Klein, Ed. (Iowa State Univ. Press, Ames, 1961), pp. 489–501.
21. ———, in *S. E. B. Seminar Series,* A. Crozier and J. R. Hillman, Eds. (Cambridge Univ. Press, Cambridge, 1984), vol. 3, pp. 17–41.
22. GA_3 is commonly used in most studies with exogenous GA treatment because it is commercially available. In most cases, GA_3 elicits the same biological response as GA_1.
23. N. H. Nickerson, *Ann. Mo. Bot. Gard.* **46**, 19 (1959).
24. D. J. B. Hansen, S. K. Bellman, R. M. Sacher, *Crop Sci.* **16**, 371 (1976).
25. H. N. Krishnamoorthy and A. R. Talukdar, *Z. Pflanzenphysiol.* **79**, 91 (1976).
26. S. B. Rood, R. P. Pharis, D. J. Major, *Plant Physiol.* **66**, 793 (1980).
27. V. R. Bommineni and R. I. Greyson, *Sex Plant Reprod.* **3**, 109 (1990).
28. ———, *Plant Sci.* **68**, 239 (1990).
29. D. F. Jones, *J. Hered.* **16**, 339 (1925).
30. B. Veit *et al.*, *Dev. Suppl.* **1**, 105 (1991).
31. E. E. Irish, J. A. Langdale, T. M. Nelson, *Dev. Genet.* **15**, 155 (1994).
32. R. A. Emerson, *J. Hered.* **11**, 65 (1920).
33. N. H. Nickerson and E. E. Dale, *Ann. Mo. Bot. Gard.* **42**, 195 (1955).
34. E. E. Irish and T. Nelson, *Plant Cell* **1**, 737 (1989).
35. A. DeLong, A. Calderon-Urrea, S. L. Dellaporta, *Cell* **74**, 757 (1993).
36. R. K. Dawe and M. Freeling, *Dev. Biol.* **142**, 233 (1990).
37. D. F. Jones, *Proc. Int. Congr. Genet.* **2**, 104 (1932).
38. C. Mariani, M. De Beukeleer, M. Truettner, J. Leemans, R. B. Goldberg, *Nature* **347**, 737 (1990).
39. C. Mariani *et al.*, *ibid.* **357**, 384 (1992).
40. R. S. Poethig, in *Maize for Biological Research,* W. F. Sheridan, Ed. (Plant Molecular Biology Association, Grand Forks, MI, 1982), pp. 9–18.
41. P. C. Cheng, personal communication.
42. Supported by a grant to S.L.D. from NIH (GM38148). A.C.U. is a recipient of a Fellowship from The Rockefeller Foundation. The artwork in Fig. 1 by M. Moreno, the helpful discussions with A. Talo, T. Nelson, E. Irish, R. S. Poethig, and critical reading by B. May, M. Ronemus, and T. Brutnell are all gratefully acknowledged. We also thank P. C. Cheng for providing Fig. 2B.

Signaling the Arrest of Pollen Tube Development in Self-Incompatible Plants

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Self-incompatibility (SI), the cellular recognition system that limits inbreeding, has served as a paradigm for the study of cell-to-cell communication in plants since the phenomenon was first described by Darwin. Recent studies indicate that SI is achieved by diverse molecular mechanisms in different plant species. In the mustard family, the mechanism of SI shows parallels to the signaling systems found in animals that are mediated by cell-surface receptors with signal-transducing protein kinase activity.

The ability to distinguish between self and nonself and to allow only legitimate cellular unions during fertilization is an essential component of sexual reproduction in plants as in other organisms. However, in flowering plants, fertilization is preceded by a series of cell-cell interactive events dictated by the anatomy of the reproductive structures (Fig. 1A). Pollen grains are released from the anther in a desiccated state. They will hydrate and germinate to produce a pollen tube when delivered to the appropriate cell surface, which is generally that of the specialized epidermal cells of the stigma, a structure located at the distal end of the pistil. In species with solid pistils, the pollen tube, which extends by tip growth, must then invade the pistil and grow in the intercellular matrix of the central transmitting tissue of the stigma, style, and ovary before gaining access to the ovule (Fig. 1B) and delivering its cargo of sperm cells that will effect the double fertilization events unique to plants (*1*).

