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Disputed Ancestry: Comments on a Model for the Origin of Incompatibility in Flowering Plants

In a recent review published in THE PLANT CELL, Bell (1995) argues that self-incompatibility (SI) in flowering plants is derived from isolating mechanisms, typically involving thickened callosic cell walls, that develop between the gametophyte and sporophyte generations of lower plants. He writes that "an interfacial reaction leading to the separation of the two generations at the time of reproduction is a fundamental property of land plants" and suggests that the recognition loci governing this isolation evolved to produce the multiallelic SI loci in angiosperms. This model requires that the first angiosperms had a genetically determined incompatibility and that all SI systems of modern plants derive from this ancestral feature. We restate here the view, based on current molecular data, that SI arose separately in different lineages of angiosperms. We also present alternative views to those of Bell concerning the function of callose in the SI response and the role of carbohydrates in mediating SI recognition.

Origins of SI

The phylogenetic origin of SI has been debated for many years, and arguments exist both for and against the early origin of SI in angiosperms (Whitehouse, 1950; Bateman, 1952). The wide distribution of SI among the 320-odd angiosperm families has been used as evidence of its ancestral nature. However, the distribution is rather sporadic, and SI is uncommon in "primitive" woody angiosperms and entirely absent from several very large families (Charlesworth, 1985).

Molecular information is now available through the cloning of SI genes from the Solanaceae (Anderson et al., 1989), Brassicaceae (Nasrallah et al., 1987), Papaveraceae (Foote et al., 1994), and Poaceae (Li et al., 1994). There is substantial evidence that these genes encode the molecules identified as *S* gene products, and their sequences give considerable insight into the evolution of SI. In three cases, the likely function of the *S* gene products can be inferred from the corresponding sequence: the *S* genes of *Brassica* encode receptor-protein kinases (SRKs) and glycoproteins (SLGs), those of the Solanaceae encode RNases, and those of *Phalaris* encode thioredoxins. Some, such as the SLGs and SRKs, are encoded by part of a multigene family present in the genome of *Brassica* and other plants, whereas others, such as the S-RNases and thioredoxins, are of more ancient lineage and are found in both eukaryotic and prokaryotic organisms (Nasrallah and Nasrallah, 1993; Green, 1994; Li et al., 1994).

The genes currently known to be present at the *S* loci in these four families have no sequence similarity—indeed, they have independent evolutionary histories. This indicates that these SI systems are unrelated at the molecular level and leads to the conclusion that SI arose independently in each family. No traces of residual similarity between SI genes have been reported, as would be required to support the idea of divergence from a single, ancestral SI system (Bell, 1995). The lack of sequence similarity between the *S* genes in these different families, coupled with the existence of related genes with other functions, indicate that the various *S* genes were independently recruited to their current functions in reproduction from previously existing genes in the genome. The molecular data therefore point to several origins of SI.

Callose: Cause or Effect of SI?

Bell argues that the expression of SI involves deposition of the polysaccharide callose as a barrier between the angiosperm gametophyte and sporophyte, with

LETTERS TO THE EDITOR

compatible pollination evading this recognition process. Compared with compatible pollen tubes, incompatible pollen tubes in the Solanaceae have thickened and irregular callosic walls and show a loss of directional growth and reduced cytoplasmic zonation. However, similar abnormalities can also be produced in pollen tubes growing in culture by altering the nutrient balance of the medium (Cresti et al., 1985, 1986; Read et al., 1993a, 1993b, and references therein). There is no evidence that SI involves the specific induction of callose deposition in pollen tubes, and the changes in callose distribution in incompatible tubes are probably just a consequence of growth inhibition. In the Brassicaceae, deposition of callose in the papillar cells of the stigma is also linked with the SI response (Nasrallah and Nasrallah, 1993). However, callose deposition in the stigma is not required for the incompatibility barrier and is induced by material leaking from already inhibited tubes (Singh and Paolillo, 1990; Elleman and Dickinson, 1994). In both gametophytic and sporophytic systems, therefore, increased callose deposition in pollen tubes or stigmas is probably an effect of SI, rather than a cause as suggested by Bell.

