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REVIEW

Tapetum-specific genes: what role do they play in male gametophyte development?

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INTRODUCTION

Plant sexual reproduction is a key process in plant development and has a great impact on agriculture. Plant sexual reproduction is established by the interaction between the male gametophyte, producing the sperm cells, and the female gametophyte harbouring the egg cell. The effectivity of this interaction largely depends on the viability and incompatibility properties of the pollen. Therefore, the key role of pollen in the fertilization process justifies a scientific effort to get a better understanding of pollen development, which is also of commercial importance. Development of pollen is initiated inside the anther locule and completed when mature pollen is released. Upon transfer to the female stigma the pollen germinates and subsequently protrudes a tube, containing the sperm cells, towards the ovule where fertilization and seed setting occur (Derksen *et al.* 1995).

Formation of viable pollen is a complex process that depends on accurate execution of developmental programmes in both the sporophytic and gametophytic anther tissues (Kamalay & Goldberg 1980; Mascarenhas 1989, 1990; McCormick 1993; Goldberg *et al.* 1993). It occurs in the anther in the pollen sac, which is surrounded by a one-cell layer tissue, the tapetum. The tapetum cells are essential for the development of the

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pollen grain. Tapetal cells arise from the same progenitor cells as the developing male gametophytes and totally enclose the latter during their development (Bhandari 1984; Scott *et al.* 1991a; Goldberg *et al.* 1993). This spatial relationship implies that the exchange of nutritional, structural, or regulatory compounds between the sporophytic anther tissues and the developing male gametophytes occurs via the tapetum. The impact of this is exemplified by several cases of male sterility where initial lesions are found in the tapetal tissue and not in the sporogenous cells (Izhar & Frankel 1971; Horner & Rogers 1974; Bino 1985; Grant *et al.* 1986; Liu *et al.* 1987; Kaul 1988; Zuberi *et al.* 1988; Chaudhury *et al.* 1994; Zhang *et al.* 1994). Conclusive evidence that the tapetum is essential for pollen development comes from experiments on transgenic *Brassica* where spatially specific expression of a cytotoxic gene in the tapetal cell layer resulted in destruction of the tapteum and, subsequently, in an arrest of pollen grain development (Mariani *et al.* 1990).

Physiological functions attributed to the tapetal cells are: (i) nourishment of the developing male gametophytes by amino acids and sugars (Heslop-Harrison 1964, 1968; Echlin 1971; Nave & Sawhney 1986; Sawhney & Nave 1986); (ii) secretion of intralocule enzymes such as β-1,3-glucanases to break down the callose wall around tetrads (Frankel *et al.* 1969; Mepham & Lane 1970; Izhar & Frankel 1971; Stieglitz 1977); (iii) participation in pollen exine wall formation by providing enzymes and exine precursors (Heslop-Harrison 1968; Echlin 1971; Vithange & Knox 1980; Bhandari 1984; Bedinger 1992); (iv) synthesis and secretion of lipoid substances, called pollen kitt, that are deposited on the pollen surface to protect the mature pollen from dehydration (Heslop-Harrison 1968; Dickinson & Lewis 1973; Weber 1992); and (v) production and secretion of proteinaceous compounds involved in rehydration during germination and tube growth after pollen grain transfer to the stigma (Dickinson & Lewis 1973; Heslop-Harrison 1975; Kandasamy 1994). The question of whether the tapetum also fulfils regulatory tasks with respect to male gametophyte development, such as induction of meiocyte meiosis or microspore mitosis, will probably be answered in the near future.

Due to the complexity of the processes in the anther, the picture of the physiological functions of tapetal cells and the underlying molecular mechanisms in pollen development is still incomplete. The isolation and characterization of anther-specific genes marks the beginning of the unravelling of these molecular mechanisms. This paper reviews recent data on genes expressed preferentially or exclusively in the tapetal cell layer of the angiosperms.

