Development 1993 Supplement, 77-84 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Molecular genetics of cell interactions in *Arabidopsis*

Robert E. Pruitt[1], Martin Hülskamp[1], Steven D. Kopczak[1], Sara E. Ploense[2] and Kay Schneitz[1]

[1]Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138, USA
[2]Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108, USA

SUMMARY

Many events in plant development are regulated by the interactions of neighboring cells. We are interested in determining what sorts of molecules act as signals and/or receptors in these interactions and how these mechanisms relate to those used in animals and fungi. We are presently working on two different types of systems to try to address this question. In one case we are starting at the molecular level and characterizing a family of receptor protein kinase genes which seem natural candidates for mediating cellular interactions. By analyzing the expression patterns of these genes as well as the phenotypes of transgenic plants bearing altered genes we hope to determine what roles these proteins play in plant development. In the second case we are starting from the organismic level and using genetics to identify genes essential to a whole range of cellular interactions which are required for proper male gametophyte development during reproduction. These interactions involve both recognition of the pollen grain to verify that it is from the correct species and also a transfer of positional information from the female to the male which first allows the pollen tube to determine the polarity of the stigmatic cell on which it has germinated and later provides 'guidance' for the elongating tube to find the ovule.

Key words: receptor tyrosine kinase, plant reproduction, pollen tube growth

INTRODUCTION

As multicellular organisms develop, their constituent cells must undergo a decision making process as they adopt their mature fates. Traditionally these decisions have been viewed as based on two types of information: information derived from cell lineage and information derived from intercellular communication. Because plant cells are encased in rigid cell walls, opportunity obviously exists for development to be based strictly on cell lineage. However, the plasticity of plant development, in particular the ability of many types of cells to regenerate the whole organism in tissue culture, argues in favor of cell-cell interactions playing an important role in regulating cell fates in development. In fact, many experiments, both classical and modern, point to the importance of cellular interactions in regulating the development of plants.

Much of the development of the adult plant is in fact due to the production of a reiterated series of organs (leaves, flowers, etc.) from a group of initial cells located at the growing apex of the plant and referred to collectively as a meristem. Individual organ primordia are produced as buttresses on the flanks of the meristem and these then differentiate into the mature organs. The cells within the meristem are organized as layers: one or more layers of single cell thickness (the tunica) and then a mass of internal cells (the corpus). Observations on experimentally induced polyploids indicate that these different layers represent populations of cells which tend to remain isolated from one another (Satina et al., 1940). This type of experiment indicates that typical meristems consist of three such cellular compartments, typically designated L1, L2 and L3 beginning with the outermost layer. The fact that these cell layers tend to remain distinct allows the formation of plants where the different cell layers are of different genetic constitutions, and these plants are known as periclinal chimeras. These chimeras can be constructed between cells derived from the same species but of different genotypes, or they can be constructed between plants of different species. Using genetically marked periclinal chimeras, it is possible to follow the fates of cells which are displaced from one cell layer to another by a cellular division perpendicular to the cell layer. These cells have the same fates as cells in the layer which they are displaced into, demonstrating that their fate is determined not by their lineage but by their position, which is presumably determined by intercellular communication (reviewed by Tilney-Bassett, 1986).

Other experiments have been used to demonstrate more directly that cell fates in different layers must be influenced by cellular communication between layers. The mutation *lateral suppressor* of tomato results in flowers which lack petals due to a failure of these organ primordia to be initiated. In a periclinal chimera where the L1 cells bore this mutation and the L2 and L3 layers were wild type, flowers were produced which initiate and produce petals normally (Szymkowiak and Sussex, 1993). The use of other genetic markers in the analysis confirms that the petal epidermal cells are derived from the mutant L1 layer, indi-

cating that these mutant cells can adopt normal petal cell fates if neighboring cells provide appropriate signals for them to do so. Another example of the same type of phenomenon comes from the analysis of the *floricaula* mutation in *Antirrhinum*. This mutation results in the conversion of the flowers into leafy shoots. Using an unstable allele induced by a transposable element, Carpenter and Coen (1990; Coen et al., 1990) isolated plants that produced fertile flowers due to the reversion of the mutation. Examination of the progeny of these flowers revealed that in some cases the progeny were still homozygous for the mutation. Because the gametes of flowering plants are derived exclusively from the L2 layer, the most probable explanation of these results is that the reversion event leading to production of the flower occurred in either the L1 or the L3 layer, again demonstrating the idea that wild-type function of a gene controlling development need only take place in a subset of the cells making up the meristem. The remaining cells, which adopt fates different from those predicted by their genotypes, presumably do so based on intercellular signals directly or indirectly dependent on the wild-type cells.

Plant cells are also capable of altering their growth or fate depending on what type of cells they come into contact with. An obvious example of this is the growth of a pollen tube through the female reproductive system, which will be considered in more detail below. Another example is the postgenital fusion of carpels to form the ovary of some flowering plants (Walker, 1975). Experiments by Siegel and Verbeke (1989) have shown that the cells along the contacting edges of the two carpels are induced to dedifferentiate and then fuse by a diffusible substance. A similar effect is observed more dramatically in the *fiddlehead* mutation of *Arabidopsis* which has been described by Lolle et al. (1992). In plants homozygous for this mutation all epidermal cells are capable of undergoing fusion when they contact other epidermal cells. This leads to gross alterations in the morphology of the plants as seen in Fig. 1, which shows a cluster of floral buds which have all fused together.