Bell is also incorrect in stating that the production of callose in pollen tubes is dependent on contact with the sporophyte. Pollen tubes of many species deposit callose as an inner wall layer during growth in culture (de Nettancourt, 1974; Cresti et al., 1980, 1985; Herrero and Dickinson, 1981; Rae et al., 1985). Pollen tubes of *Nicotiana* rapidly extending in an optimized culture medium have a morphology resembling that of tubes growing through compatible styles and produce an even layer of callose behind their growing tips as well as regular transverse callose plugs (Read et al., 1993b). Indeed, callose constitutes over 80% of the wall carbohydrate of cultured pollen tubes of *Nicotiana*, and the rate of callose deposition in the region behind the growing tip is extremely high and comparable with the rate of cellulose synthesis in the secondary wall of cotton fibers (Schlüpmann et al., 1994).

Bell interprets the evolution of multiallelic SI loci in angiosperms from recognition loci in lower plants by reference to the sixth "cardinal principle of morphology," that of metamorphosis by transformation (Ganong, 1901). The function and disposition of callose in pollen tubes could also be viewed in these terms. Callose could be considered to have been transformed from a role as an intergenerational barrier to a function in radial strengthening of the older portions of the pollen tube wall, with the transverse callose plugs allowing turgor pressure to be maintained at the growing tip. Consistent with this role, the developmentally regulated callose synthase in pollen tubes, in contrast with the enzyme responsible for callose deposition on cell perturbation, does not require activation by the cellular response signal of Ca^{2+} ions (Schlüpmann et al., 1993).

The Role of Carbohydrates in SI Signaling

The dominant role of polysaccharides and glycoproteins in forming cell wall barriers, together with the proposed informational role of glycoproteins and oligosaccharides in other cell–cell signaling pathways, led Bell (1995) to propose that carbohydrates are involved in the recognition reaction of SI. A specific role for carbohydrates in signaling in SI, involving stylar S-lectins and pollen S-glycosyl transferases, was also suggested by Heslop-Harrison (1983); alternatively, a stylar S-glycosidase could produce an allele-specific oligosaccharide that acts as a ligand and activates the corresponding pollen S-receptor.

The secreted products of the *S* locus in different groups of angiosperms are indeed glycoproteins, but this is not surprising, because almost all secreted proteins in animals and plants are glycosylated. There is also direct evidence against a specific role for glycosyl groups in SI. First, the carbohydrate side-chains attached to S-proteins in two unrelated SI species (*Petunia inflata* and *Papaver rhoeas*) are not required for pollen inhibition or rejection (Foote et al., 1994; Karunanandaa et al, 1994). Second, there are no significant differences between the structure of glycosyl chains attached to different alleles of the S-RNases of *N. alata* (Woodward et al., 1992). The glycoprotein nature of the interacting molecules is thus not of itself sufficient evidence to support an informational role for carbohydrates in the SI response.

Current models for SI derive instead from other conserved structural features of these genes and their products. Concerning the Solanaceae, Bell asserts that "the RNase activity of the S-RNases is, in the context of incompatibility, irrelevant," but the molecular evidence points to a contrary view. The RNase active site residues are strikingly conserved against a background of sequence that is otherwise extremely divergent between alleles (Tsai et al., 1992). Furthermore, *P. inflata* plants transformed with DNA constructs encoding an inactive form of the S_3-RNase fail to reject pollen carrying the S_3 allele, whereas plants transformed with DNA constructs encoding the unmodified S_3-RNase reject this pollen (Huang et al., 1994). Kowyama et al. (1994) also demonstrated that a nonfunctional *S* allele present in a normally self-incompatible species of *Lycopersicon* encodes an inactive RNase. Indeed, RNase activity probably plays a central role in the mechanism of pollen tube arrest (McClure et al., 1990). In the Brassicaceae, the structural features of the SLG and SRK proteins have led to the proposal that signaling involves the binding of peptide ligands to extracellular receptors (Nasrallah and Nasrallah, 1993). The nature of the mechanism of allelic recognition in the Papaveraceae and Poaceae is still unclear, but there is also no evidence in these families to support the idea of the primary role of carbohydrates in cell–cell signaling in SI.