The pistil acts as an efficient screen of pollen grains, promoting the germination and growth of "appropriate" pollen tubes but not of "inappropriate" pollen tubes. Thus, gametes are preserved by interspecific pollination barriers that prevent the activation or growth of foreign pollen that is indiscriminately disseminated by wind, insect, or animal pollinators. Because the proximity of pistil and anther in plants with perfect flowers renders self-pollination more likely than cross-pollination, intraspecific barriers to pollination have also evolved, presumably as a means to avoid the deleterious effects of inbreeding and to promote outbreeding. One class of such barriers, namely genetic self-incompatibility (SI), is a prezygotic barrier that is widely distributed in both dicots and monocots. In self-incompatible plants, highly polymorphic loci control the ability of the pistil to inhibit the germination or subsequent development of self-related, but not genetically unrelated, pollen. SI systems can vary greatly between plant families with respect to site of pollen inhibition (in stigma, style, or ovary) and mode of genetic control (by one or more loci; sporophytic or gametophytic control of pollen phenotype) (*2*).

The specificity of self-incompatible pollen-pistil interactions has provided plant biologists with the opportunity to investigate the basis of plant cell-cell communication. In this article, we present an overview of our current understanding of the molecular basis and diversity of genetic SI systems, with a particular emphasis on the SI system of the crucifer (mustard) family. A consideration of the underlying molecular mechanisms is not only critical to the understanding of plant reproduction, but is also likely to shed light on the ways in which a plant cell interacts with neighboring plant cells during differentiation and morphogenesis, as well as with pathogens and symbionts.

Pollination Responses and Self-Incompatibility in Crucifers

The sequence of postpollination events that lead to successful fertilization is conserved in all cruciferous plants (*3*, *4*), whether they are members of self-compatible genera in which no SI has been reported, such as *Arabidopsis*, or of largely self-incompatible genera, such as *Brassica*. In *Arabidopsis*, the four phases of pollen tube growth proceed at an astoundingly rapid rate, and the pollen tube reaches the ovary 2 hours after the pollen grain is delivered to the stigma surface (Fig. 1B). Particularly rapid are the very early events that take place at the pollen–papillar cell interface, including the establishment of a zone of adhesion between the pollen grain and the "dry" surface of a papillar cell (Fig. 2A), hydration of the pollen grain, establishment of polarity within the pollen grain in preparation for germination (Fig. 2B), tip growth and elaboration of the pollen tube, and ingress of the pollen tube into the papillar cell wall, presumably through the action of hydrolytic enzymes located at the pollen tube tip.

It is during this one-on-one interaction between a pollen grain and papillar cell that the SI reaction is manifested: A pollen tube is either not formed or is unable to invade the papillar cell wall. The SI reaction is highly localized to the site of contact, because in mixed pollinations of a papillar cell with incompatible and compatible pollen grains, the latter are not arrested (*5*).

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Receptor-Mediated Cell-Cell Signaling in the Recognition of Self

The specificity of the incompatible pollen-stigma interaction in crucifers is controlled by a single, highly polymorphic Mendelian locus, the *S* locus: Inhibition of pollen occurs when the same allele of the *S* locus is active in the pistil and during pollen development, whether pistil and pollen are derived from the same plant or from different plants that carry the same allele. However, this apparently simple genetic basis belies the underlying complexity of the phenomenon and its controlling locus (6). Recent molecular analyses of the *S*-locus region in *Brassica* have identified a region of ~200 kb containing several physically linked transcriptional units that cosegregate perfectly with SI phenotype (7, 8) and that comprise an "S haplotype." A subset of genes within the *S*-locus complex is highly polymorphic, and specific combinations of allelic forms of each of these genes may define different SI specificities. In this respect, the *Brassica S* locus is similar to other complex recognition loci, such as the major histocompatibility complex of mammals that comprises many genes, some polymorphic and others not, all involved in the immune response.