TAPETUM AND MALE GAMETOPHYTE DEVELOPMENT: FINELY TUNED PROCESSES

The expression of tapetum-specific genes and their protein products is linked to certain cytological and morphological stages of gametophyte development (Fig. 1). Male gametophyte development begins when the archesporial cells appear within the anther primordium. The archesporial cells divide mitotically to form a primary parietal layer, producing the tapetum, and an inner sporogenous cell mass. The sporogenous cells proliferate to form meiocytes, or pollen mother cells, which undergo meiosis and produce tetrads of haploid microspores totally enclosed by a callose wall. After dissolution of the callose wall, the freed microspores enter a period of growth and differentiation leading to an asymmetric mitotic division. A large vegetative and a small generative cell are formed in the maturing pollen grain. The generative cell divides

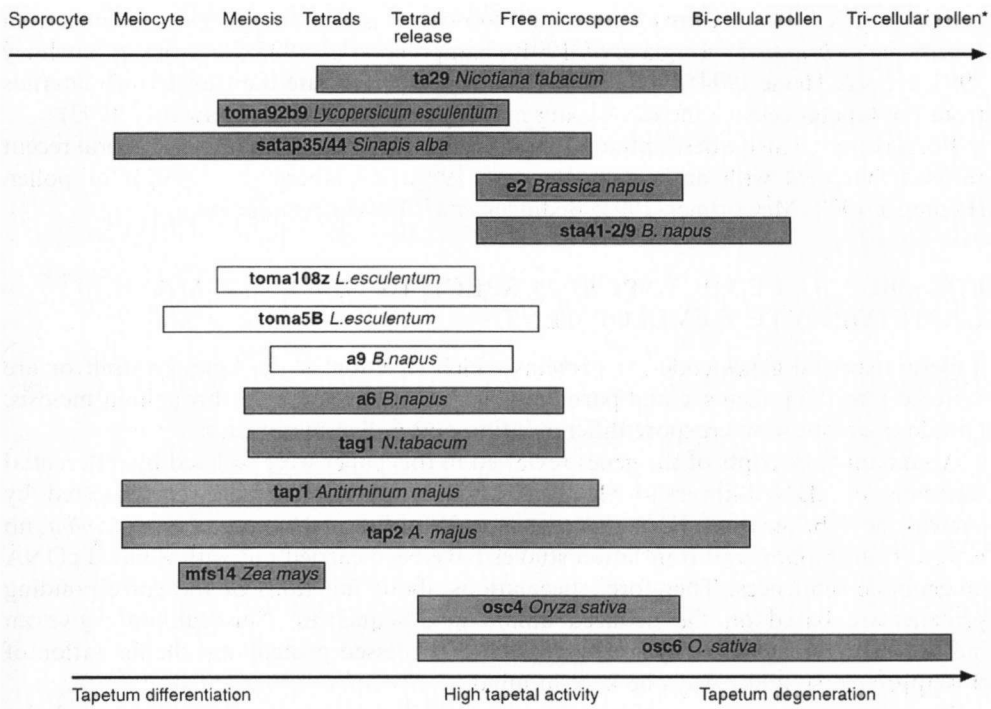

Fig. 1. Presence of 17 specific mRNAs in the tapetum of various angiosperms during the development of the male gametophyte (*where present).

mitotically yielding two sperm cells. Depending on the plant species, this second pollen mitosis takes place either within the anther before anthesis or during pollen tube growth in the style. Intra-anther development of the male gametophyte is finished when pollen grains become dehydrated and are released from the microsporangium.

The primary parietal layer differentiates into the tapetum that completely encloses the sporogenous cells. The tapetal layer becomes morphologically distinguishable from the other anther tissues before the meiocyte stage, by its meristematic morphology (Bhandari 1984). At the onset of meiosis in the meiocytes, the tapetal cells enter a period of extensive differentiation with polyploidisation of their nuclei. The meristematic morphology gradually changes into that of polar secretory cells. The ER-Golgi network becomes more pronounced and differentiated, a process accompanied by an increase in the number of secretory vesicles, especially towards the anther locule, and by the appearance of small vacuoles containing lipophilic substances. The secretory activity of the tapetum peaks after dissolution of the callose wall around the tetrads after which the free haploid microspores enter a period of growth and differentiation. Finally, the tapetal cells degenerate and release their cytoplasmatic content into the anther locule between pollen mitosis and anthesis, the exact moment depending on the plant species (Bhandari 1984; Koltunow *et al.* 1990; Murgia *et al.* 1991; Weber 1992; Bedinger 1992; Ciampolini *et al.* 1993; Polowick & Sawhney 1993; Majewska-Sawka *et al.* 1993; Hess & Hesse 1994). The polar secretory character of tapetal cells is also apparent from a strong modification of the cell wall, especially towards the locule containing the developing microspores. When the tapetal cells enter the phase of high secretory