Thus plant cells, like animal cells, respond developmentally to signals from neighboring cells in their environment. Little is known however about what types of signaling systems mediate these developmentally important interactions. Two approaches are outlined below which our laboratory is presently using to try to determine the molecular nature of these signals and their receptors. The first system involves a genetic analysis of the reproductive system of *Arabidopsis thaliana*. By isolating mutations that disrupt fertility without altering morphology we hope to identify genes that are involved in the cell signaling processes between the male and female reproductive systems. Subsequent characterization of these genes will provide molecular information about the types of signaling systems involved. The second system involves the characterization of a group of genes which encode receptor protein kinases; logical candidates for involvement in cell signaling systems. We hope to identify the roles of these genes in the biology of the organism using a combination of molecular biology and reverse genetics.

CELL-CELL INTERACTIONS IN THE FERTILIZATION PROCESS

The life cycle in plants is an alternation of diploid (sporophyte) and haploid (gametophyte) forms of the organisms. It is the male and female gametophyte that develop the gametes and procure the union of the two haploid gametes to form the zygote. Although the gametophytes are very much reduced in higher plants, their functions remain the same. The female gametophyte develops into the embryo sac, which usually consists of a seven celled structure including the actual egg cell. The male gametophyte is composed of the pollen grain, which upon pollination grows a pollen tube to deliver the sperm cells to effect fertilization. Although the botanical term 'fertilization' is defined as the actual fusion of the gametes we will refer to the whole process from the initial landing of the pollen grain on the stigma to the fertilization of the egg cell as the 'fertilization process'.

The process begins with the landing of a pollen grain on a stigmatic papillar cell. This triggers a water transfer from the papillar cell to the pollen grain resulting in the hydration of the pollen grain. This is a prerequisite for the germination and growth of the pollen tube. The pollen tube penetrates the stigma surface, enters a specialized tissue, the

Fig. 1. Photograph of a *fiddlehead* mutant plant. Note the compact appearance of the inflorescence due to the fusion of the flower buds.

transmitting tract, where it grows basally. Eventually it leaves the transmitting tract and emerges to its surface, where it grows across the surface towards the ovules. The tube grows up the funiculus, enters the micropyle and delivers the two sperm cells to the embryo sac. One of the sperm cells fuses with the egg cell while the second sperm cell fuses with the central cell, an event termed 'double fertilization'.

Thus, the fertilization process is a successive series of developmental steps that require a number of different cellular interactions between the male gametophyte and the sporophytic or female gametophytic tissues. As sketched in Fig. 2 different steps can be distinguished (Pruitt and Hülskamp, 1994). We will discuss these steps in turn with a special focus on the fertilization process in *Arabidopsis thaliana*.

STEPS IN THE FERTILIZATION PROCESS

The first step of the fertilization process is the recognition of the pollen grain by the stigmatic cell. The mature pollen grain is almost desiccated and needs to hydrate again before it can proceed with its development. Upon landing on the stigma the papillar cell releases water and thereby triggers the next step of pollen development, the germination of the pollen tube. The release of water by the papillar cell, however, is a highly regulated step rather than a passive osmotic water exchange. One line of evidence for this is

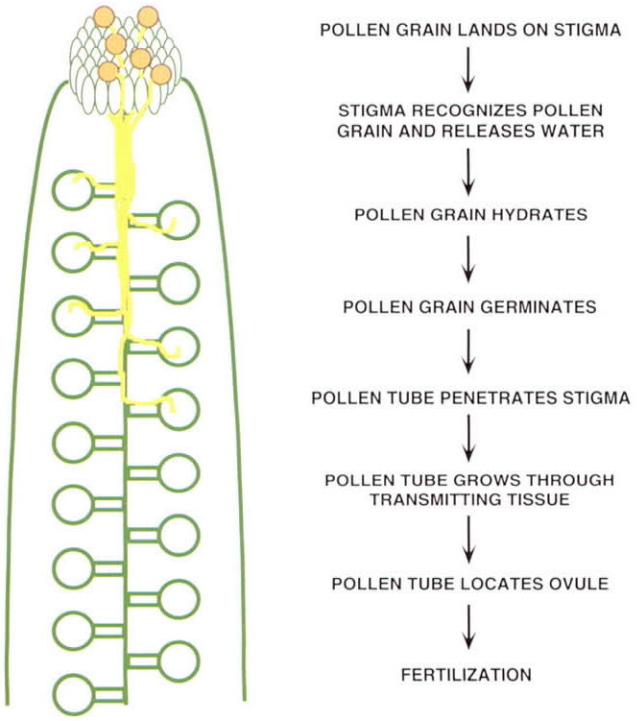

Fig. 2. Summary of the fertilization process. The left side shows a schematic drawing of the reproductive system of *Arabidopsis*. The right panel illustrates the various steps which take place during fertilization.

derived from the analysis of plant species that are self-incompatible. In such plant species, sporophytic self-incompatibility systems prevent self-fertilization and thus promote outcrossing. In some of these species (e.g. *Brassica*) pollen from genetically identical plants fails to hydrate. This implies, that the recognition and the resulting hydration of the pollen is the primary site of regulation. The molecular characterization of genes involved in the self-incompatible system of *Brassica oleracea* has revealed a number of genes which are thought to be involved in the recognition of the pollen grain by the papillar cell (reviewed by Nasrallah et al., 1991; Dzelzkalns et al., 1992). As detailed below, these genes encode receptor protein kinases, thus suggesting a recognition system that is based on specific signals and the corresponding receptor proteins.