Two of the highly polymorphic *S*-locus genes represent a pair of related but not identical genes. The *S*-locus receptor kinase (*SRK*) gene is predicted to encode a transmembrane serine-threonine protein kinase (7) with structural similarity to the receptor protein kinases that have been well characterized in animal systems. The *S*-locus glycoprotein (*SLG*) gene encodes an abundant glycoprotein that is secreted into the papillar cell wall. *SLG* exhibits a high degree of sequence identity to the extracellular domain of *SRK* and was apparently generated by duplication of the *SRK* gene. The *SLG-SRK* gene pair can diverge by >30% between different *S* haplotypes; however, within an *S* haplotype, SLG and the extracellular domain of SRK are >90% identical, suggesting that the two genes coevolve. The two genes are coordinately regulated (9, 10), and the stigmatic papillar cells are the major site of *SRK* (Fig. 3) and *SLG* (10) promoter activity. Both promoters also exhibit a lower level of activity in anthers (9, 10), although the significance of this activity is not clear. In contrast, there is strong genetic evidence that the expression of the *SLG* and *SRK* genes in papillar cells is required for the operation of SI. Several self-compatible mutant strains of *Brassica* have been identified in which self-compatibility is associated with spontaneous mutations at the *S* locus that disrupt the *SRK* gene (11, 12). In addition, spontaneous mutations at loci unlinked to the *S* locus that down-regulate the *SLG* gene (13), and transgene-induced mutations that down-regulate the *SLG* (14) and *SRK* genes (15), are associated with the loss of the pistil's ability to inhibit self pollen.

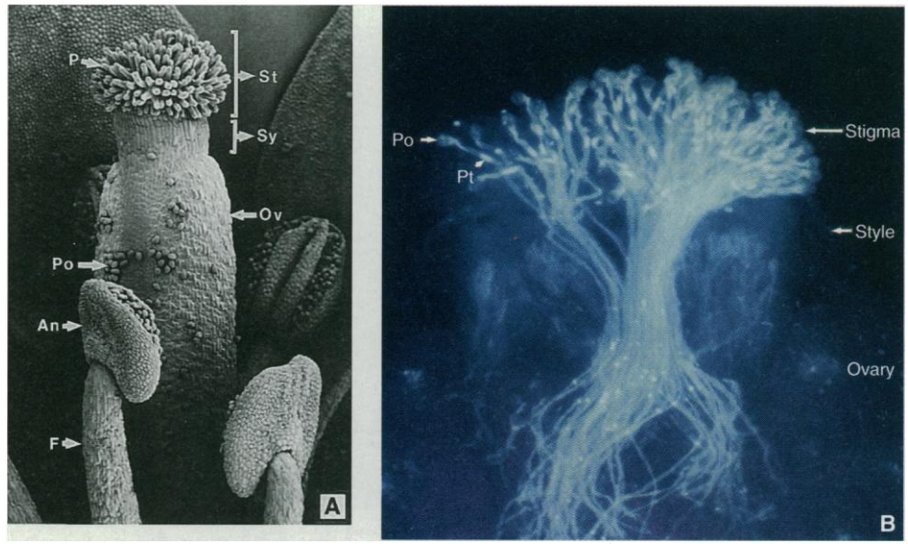

Fig. 1. Reproductive structures and pollination in crucifers. The scanning electron micrograph (**A**) shows an *Arabidopsis* flower at 75× magnification. The female reproductive structure, the pistil, consists of the stigma (St) crowned by papillar cells (P), the style (Sy), and the ovary (Ov). The male reproductive structure, the stamen, consists of the filament (F) and anther (An) shown here releasing pollen grains (Po). The ultraviolet-fluorescence micrograph (**B**) shows the path of pollen tube growth in the crucifer pistil. A pollinated *Arabidopsis* pistil was treated with decolorized aniline blue to stain the pollen tubes (Pt). Adhesion of the pollen grain to the stigma surface as well as the establishment of polarity and pattern of tip growth occur within 15 min after pollen comes in contact with the stigma. The next 25 min are characterized by pollen tube growth within the papillar cell wall, and the following 60 min by basipetally oriented intercellular growth in the central region of the stigma, style, and ovary. Once in the ovary, the pollen tubes fan outward as they are targeted toward the ovules. A whole mount of a 1-mm upper segment of the pistil is shown in (B).