activity, the cellulose texture of these walls loosens or is even totally dissolved in certain species, as in *Brassica* (Murgia *et al.* 1991; Ciampolini *et al.* 1993; Polowick & Sawhney 1993; Hess & Hesse 1994). These wall modifications facilitate the transfer of materials from the tapetal cells to the developing microspores (Polowick & Sawhney 1993).

For a comprehensive description of these events the reader is referred to several recent reviews that deal with anther (Scott *et al.* 1991a; Goldberg *et al.* 1993) or pollen (Bedinger 1992; McCormick 1993; Bedinger *et al.* 1994) development.

POSSIBLE ROLE OF TAPETUM-SPECIFIC GENES IN MALE GAMETOPHYTE DEVELOPMENT

Tapetum-specific genes code for proteins which function in the tapetum itself or are secreted into the pollen sac and participate in pollen development throughout meiosis, tetrade dissolution, microspore differentiation and pollen maturation.

Abundant transcripts of the genes reviewed in this paper were isolated by differential screening of cDNA libraries, whereas less abundant transcripts were detected by subtractive hybridization. Except for the satap35 and satap44 genes of *Sinapis alba*, no *in vitro* transcription and translation studies have been carried out with isolated cDNA or genomic sequences. Therefore, speculations about functions of the corresponding proteins are based on the deduced amino acid sequences. This can only give an indication of the actual *in vivo* structure of the processed protein and the limitation of this approach should always be kept in mind.

The expression of tapetum-specific genes is tightly regulated in time, which excludes an involvement in general metabolic processes (Fig. 1). The temporal expression pattern shown in Fig. 1 indicates that the transcription of these genes during tapetal life span is turned on and off at specific points. The finely tuned temporal expression and its spatial restriction to one cell layer; known to be involved in support of male gametophyte development, suggest that these genes and their protein products may participate in pollen formation. This idea is strengthened by the presumed properties of their protein products (Table 1). Fifteen of the 17 genes examined encode proteins with a N-terminal signal peptide as typical for proteins that enter the secretory pathway. The lack of hydrophobic intra-membrane domains in 16 of the 17 encoded proteins (Kyte & Doolittle 1982; Engelman *et al.* 1986) supports the idea that most of these proteins are secreted rather than inserted into the tapetal cell membrane. Some of the gene products (TA29, A6, OSC6 and TAG1) contain aspartic-linked (N-linked) glycosylation sites (Alberts *et al.* 1989). This type of glycosylation is restricted to proteins in the ER-Golgi lumen. Therefore, gene products with N-linked glycosylation sites are likely to be secreted proteins if they are really glycosylated. Thus, there is much evidence that 15 of the 17 genes encode proteins that are eventually secreted into the extracellular space of the tapetal cells. Because of the assumed polarity of secretion, the proteins are likely to enter the anther locule and to play a role in the male gametophyte development.

The fact that 15 of 17 tapetum-specific gene products may enter the secretory pathway indicates that the transcripts encoding these proteins are very abundant in the pool of mRNAs. Another interesting finding is that 12 of the 17 encoded polypeptides have a molecular weight below 20 kDa, so it is unlikely that they possess an enzymatic activity. The average diameter of the spaces between the cross-linked macromolecules in most plant cell walls is about 5 nm, small enough to make the movement of any globular protein with a molecular weight above 20 kDa extremely slow (Carpita *et al.* 1979;