However, the mechanisms found in species with a self-incompatibility system are only relevant generally if we assume that the steps blocked in the self-incompatible system are part of the normal fertilization process. The genetic analysis of the fertilization process in *Arabidopsis thaliana* has allowed the identification of mutants which superficially resemble the situation described for sporophytic self-incompatibility (Pruitt et al., 1991; Preuss et al., 1993; Pruitt et al., unpublished results). Pollen from these mutants fails to hydrate on the stigma surface (Fig. 3). This block to development, however, can be bypassed by increasing the humidity or by a mixed pollination with wild-type pollen, indicating that the pollen is specifically blocked only at the recognition step. Moreover, these data suggest that a specific recognition substance is normally provided on the outer surface of the pollen grain. Although nothing is known about the nature of this recognition substance some light is shed on these mutants by their pleiotropic phenotype. In addition to the hydration phenotype these mutants show an eceriferum phenotype: the outer wax layer which normally covers the epidermis of the whole plant is altered (Koornneef et al., 1989). Some eceriferum mutants also lack the outermost layer of the pollen grain, the tryphine layer, which is composed of lipids and proteins (Preuss et al., 1993). This observation opens the possibility that the hydration phenotype is either caused by the fact that lipids or waxes are required as an embedding medium for the recognition substance on the outer surface of the pollen grain or that a member of this class of molecules represents the actual signaling molecule.

As an immediate consequence of the hydration, the germination of the pollen tube is initiated. When pollen is germinated in vitro the first visible change in the pollen grain is the rearrangement of the actin microfilament network from a symmetric to an asymmetric distribution with a higher concentration of actin at the site where the pollen tube will emerge (reviewed by Pierson and Cresti, 1992). Thus the initially triaxially symmetrical pollen grain attains an asymmetry with respect to its inner architecture. The actual position of the point of emergence seems to be determined by the papillar cell. As a result, the pollen tube emerges from one of the three possible apertures of the pollen grain, choosing the one which most closely faces the papillar cell. Although nothing is known about the mechanism of the signaling it is obvious that the position of the papillar cell

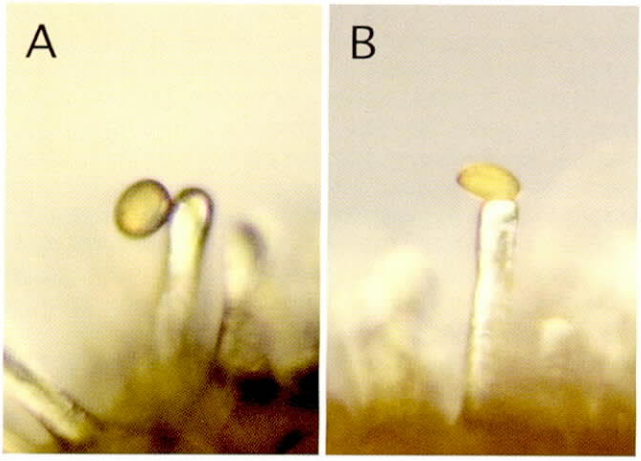

Fig. 3. Comparison of the hydration behaviour of wild-type pollen with respect to a hydration-mutant pollen. (A) Fully hydrated wild-type pollen located on a papillar cell. Note the balloon-like shape of the pollen. The furrows have disappeared. (B) A hydration-mutant pollen on a papillar cell. No hydration occurs and therefore the pollen keeps its ellipsoid shape.

relative to the pollen grain is used as a positional reference system to induce a polarity in the pollen grain.

The emerging pollen tube grows through the 'foot' of coating material and penetrates the stigmatic cuticle, where it enters the space between layer I and layer II of the primary cell wall (Elleman et al., 1992). The pollen tube grows basally and eventually enters the transmitting tissue, where it commences intercellular growth. Virtually nothing is known about the regulation of these early growth steps.

The micrograph shown in Fig. 4D shows an overview of the general path of pollen tube growth in the ovary. In this photograph an ovary is shown from which the ovary walls have been removed and the ovules folded to the side to allow a view of the surface of the transmitting tract. The pollen tubes were visualized by staining with aniline blue. As can be easily seen, the paths of the pollen tubes are complex and irregular. Despite the excess number of pollen tubes in the ovary, a single pollen tube can be seen associated with each ovule. The mechanisms that control the directional growth of the pollen tubes and result in their guidance to the ovules are poorly understood but will be discussed further below.

The growth pattern in the ovary can be subdivided into two phases : (1) intercellular growth in the transmitting tract and (2) growth on the surface of the transmitting tract. The initial growth inside the transmitting tract is characterized by a very straight growth of the pollen tube (Fig. 4A). Although little is known about the growth mode and the regulation of the directionality in *Arabidopsis thaliana*, some very interesting data are available for another crucifer, *Raphanus raphanistrum* (Sanders and Lord, 1989). Experiments of Sanders and Lord (1989) indicate that the growth force and thereby also the guidance for the pollen tube inside the transmitting tract is provided by the female sporophytic tissue. This is suggested by the finding that latex beads of a similar diameter to a pollen tube are translocated in the transmitting tissue at the same speed as pollen tubes would normally grow. According to a model by Sanders and Lord (1989) the pollen tube growth is governed by the interaction of the pollen tube with the extracellular matrix of the transmitting tract. They propose that the interaction between the pollen tube and the transmitting tract tissue is mediated by substrate adhesion molecules like vitronectin in an analogous way to that described by Dufour et al. (1988) for cell motility in animal cells.

