locus-encoded enzyme — a ribonuclease — to inhibit the growth of self pollen. What isn't clear is the *S*-locus product that identifies self pollen. Two tantalizing reports have shown that a gene encoding another component of

the ubiquitination machinery is near the *S*-locus ribonuclease gene in the Rosaceae^{9,10}. Possibly, then, ubiquitination helps to control self-incompatibility in both the Rosaceae and Brassicaceae, the family to which brassicas belong. If it does, we shall have to elevate ubiquitin to an even loftier place in the pantheon of plant regulatory molecules. ■

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Cosmology

A just-so story

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Physicists are learning to live with Einstein's 'fudge factor', the cosmological constant. New thinking attempts to tie its value to other fundamental constants in elementary particle physics.

The recognition, in the light of observational data, that Einstein's infamous cosmological constant might not be zero^{1–3} has changed almost everything about the way we think about the Universe, from reconsidering its origin⁴ to re-evaluating its ultimate future⁵. But perhaps the most significant change in cosmological thinking involves a new willingness to discuss what used to be an idea that was not normally mentioned in polite company: the anthropic principle. This idea suggests that the precise values of various fundamental parameters describing our Universe might be understood only as a consequence of the fact that we exist to measure them. To paraphrase the cosmologist Andrei Linde, "If the Universe were populated everywhere by intelligent fish, they might wonder why it was full of water. Well, if it weren't, they wouldn't be around to observe it!"

The reason that physicists have been so reluctant to consider the anthropic principle seriously is that it goes against the grain. Most physicists have hoped that an ultimate physical explanation of reality would explain why the Universe must look precisely the way it does, rather than why it more often than not would not. Into the fray has entered James Bjorken. In a paper⁶ published in *Physical Review D*, entitled "Cosmology and the Standard Model", Bjorken proposes a new 'scaling' ansatz, based on well-established notions in particle theory, for exploring how anthropically viable a small cosmological constant might be.

The realization that an extremely small, but non-zero, cosmological constant might exist has changed physicists' interest in anthropic explanations of nature precisely because the value it seems to take is otherwise

so inexplicable. In 1996, physicist Steven Weinberg and his colleagues Hugo Martel and Paul Shapiro argued that, if the laws of physics allow different universes to exist with a cosmological constant chosen from an underlying probability distribution, then galaxies, stars and presumably astronomers might not ultimately evolve unless the cosmological constant were not much larger than the one we apparently observe today⁷.

Although this suggestion has spurred several authors to reconsider anthropic arguments, the problem in cosmology is that without a fundamental theory underlying such a probability distribution, very few concrete calculations can be performed. Moreover, while the discussion may centre on fundamental parameters, many of the authors of these discussions are cosmologists, so that little explicit use is made of existing notions from the theory of elementary particles.

However, Bjorken — a particle theorist — notes that a cosmological constant provides a fundamental dimensional parameter that asymptotically characterizes any universe: the so-called de Sitter horizon, R_H (what Bjorken calls R_c). In a universe dominated by a cosmological constant, distant objects recede from an observer at a speed proportional to their distance. Ultimately, beyond a certain, fixed distance, all objects will recede at velocities greater than that of light, and so causal contact will be lost. This distance, the de Sitter horizon, therefore characterizes the effective operational 'size' of an infinite universe undergoing de Sitter-like expansion.

Bjorken suggests that all fundamental dimensional quantities are related to this