Table 1. Properties of 17 proteins encoded by tapetum-specific genes

Gene	MW (kDa)	Sequence similarity to known proteins; important properties	N-terminal secretory signal	Putative function	Reference
ta29	33	Similarity to members of a family of glycine-rich cell wall proteins	+	Transfer of apolar compounds; involvement in pollen exine wall formation	Seurinck *et al.* 1990 Koltunow *et al.* 1990
toma92b9	15	Idem	+	Idem	Chen *et al.* 1994
e2	12·5	Cysteine-rich protein with 55–60% amino acid homology to members of a family of plant phospholipid transfer proteins	+	Idem	Foster *et al.* 1992
Sta41-2/ sta41-9	42/37·6	71–97% similarity in one domain to oleosins	−	Idem	Robert *et al.* 1994b
satap35/ satap44	12·7/12·4	Presence shown in the mature pollen exine	+	Idem	Staiger & Apel 1993; Staiger *et al.* 1994
a9	10·3	Similarities to members of a family of seed storage proteins, protease- and α-amylase inhibitors	+	Intra-locule callase inhibitor	Paul *et al.* 1992; Turgut *et al.* 1994
toma5b	11·1	Idem	+	Idem	Aguirre & Smith 1993
toma108z	10·6	Idem	+	Idem	Chen & Smith 1993
a6	53	Homology to β-1,3-glucanases	+	Part of the callase complex degrading the tetrad callose wall	Hird *et al.* 1993
tag1	34·7	Idem	+	Idem	Bucciaglia & Smith 1994
tap1/tap2	>20	No similarities to known proteins	+	Unknown	Nacken *et al.* 1991a,b
mfs14	13	Idem	+	Unknown	Wright *et al.* 1993
osc4/osc6	10·3/14	Idem	+	Unknown	Tsuchiya *et al.*1992, 1994

Milburn 1979). Only in the case of the SATAP proteins in mustard is there direct evidence of proteins secreted by the tapetum being incorporated into pollen structures (Staiger *et al.* 1994).

GENE PRODUCTS THAT MAY BE INVOLVED IN POLLEN EXINE FORMATION

When it is assumed that (i) the protein products of the tapetum-specific genes are secreted into the anther locule; and (ii) they are temporarily present (Fig. 1) to facilitate development at specific stages, the question arises of how they would affect pollen development. Although this question cannot be answered at present, indications for their function can be deduced from the data (Table 1) reviewed in this paper, which allow the formulation of some hypotheses.

An important aspect of pollen development in which the tapetal cell layer is involved is pollen exine wall formation. Tapetal cells are thought to provide the hydrophilic exine precursors and enzymes that catalyse their oxidative polymerization (Heslop-Harrison 1968; Echlin 1971; Vithange & Knox 1980; Bhandari 1984; Bedinger 1992). Tapetum-supported synthesis of the exine wall begins after dissolution of the tetrad callose wall (Bhandari 1984; Gabarayeva 1993; Perez-Munõz *et al.* 1993). The tuning of this process by the tapetum is unknown.

Recently isolated tapetum-specific transcripts (Table 1) encode proteins that might be involved in the synthesis of the pollen exine wall. These genes are ta29 in tobacco, toma92b9 in tomato, the mustard genes satap35, satap44 and in rape seed e2, sta41-2, sta41-9. The hypothesis on their functions is based on the particular expression pattern (Fig. 1) and the finding that the corresponding proteins exhibit similarities either to plant cell wall proteins (TA29, TOMA92B9) or to polypeptides that interact with apolar compounds in an aqueous environment (E2, STA41-2, STA41-9). Of these proteins, the satap gene products have been demonstrated, by immunolocalization, to be taken up in the growing exine wall of developing microspores. The ta29 transcript accumulates in the tapetum of tobacco at the tetrad stage and declines at the stage of microspore mitosis when the tapetum degenerates (Fig. 1) (Koltunow *et al.* 1990). Isolation and sequencing of a genomic fragment containing the ta29 gene revealed one continuous open reading frame encoding a 33 kDa glycine-rich protein that has several potential N-glycosylation sites. It contains two similar highly hydrophobic domains and an N-terminal signal peptide (Seurinck *et al.* 1990). Amino acid sequence comparison between TA29 and known proteins placed this polypeptide in a family of glycine-rich proteins with a structural function in the cell wall (Koltunow *et al.* 1990; Showalter 1993). Because of the presence of two hydrophobic domains, TA29 might serve as a carrier for the apolar exine. After incorporation of the TA29-precursor complex in the growing exine wall, TA29 could function as a catalyst of oxidative polymerization of sporopollen in precursors. This assumption is based on certain characteristics of the protein family to which the TA29 protein belongs. Some of these glycine-rich proteins are found in the cell wall of cells destined to become lignified. They may serve as nucleation sites in the oxidative lignin polymerization during lignin synthesis (Keller *et al.* 1989). The TA29 protein could fulfil a similar function in sporopollenin biosynthesis. However, there is no evidence on TA29 location in the anthers.