Once the pollen tube emerges on the surface of the transmitting tract its growth behavior changes dramatically (Pruitt and Hülskamp, 1994; Hill and Lord, 1987). The micrograph shown in Fig. 4B shows the pollen tube growth pattern at the surface of the transmitting tract. The growth path is no longer straight as found inside the transmitting tract but now curves back and forth along its path. The growth direction appears initially random, but eventually the pollen tube is directed towards the funiculus and ultimately to the micropyle of the ovule (Fig. 4C). Three different models have been suggested to explain how the pollen tube is guided inside the ovary (reviewed by Heslop-Harrison, 1987). The first model predicts that the pollen tube is exclusively guided by the female reproductive system which pulls the pollen tube in the direction of the ovules. The second model postulates defined tracks of signaling molecules which lead individual pollen tubes to their destination. The third model explains the pollen tube guidance by a chemotactic signal which directs the pollen tube towards the ovules.

None of the three models easily account for the available data. It is conceivable, that the straight growth of the pollen tubes inside the transmitting tract can be explained by a pulling force provided by the surrounding tissue. However, the growth behavior outside the transmitting tract tends to support either the second or third model. The second type of model would easily explain the observed exclusion of other pollen tubes from the same path and thereby the observed limitation of one pollen tube per ovule. By the same token, the path of the pollen tube would be expected to be much more invariant than observed. The variability seen in the growth paths of the pollen tubes, however, would favor the third model in which the ovules would provide a chemotactic signal to guide pollen tube growth.

MOLECULAR APPROACH

As a complementary strategy to investigate intercellular communication in *Arabidopsis*, we have taken a molecular approach. We isolated a class of genes, named the Sigma genes, based on homology to a gene encoding the *S*-locus-specific glycoprotein (SLG) first identified in *Brassica* (Nasrallah et al., 1985). SLG is believed to be involved in the control of sporophytic self-incompatibility (for a recent review on self-incompatibility see Dzelzkalns et al., 1992).

Self-incompatibility is a common feature among many angiosperms. These systems ensure that only compatible pollen grains, i.e. of different genetic make-up than the female parent, are able to effect fertilization. This is thought to safeguard the genetic variability of the species and to be controlled by a different mechanism than inter-specific incompatibility (de Nettancourt, 1977). There are principally two different self-incompatibility systems, which are