Although the *SLG* and *SRK* genes are necessary, it is not clear if they are sufficient for the operation of the SI response in the pistil. Attempts to modify SI specificity by transformation experiments have so far been unsuccessful, largely as a result of transgene-induced cosuppression (14, 15). Nevertheless, the data are consistent with a mechanism based in the papillar cell whereby the SRK protein kinase is activated by contact between a papillar cell and self pollen (Fig. 4). By phosphorylating intracellular substrates, the SRK protein would couple the initial molecular recognition events at the papillar cell–pollen interface to the signal-transduction chain that leads ultimately to pollen rejection. Because SLG and SRK are both expressed in papillar cells in the absence of pollen, a pollen-borne component, possibly a ligand for SRK, is postulated (Fig. 4). Such an extracellular ligand would be highly polymorphic and encoded within the *S*-locus complex. It would activate the receptor in a haplotype-specific manner, thus providing the specificity inherent in "self" recognition. SLG, which is freely diffusible in the cell wall,

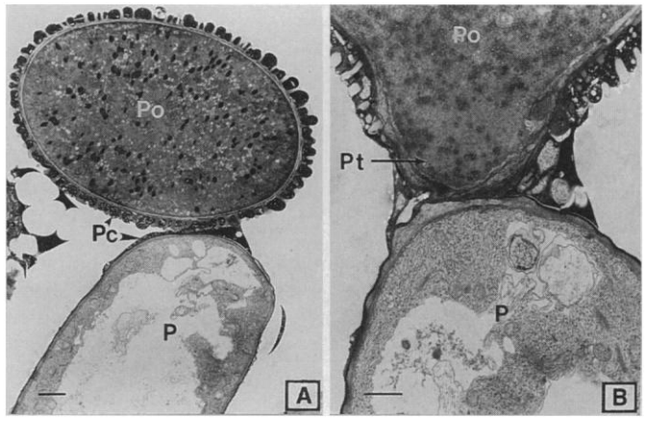

Fig. 2. Early pollination events at the pollen-papillar cell interface in *Arabidopsis*. The transmission electron micrographs show (**A**) the formation of an adhesion zone derived from pollen coat, evident 5 min after pollination, and (**B**) the establishment of polarity and the initiation of tip growth 10 min after pollination. P, papillar cell; Po, pollen grain; Pc, pollen coat; and Pt, emerging pollen tube. Bars, 2 μm (A) and 1 μm (B).

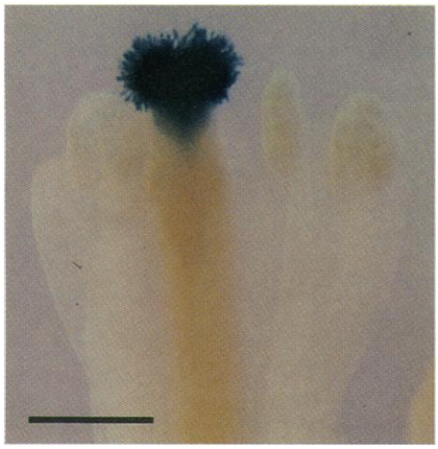

Fig. 3. Localization of *SRK* promoter activity to the papillar cells of the stigma. *Arabidopsis* was transformed with a chimeric gene consisting of a 450–base pair *SRK* promoter fragment fused to the β-glucuronidase reporter gene (*9*). The transgenic flower shows intense blue staining of papillar cells after histochemical staining for β-glucuronidase activity. Bar, 600 μm.

would be essential, either by acting as an extracellular regulator of ligand access to the signaling receptor or by being an integral part of a functional receptor complex.