Conclusions

The radiation of the angiosperms closely followed their initial appearance, but it is difficult to establish the evolutionary forces that led to their success. A reduced

LETTERS TO THE EDITOR

and enclosed female gametophyte, competition between rapidly growing male gametophytes, the rise of insect pollinators and increased outcrossing, and a genetically determined system of incompatibility have all been cited as possible advantages of early angiosperms. The model proposed by Bell (1995) suggests that the evolution of SI from a preexisting gametophyte–sporophyte recognition system was the important step. We argue that, although no single SI system is as yet completely characterized, the current molecular data are not consistent with Bell's concept of a single origin of SI. The precise origins of the different SI systems, and their significance in the success and subsequent diversification of the various angiosperm lineages, therefore remain to be determined.

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Reply

The letter from Read et al. in response to my review article (Bell, 1995), which suggested an origin of self-incompatibility (SI) from a gametophyte–sporophyte antagonism persisting from the ancient land plants, raises many interesting points. I confine myself here to those that appear to be of greatest general interest.

The lack of sequence similarity in the genes of the *S* loci of the Solanaceae, Brassicaceae, Papaveraceae, and Poaceae does not invalidate the argument presented. It seems very likely that the angiosperms emerged not uniquely, as Adam and Eve in the Garden of Eden, but sporadically from a complex of gymnospermous seed plants in the Late Jurassic or very Early Cretaceous. It is not unreasonable to assume that, in the 250 or more million years of land plant evolution before the condition of angiospermy, typified by penetrative siphonogamy, was attained, variation had arisen in the genes determining the composition of the barriers separating the gametophytic and sporophytic generations. These variations would have been carried into the emergent angiosperms and would be expected to persist in current families. In addition, it is not wholly true to say that SI systems are unrelated at the molecular level; so far as the nature of the gene products is concerned, there are striking similarities (that is, glycoproteins play a conspicuous role). The notion that the genes involved in SI, basically an intergenerational response of a kind as old as the land plants themselves and probably already present in the antecedent algae (witness the thickened boundary of the endogametophytic zygote of *Coleochaete* [Delwiche et al., 1989]), were "independently recruited to their current function in reproduction" seems altogether bizarre.

Read et al. also point out that callose is unlikely to be a "cause" of SI, but my suggestion was not that callose causes SI but rather that its regular production in the SI response betrays an origin from an intergenerational reaction in which the isolating barriers (not confined to lower plants) are typically callosic. Incidentally, Elleman and Dickinson (1994) did not state that the production of callose was dependent on leakage from already inhibited tubes but that its production was promoted by leakage, as would be expected if the response was intergenerational. I accept that callose is produced by pollen tubes in culture, but, because callose tends to appear when a metabolizing cell is subjected to insult or injury (sieve tubes providing a familiar example), the production of a regular lattice, depending, in *Nicotiana*, on the presence of copper ions in the medium (Read et al., 1993), throws doubt on the presence of the lattice in these conditions representing the behavior of an unstressed tube. In compatible pollinations, stress may be provided by residual gametophyte/sporophyte antagonism. That the callose lattice may play a role in maintaining the structural integrity of the tube conforms with the general experience that natural selection customarily results in sequestered metabolic products being disposed in the most beneficial fashion; an example is the manner in which lignin is deposited in conducting elements.

The evidence for the RNase activity of the S-glycoproteins of *Nicotiana* being responsible for the elimination of the incompatible tube is unconvincing. It is true that the mutant S_3 allele created by Huang et al. (1994) both lacks RNase activity and is unable to confer on transgenic plants the ability to reject S_3 pollen, but the lack of pollen rejection could be due to a structural alteration rather than to the loss of RNase activity per se. That is, because of conformational change, the product of the altered S_3 allele may fail to dimerize with the stylar partner, so preventing the release of an elicitor. The situation will become clearer when the nature of the pollen product that interacts with the "S-RNase" is known. Further, confidence in the RNAse hypothesis in its simple form is weakened by the cytology of arrested tubes, which give no evidence of RNase digestion. The conspicuous density of ribosomes in these tubes and the pycnotic nuclei are features suggestive of the activation of an apoptotic program. The nature of the agent that brings about self-destruction is so far unknown. It is presumably effective also in poppy, in which the glycoproteins involved are not ribonucleases.

Recognition of the role of carbohydrates in SI is of course not new. What is new