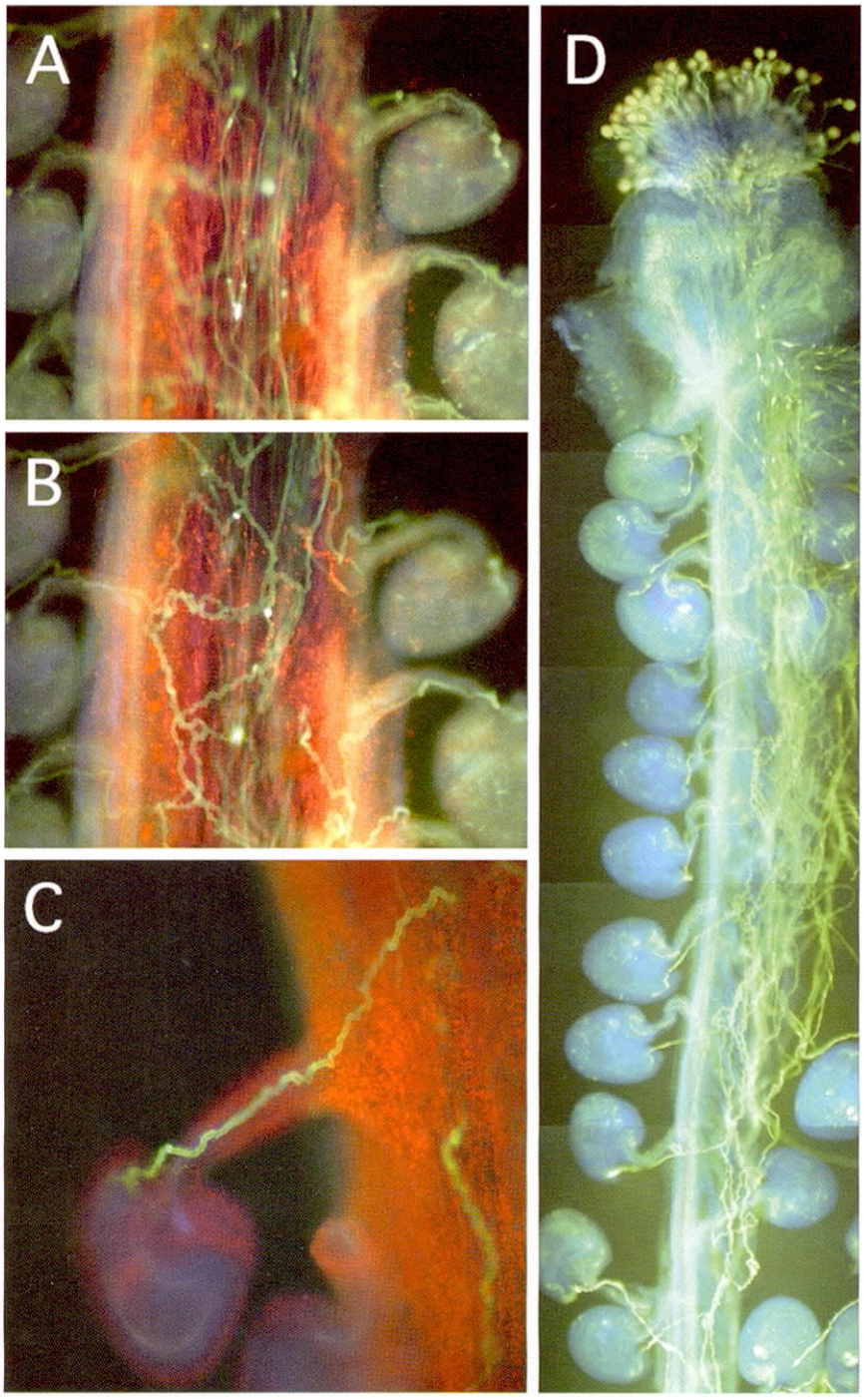

Fig. 4. Composite fluorescent micrograph. The path of the pollen tube in the ovary can be seen by the yellow-green fluorescence of the tubes. The ovary is dissected free and the single ovules are folded to the side of the transmitting tract. (A) View of a focal plane inside the transmitting tract. The pollen tubes appear as straigth lines. (B) Different focal plane than in (A) showing the zigzag path of the pollen tubes on the surface of the transmitting tract. (C) Same plane as in (B). The path of a single pollen tube can be followed from the point of its emergence on the outer surface of the transmitting tract to the micropyle of the ovule. (D) Overview of one half of an ovary is depicted. Pollen tubes grow from the papillar cells basally through the stigma and the transmitting tract. Each ovule is associated with a single pollen tube only. This figure has been previously published in the book *Arabidopsis* published by Cold Spring Harbor Laboratory Press and is reprinted with permission.

referred to as sporophytic and gametophytic. In the former process the identity of the individual pollen grains depends on the parental genotype, while in the latter system identity is determined by the genotype of the pollen grain itself. In both cases, genetic control of self-incompatibility resides in a single co-dominant locus, the *S*-locus. In a typical sporophytic self-incompatible reaction the pollen does not hydrate or only poorly hydrates on the papillar cell surface. Subsequent pollen tube germination is either absent or the elongation of the pollen tube is inhibited. These features are very reminiscent of some of the phenotypes shown by the hydration mutants, described above. *Arabidopsis thaliana* is a self-compatible plant. Nevertheless, due to the similarity of the phenotypes, it is reasonable to assume that putative SLG homologues might be involved in the early steps of the recognition process between the pollen and a stigmatic papillar cell in *Arabidopsis*.

In *Brassica*, the *S*-locus encompasses at least two genes, the SLG and the SRK genes (Nasrallah et al., 1985; Stein et al., 1991). The SLG protein is a secreted protein and can have different relative molecular masses in the range of 55-65×10^3. It carries a single so-called S-domain and is