The operation of a signaling receptor is consistent with the rapidity of the SI reaction in crucifers and the inhibition of pollen at the papillar cell surface. Because of the highly localized nature of the SI response, it is anticipated that the signal-transduction pathway initiated by the activation of SRK is spatially limited to the region immediately subjacent to the incompatible pollen grain. Self-pollination may induce the localized release by the papillar cell of a preformed or newly synthesized pollen-inhibitory susbstance (*16*). Alternatively, stigmatic wall components such as "adhesion factors" required for interaction with the pollen coat may be modified or inactivated (Fig. 4). The existence of such factors has been predicted by microscopic observations of early pollination events in crucifers (*4*) and by studies in which adhesion was inhibited on stigmatic surfaces treated with protease (*17*). We propose that once adhesion, which may be a prerequisite for the establishment of cellular polarity in the pollen grain, is inhibited or weakened, the initiation of normal tip growth and the directed growth of the pollen tube into the papillar cell wall would be aborted.

Relation to Other Self-Incompatibility Systems

Does receptor-mediated signaling operate in other SI systems as well? A definitive answer is not available, as only a few SI systems have been analyzed at the molecular or biochemical levels. The involvement of kinase-based mechanisms has been suggested by biochemical studies in two species that also exhibit stigmatic inhibition: *Papaver* (poppy), where a calcium-mediated signaling pathway is apparently activated in pollen by an SI-specific stigmatic glycoprotein (*18*); and *Secale* (rye), where the SI response is inhibited by treatment of pistils with protein kinase inhibitors (*19*).

A very different mechanism has been proposed for the extensively studied SI system of the Solanaceae (the nightshade family, which includes ornamental *Nicotiana* and *Petunia*), and also for the Rosaceae (the rose family, which includes fruit trees) and the Scrophulariaceae (the snapdragon family) (*20*). In these families, the SI reaction occurs within the style after pollen germination and pollen tube ingress into the pistil. The arrest of pollen tube growth and bursting of pollen tube tips observed after an incompatible pollination has been postulated to result from the cytotoxic action of a class of polymorphic nonspecific ribonucleases (RNases), called S-RNases because they are associated with the single multiallelic SI locus (*20*). S-RNases are secreted into the intercellular matrix within the styles and are presumably taken up into the pollen tube where they degrade cytoplasmic RNA (*20*). Transgenic experiments reporting on loss and gain of the pistil SI response in *Nicotiana* and *Petunia* by transformation with antisense and sense S-RNase genes (*21*) suggest that the S-RNase is the sole determinant of SI specificity in the pistil, although rigorous progeny analysis demonstrating the cosegregation of the modified SI response with the transgene is as yet unavailable. In addition, and as in the *Brassica* system, information relating to the nature of the pollen component of the SI response is lacking, and the basis of allelic specificity is not understood. A favored hypothesis is based on the operation of an allele-specific translocator system that would operate in pollen tubes to allow the uptake of self RNase but not of nonself RNase. The identification of the relevant pollen molecules is clearly crucial and will no doubt shed light on some puzzling observations, such as the detection of S-RNase transcripts and proteins in pollen (*22*) and the apparently nonspecific uptake of nonself as well as self S-RNase by pollen tubes grown in vitro (*23*).

Thus, the current data favor the conclusion that SI evolved several times during the expansion of the angiosperms. Although it is not possible to estimate how many different mechanisms of intraspecific pollen inhibition might exist, at least two broad classes have emerged: One class, based on the operation of protein kinases within cells of the pistil or pollen, may operate in species with a rapid stigmatic inhibition of pollen; another class, based on cytotoxic effects, may be characteristic of species in which inhibition is delayed until after the pollen tube has grown into the pistil.

Relation to Other Cellular Communication Phenomena in Plants

Another important evolutionary question has to do with the origin of SI within each plant family. It is likely that the genes involved in these highly specific pollen-pistil interactions were recruited from genes that function in other plant processes. An often formulated hypothesis relates SI to host-pathogen interactions because of parallels between pollen rejection and the inhibition of invading pathogens (*24*). The recent cloning from tomato of a cytoplasmic serine-threonine kinase involved in disease resistance may be taken as support for the evolutionary relatedness of host-pathogen responses to the *Brassica* SI system, but could simply reflect the requirements for a rapid response in the two systems. An alternative hypothesis is suggested from the analysis of several recently identi-