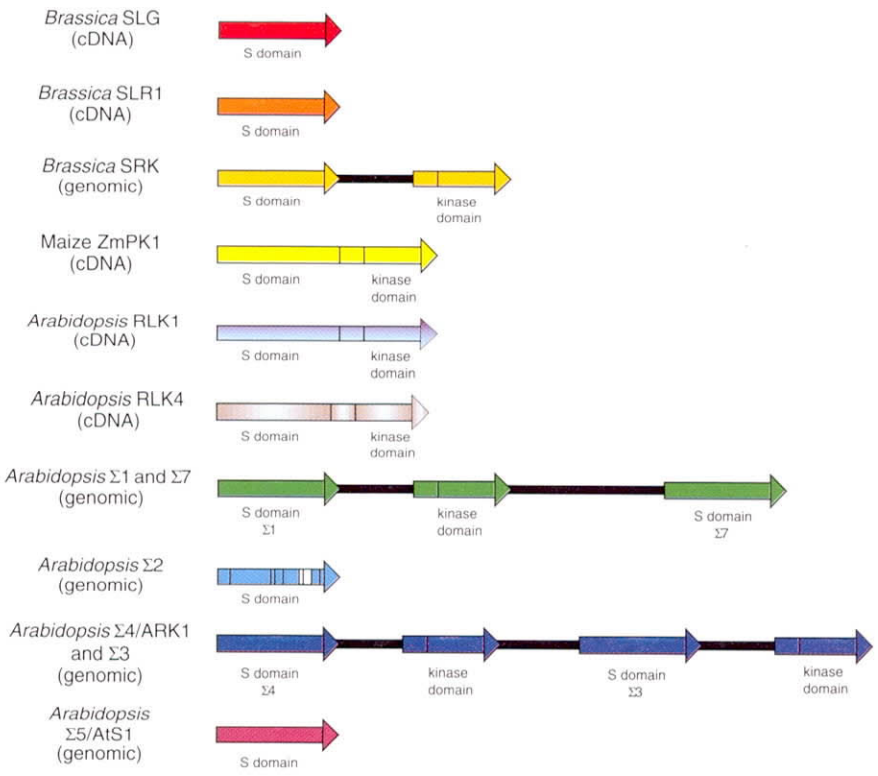

Fig. 5. Scheme showing the structure of the known members of the S-locus superfamily. The canoncial member, SLG, bears only a S-domain. This structure is shared by the SLR1, Σ2, Σ5/AtS1 and Σ7 genes. All others carry an additional kinase domain. In the case of Σ1, Σ3 and Σ4/ARK1 an alternative splice event yields two distinct forms of the protein: one which bears the S-domain alone and one which includes a transmembrane and a kinase domain. Σ2 is a pseudo-gene as judged from its sequence.

expressed at high levels in the cell wall of the papillar cells (Kandasamy et al., 1989). The SRK gene encodes a putative transmembrane receptor protein kinase. Its extracellular domain shares over 90% identity with the S-domain of SLG and it is expressed in the reproductive organs as well. Even though the particular function of the two proteins has not yet been elucidated these findings shed some light on the molecular mechanism involved in self-incompatibility. The SLG gene is the canonical member of a larger, *S*-locus superfamily. To date, representatives have been found in *Brassica*, maize, and *Arabidopsis* (Dzelzkalns et al., 1992).

A schematic representation of the genomic structure of six of the genes we isolated from *Arabidopsis* is given in Fig. 5 and compared to other members of the *S*-locus superfamily. Some of these genes have been isolated by others as well (Dwyer et al., 1992; Tobias et al., 1992). Σ2 appears to be a pseudo-gene as judged from the sequence analysis. Interestingly, three of the Sigma genes isolated give rise to two alternative transcripts (Σ1, Σ3 and Σ4). The smaller 1.5 kb transcript corresponds to a secreted version of the S-domain alone and the longer 3.0 kb corresponds to a transmembrane receptor kinase similar to the SRK gene. Northern analysis using probes corresponding to single members of the Sigma gene family show them to be expressed in a variety of the aerial tissues of the plant (S.E. Ploense and R.E. Pruitt, unpublished results). This suggests that, besides having a potential role in the fertilization process, at least some of these genes may function in other aspects of the plants life-cycle as well.

As an example, a scheme of the protein structure of Σ3 is presented in Fig. 6. A very similar, but not identical gene from *Arabidopsis*, has recently been described (Tobias et al., 1992). Based on sequence comparisons a couple of interesting features stand out. The overall structure of the transmembrane version of Σ3 follows the general scheme of

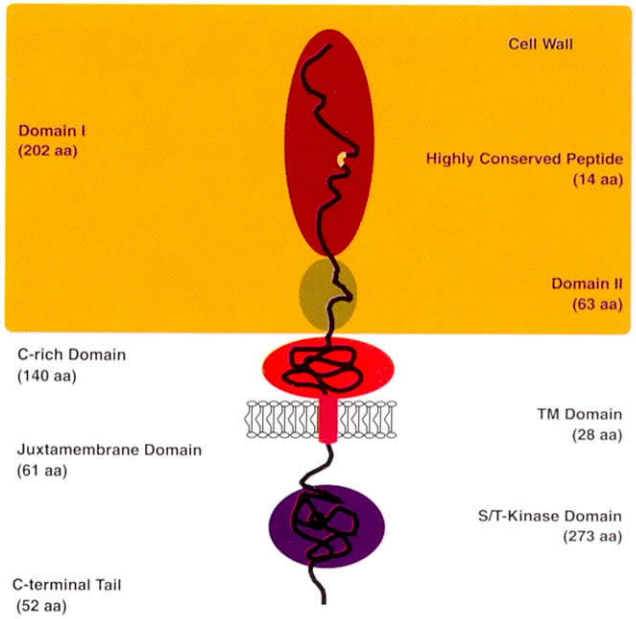

Fig. 6. Speculative scheme depicting the structure of Σ3. The cell wall is presented as a large rectangle. The extracellular S-domain is shown with its putative three-fold subdivision. The conserved 14 residues stretch is indicated. The extent to which the S-domain is penetrating the cell wall is unknown. The total length of the mature receptor version of the protein is 819 amino acids.

receptor tyrosine kinases (reviewed by Yarden and Ullrich, 1988; Ullrich and Schlessinger, 1990). After cleavage of a potential 26 residue signal sequence it consists of an extracellular domain of 413 amino acids followed by a putative transmembrane domain, a juxtamembrane region of about 61 residues, a kinase domain encompassing 273 residues and finally a carboxyl terminal tail of 52 amino acids. Based on the degrees of conservation among homologues as well as on characteristic sequence motifs one can distinguish at least three subdomains within the S-domain. At its N-terminal end domain I is found with a length of about 202 amino acids. This region exhibits an elevated degree of conservation (69-85%) among the various S-domains of the *Brassica* and *Arabidopsis* Sigma genes. In particular it harbors a very strongly conserved 14 residue peptide. Seven of the 14 residues are identical in all S-domains described to date and in each individual S-domain sequence at least 11 of the 14 residues are identical to the consensus peptide sequence. This short stretch of amino acids may represent the putative ligand binding site, a multimerization site, an enzymatically active site or an important structural feature necessary for the secondary and tertiary structure of the domain. A block of about 63 residues of less conservation (domain II) separates an 140 amino acid cysteine-rich region (C-rich domain) from domain I. The latter domain is characterized by a cluster of 12 cysteines, the positions of which are strictly conserved among *Brassica* and *Arabidopsis* Sigma gene S-domains. In the case of the more distantly related genes ZmPk1, RLK1 and RLK4 (Walker and Zhang, 1990; Walker, 1993) from maize and *Arabidopsis* respectively, 10 of these cysteines are still maintained. Some of the cysteines most likely undergo disulfide-bonding and help to determine the three-dimensional structure of the domain. The intracellular portion of the protein carries a protein kinase domain, the sequence of which predicts that it would be of the serine/threonine type. The protein kinase encoded by the SRK gene has been shown to have serine/threonine kinase activity when produced in *E. coli* (Goring and Rothstein (1992).

It is presently not known if the sub-domain structure of the S-domain, as revealed by an analysis of the sequence alignment, translates into a corresponding structural and/or functional subdivision. We also do not know what the ligands are that bind to the extracellular portion of the receptors. If the conserved 14 amino acid sequence does indeed correspond to the ligand binding site, then this suggests that the different ligands are possibly related among themselves and may constitute an entire class of molecules. A similar argument can already be made taking the overall homology of the S-domain into account.

Generally, plant cells are surrounded by a thick and supposedly rigid primary or even secondary cell wall, depending on the cell type. Therefore, the existence of potential signal receptor proteins with large extracellular domains raises a number of interesting structural and functional questions. How, for example, does the S-domain interact with the cell wall? Does it stick into the wall or does it stay beneath? Does it undergo interactions with components of the cell wall? Does the cell wall exert restrictions on lateral diffusion of these molecules, thereby making, for example, a putative dimerization of the proteins impossible? Another problem is raised by the question of how two transmembrane receptor proteins on neighboring cells may interact. Theoretical considerations suggest direct homophilic interactions between the two molecules to be unlikely. The average primary cell wall has a thickness of roughly about 50 nm. Therefore, the cell membranes of two cells next to each other are separated by at least 100 nm. Assuming that the whole S-domain of Σ3 exists as a single α-helix in vivo leads to the idea of the S-domain being a rod of about 62 nm in length. This would, in principle, enable the two molecules to directly contact one another. However, such a structure of the molecule is unlikely. A model involving some kind of even slightly globular structure reduces the longitudinal diameter of the sphere, therefore making direct contacts impossible. However, as mentioned earlier some of the Sigma genes give rise to alternative transcripts one of which encodes the S-domain alone. It is therefore tempting to speculate that this shorter molecule might serve as the ligand in what could be called indirect homophilic interactions. This smaller protein molecule might be capable of passing through the wall. Hence, the S-domain alone might serve as a shuttle which passes through the cell wall and docks to the neighboring receptor allowing signal transduction to occur. Alternatively of course, the receptors may undergo heterophilic interactions and the secreted version of the S-domain may be involved in binding to some other kind of receptor.

As mentioned above a whole range of exciting and as yet unsolved problems are associated with respect to the biological and biochemical functions of these genes. One particular strategy we are currently invoking uses a reverse genetic approach to unravel the function of the Sigma genes. By introducing a variety of different mutant copies back into the plant we hope to be able to interfere with the corresponding wild-type functions through dominant-negative effects. In addition, we are also performing more refined expression studies in order to learn more about the distribution of these proteins at the cellular level. The results of such a combined analysis should give us some indication of what biological process a particular gene is involved in. Complementary to these experiments, genetic mapping studies will tell us if any of the Sigma genes is a good candidate for a particular hydration mutant isolated in the screen. A potential candidate will then be tested by genomic rescue experiments for complementation of the mutant phenotype.

We wish to thank Susan Lolle for the *fiddlehead* photograph used in Fig. 1, in addition to many useful scientific discussions. This work was supported by NSF grants DMB-8718570 and DCB-9018889 to R. E. P. M. H. was supported by an EMBO long term postdoctoral fellowship, K. S. by a Swiss National Science Foundation postdoctoral fellowship and S. D. K. by a USDA postdoctoral fellowship.

REFERENCES

Carpenter, R. and Coen, E. S. (1990). Floral homeotic mutations produced by transposon mutagenesis in *Antirrhinum majus*. *Genes Dev.* **4**, 1483-1493.

Coen, E. S., Romero, J. M., Doyle, S., Elliot, R., Murphy, G. and

Carpenter, R. (1990). *Floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. *Cell* **63**, 1311-1322.

de Nettancourt, D. (1977). *Incompatibility in the Angiosperms*. Berlin: Springer-Verlag.

Dufour, S., Duband, J.-L., Kornblihtt, A. and Thiery, J. P. (1988). The role of fibronectins in embryonic cell migrations. *Trends Genet.* **4**, 198-203.

Dwyer, K. G., Lalonde, B. A., Nasrallah, J. B. and Nasrallah, M. E. (1992). Structure and expression of *AtS1*, an *Arabidopsis thaliana* gene homologous the the *S*-locus related genes of *Brassica*. *Mol. Gen. Genet.* **231**, 442-448.