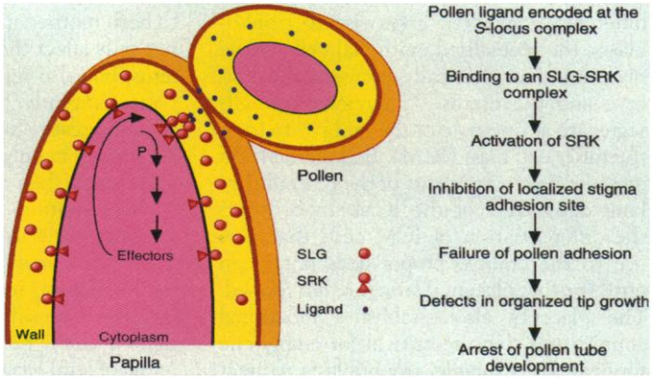

Fig. 4. A model of papillar cell–based signaling in the SI response of *Brassica*. The diagram shows the localization of the SLG and SRK molecules at the papillar cell surface. A phosphorylation cascade is postulated to be precipitated within the papillar cell by the binding of a pollen-borne ligand to the receptor. The sequence of events proposed to culminate in localized modification of the papillar cell surface and the arrest of pollen tube development is outlined to the right.

fied *SLG*- and *SRK*-related genes. Sequences encoded by this family of genes have been cloned from diverse plants and tissues, such as maize roots, carrot cell cultures, and *Arabidopsis* shoots and roots (6). The cell type–specific pattern of expression exhibited by the vegetatively expressed *SRK*-like genes of *Arabidopsis* (25) is consistent with the hypothesis that the *Brassica S*-locus genes are derived from ancestral genes having a very basic role in intercellular communication during plant development.

Characterization of these genes will determine how far the lessons learned from the study of SI can be applied to other plant systems. This question is of more than academic interest, for it will determine if the mechanism by which a plant controls the formation and growth of a pollen tube bears similarity to how it controls the growth of invading pathogens or the growth of its own cells in various phases of its development. Thus, further refinement of the mechanism of SI, including identification of activators and targets of the stigmatic proteins, will be important for understanding the fundamental nature of cell-cell signaling during the development of the plant body.

REFERENCES AND NOTES

1. C. Dumas and H. L. Mogensen, *Plant Cell* **5**, 1337 (1993).
2. J. Heslop-Harrison, *Proc. R. Soc. London Ser. B* **190**, 275 (1975).
3. J. P. Hill and E. M. Lord, *Am. J. Bot.* **74**, 988 (1987); C. J. Elleman, V. Franklin-Tong, H. G. Dickinson, *New Phytol.* **121**, 413 (1992).
4. M. K. Kandasamy, J. B. Nasrallah, M. E. Nasrallah, *Development*, in press.
5. R. H. Sarker, C. J. Elleman, H. G. Dickinson, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4340 (1988).
6. J. B. Nasrallah and M. E. Nasrallah, *Plant Cell* **5**, 1325 (1993).
7. J. C. Stein, B. Howlett, D. C. Boyes, M. E. Nasrallah, J. B. Nasrallah, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8816 (1991).
8. D. C. Boyes and J. B. Nasrallah, *Mol. Gen. Genet.* **236**, 369 (1993).
9. J. C. Stein, thesis, Cornell University (1994).
10. T. Sato *et al.*, *Plant Cell* **3**, 867 (1991); K. Toriyama, M. K. Thorsness, J. B. Nasrallah, M. E. Nasrallah, *Dev. Biol.* **143**, 427 (1991).
11. D. R. Goring, T. L. Glavin, U. Schafer, S. J. Rothstein, *Plant Cell* **5**, 531 (1993).
12. J. B. Nasrallah, S. J. Rundle, M. E. Nasrallah, *Plant J.* **5**, 373 (1994).
13. M. E. Nasrallah, *Genetics* **76**, 45 (1974); ———, M. K. Kandasamy, J. B. Nasrallah, *Plant J.* **2**, 497 (1992).
14. K. Toriyama, J. C. Stein, M. E. Nasrallah, J. B. Nasrallah, *Theor. Appl. Genet.* **81**, 769 (1991).
15. T. Tantikanjana, thesis, Cornell University (1994).
16. T. Hodgkin and G. D. Lyon, *J. Exp. Bot.* **37**, 406 (1986).
17. A. D. Stead, I. N. Roberts, H. G. Dickinson, *J. Cell Sci.* **42**, 417 (1980).
18. V. E. Franklin-Tong and F. C. H. Franklin, *Trends Cell Biol.* **3**, 340 (1993).
19. P. Wehling, B. Hackauf, G. Wricke, *Sex. Plant Reprod.* **7**, 67 (1994).
20. E. Newbigin, M. A. Anderson, A. E. Clarke, *Plant Cell* **5**, 1315 (1993).
21. H.-S. Lee, S. Huang, T.-H. Kao, *Nature* **367**, 560 (1994); J. Murfett, T. L. Atherton, B. Mou, C. S. Gasser, B. A. McClure, *ibid.*, p. 563.
22. P. N. Dodds *et al.*, *Plant Cell* **5**, 1771 (1993).
23. J. E. Gray, B. A. McClure, I. Bonig, M. A. Anderson, A. E. Clarke, *ibid.* **3**, 271 (1991).
24. C. J. Lamb, *Cell* **76**, 419 (1994).
25. K. G. Dwyer *et al.*, *Plant Cell, in press.*

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Implantation and the Placenta: Key Pieces of the Development Puzzle

James C. Cross,* Zena Werb, Susan J. Fisher

The mammalian embryo cannot develop without the placenta. Its specialized cells (trophoblast, endoderm, and extraembryonic mesoderm) form early in development. They attach the embryo to the uterus (implantation) and form vascular connections necessary for nutrient transport. In addition, the placenta redirects maternal endocrine, immune, and metabolic functions to the embryo's advantage. These complex activities are sensitive to disruption, as shown by the high incidence of early embryonic mortality and pregnancy diseases in humans, as well as the numerous peri-implantation lethal mutations in mice. Integration of molecular and developmental approaches has recently produced insights into the molecules that control these processes.

In a remarkable series of events, implantation and placental development physically connect the mammalian embryo to its mother. Establishing this connection is the embryo's first priority, which is essential for its subsequent development. The importance of this simple fact is often overlooked, but is underscored by the temporal sequence in which the differentiated embryonic cell types appear. In mammals, the initial developmental decisions set aside three unique extraembryonic lineages that are the precursors of the placenta. The first differentiation event gives rise to trophoblasts, the specialized epithelial cells of the placenta that physically connect the embryo and the uterus. The remaining cells segregate at one pole of the embryo to form the inner cell mass (ICM). Endodermal and mesodermal components of the placenta are later derivatives of the ICM. In contrast, the differentiation of ICM cells that give rise to the embryo proper does not begin until the first placental structure has formed. The placenta also establishes functional connections that are critical for embryonic survival. For example, trophoblasts redirect the maternal endocrine system to create the hormonal milieu that directs changes in the uterus necessary for pregnancy to continue. Trophoblasts express paternal proteins and interact directly with maternal immune cells but somehow avoid rejection. Another critical step is the establishment of a hybrid vasculature in which the fetal trophoblasts, acting like endothelial cells, are in direct contact with maternal blood, where they transport nutrients and gases.

Failures in implantation and placental development are clinically important. About one-third of normal human pregnancies end in spontaneous abortion; 22% of such abortions occur before pregnancy is detected clinically (*1*). Similarly, failures in development during the peri-implantation period account for almost 80% of the embryonic loss that occurs in farm animal species (*2*, *3*). Even seemingly minor defects in placentation can have severe negative consequences. In humans, for example, abnormalities in the vascular connections result in preeclampsia, a disease of pregnancy with significant morbidity and mortality to both mother and fetus (*4*). Such disorders not only affect the health of the mother and fetus, but also represent significant societal costs. Currently, the approaches for diagnosis and treatment of diseases of pregnancy are limited mainly because of our inability to understand their causes.

Implantation and development of the placenta occur in a stepwise manner (Table 1). Recent analyses of naturally occurring mouse mutants and several mutants created by gene targeting experiments have highlighted these processes as major determinants of fetal growth and development (Table 2). The important conclusion from

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