Dzelzkalns, V. A., Nasrallah, J. B. and Nasrallah, M. E. (1992). Cell-cell communication in plants: self-incompatibility in flower development. *Dev. Biol.* **153**, 70-82.

Elleman, C.J., Franklin-Tong, V. and Dickinson, H. G. (1992). Pollination in species with dry stigmas: the nature of the early stigmatic response and the pathway taken by pollen tubes. *New Phytol.* **121**, 413-424.

Goring, D. R. and Rothstein, S. J. (1992). The S-locus receptor kinase gene in self-incompatible *Brassica napus* encodes a functional serine/threonine kinase. *Plant Cell* **4**, 1273-1281.

Heslop-Harrison, J. (1987). Pollen germination and pollen-tube growth. *Int. Rev. Cytol.* **107**, 1-78.

Hill, J. P. and Lord, E. M. (1987). Dynamics of pollen tube growth in the wild radish *Raphanus raphanistrum* (Brassicaceae). II. Morphology, cytochemistry and ultrastructure of transmitting tissues, and path of pollen tube growth. *Amer. J. Bot.* **74**, 988-997.

Kandasamy, M. K., Paolillo, D. J., Faraday, C. D., Nasrallah, J. B. and Nasrallah, M. E. (1989). The S-locus specific glycoproteins of *Brassica* accumulate in the cell wall of developing stigma papillae. *Dev. Biol.* **134**, 462-472.

Koornneef, M., Hanhart, C. J. and Thiel, F. (1989). A genetic and phenotypic description of eceriferum (*cer*) mutants in *Arabidopsis thaliana*. *J. Hered.* **80**, 118-122.

Lolle, S. J., Cheung, A. Y. and Sussex, I. M. (1992). *Fiddlehead*: An *Arabidopsis* mutant constitutively expressing an organ fusion program that involves interactions between epidermal cells. *Dev. Biol.* **152**, 383-392.

Nasrallah, J. B., Kao, T.-H., Goldberg, M. L. and Nasrallah, M. E. (1985). A cDNA clone encoding an S-locus-specific glycoprotein from *Brassica oleracea*. *Nature* **318**, 263-267.

Nasrallah, J.B., Nishio, T. and Nasrallah, M. E. (1991). The self-incompatibility genes of *Brassica*: Expression and use in genetic ablation of floral tissues. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 393-422.

Pierson, E.S. and Cresti, M. (1992). Cytoskeleton and cytoplasmic organization of pollen and pollen tubes. *Int. Rev. Cytol.* **140**, 73-125.

Preuss, D., Lemieux, B., Yen, G. and Davis, R. W. (1993). A conditional sterile mutation eliminates surface components from *Arabidopsis* pollen and disrupts cell signalling during fertilization. *Genes Dev.* **7**, 974-985.

Pruitt, R.E., Horejsi, T. F., Pierskalla, B. K. and Ploense, S. E. (1991). Genetic analysis of cellular interactions during fertilization of *Arabidopsis thaliana*. *J. Cell. Biochem.* **15A**, 137.

Pruitt, R. E. and Hülskamp, M. (1994) From pollination to fertilization in *Arabidopsis*. In: *Arabidopsis* (ed. E. M. Meyerowitz and C. R. Somerville). New York: Cold Spring Harbor Laboratory Press.

Sanders, L.C. and Lord, E. M. (1989). Directed movement of latex particles in the gynoecia of three species of flowering plants. *Science* **243**, 1606-1608.

Satina, S., Blakeslee, A. F. and Avery, A. G. (1940). Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. *Am. J. Bot.* **44**, 311-317.

Siegel, B. A. and Verbeke, J. A. (1989). Diffusible factors essential for epidermal cell redifferentiation in *Catharanthus roseus*. *Science* **244**, 580-582.

Stein, J. C., Howlett, B., Boyes, D. C., Nasrallah, M. E. and Nasrallah, J. B. (1991). Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. *Proc. Natl. Acad. Sci. USA* **88**, 8816-8820.

Szymkowiak, E. J. and Sussex, I. M. (1993). Effect of *lateral suppressor* on petal initiation in tomato. *Plant J.* **4**, 1-7.

Tilney-Bassett, R. A. E. (1986). *Plant Chimeras*. London: Edward Arnold Publishers.

Tobias, C. M., Howlett, B. and Nasrallah, J. B. (1992). An *Arabidopsis thaliana* gene with sequence similarity to the S-locus receptor kinase of *Brassica oleracea*. *Plant Physiol.* **99**, 284-290.

Ullrich, A. and Schlessinger, J. (1990). Signal transduction by receptors with tyrosine kinase activity. *Cell* **61**, 203-212.

Walker, D. B. (1975). Postgenital carpel fusion in *Catharanthus roseus* (Apocynaceae). I. Light and scanning electron microscopic study of gynoecial ontogeny. *Am. J. Bot.* **62**, 457-467.

Walker, J. C. (1993). Receptor-like protein kinase genes of *Arabidopsis thaliana*. *Plant J.* **3**, 451-456.

Walker, J. C. and Zhang, R. (1990). Relationship of a putative receptor protein kinase from maize to the S-locus glycoprotein of *Brassica*. *Nature* **345**, 743-746.

Yarden, Y. and Ullrich, A. (1988). Growth factor receptor tyrosine kinases. *Ann. Rev. Biochem.* **57**, 443-478.