In tomato, toma92b9 mRNA accumulates in the tapetum at the onset of pollen mother cell meiosis, peaks during the tetrad stage when the tapetal cells are

physiologically most active in tomato (Smith *et al.* 1992; Chen *et al.* 1994) and disappears prior to tapetum degeneration (Fig. 1). The study of the toma92b9 cDNA sequence revealed that the deduced protein displays amino acid sequence similarity to the tobacco TA29 polypeptide, as does the content of glycine residues (Chen *et al.* 1994). However, several characteristic amino acid motifs and the temporal expression pattern are different (Fig. 1). Thus, there is virtually no evidence for a homologous function for these two genes.

The expression of the satap genes in mustard starts when the tapetum is fully differentiated, presumably at the early meiocyte stage, reaches a maximum at the microspore release stage, and decreases at the free microspore stage before the tapetum disintegrates (Fig. 1). Sequence analysis of the cDNAs of satap35 and satap44 showed an identity of 86% at the nucleotide and 76% at the amino acid level. Database searches on the nucleotide sequences of the satap35 and satap44 cDNAs did not reveal significant homology to known sequences so that no function can be assigned to the proteins by this analysis. There is evidence for translocation of these proteins from tapetal cells to the exine layer (Staiger *et al.* 1994). Their localization in the young exine wall was confirmed with SATAP-specific antibodies. Immunolabelling was also observed within the anther locule in association with globules that appear at the tetrad stage and presumably contain sporopollenin precursors. *In vitro* transcription of the cDNAs and translation of the resulting mRNAs in the presence of pancreatic microsomes showed that the two proteins are translocated into the microsomes and that signal peptides are proteolytically cleaved off during processing. These observations indicate that SATAP proteins enter the secretory pathway of the tapetal cells and are secreted into the anther locule.

The SATAP44 protein was immunologically detected by Western blotting in protein extracts from anthers at approximately the tetrad stage but not in extracts from later stages. This indicates that after termination of satap44 gene expression also its product disappears (Staiger *et al.* 1994). Perhaps the SATAP44 protein becomes immobilized in the pollen exine wall and is no longer detectable. Staiger *et al.* (1994) speculate that the SATAP proteins are exclusively produced by the tapetum, secreted into the anther locule and incorporated into the pollen exine wall. Whether these particular proteins are actually carriers of sporopollenin precursors, involved in precursor polymerization, as hypothesized for TA29, and whether these proteins do become part of a structural framework in the pollen wall remains to be elucidated.

Gene transcript e2 accumulates in tapetal cells of *Brassica napus* from the stage of microspore release until microspore mitosis (Fig. 1) (Scott *et al.* 1991b). It encodes a polypeptide similar to plant phospholipid transfer proteins (Foster *et al.* 1992), and this E2 protein may be involved in the transport of lipophilic substances from the tapetum to the microspores. After dissolution of the tetrad callose wall, the pollen exine wall is synthesized (Bhandari 1984; Perez-Muñoz *et al.* 1993). This marks the beginning of the free microscore stage and coincides with the onset of e2 gene expression (Foster *et al.* 1992).

The tapetum and the developing microspores are assumed to participate in the synthesis of this sporopollenin wall by providing precursors and enzymes (Bhandari 1984; Foster *et al.* 1992). These precursors are highly hydrophobic carotenoids or carotenoid esters which are secreted by the tapteum and the developing microspores. After transport through the aqueous locular fluid to the sites of sporopollenin synthesis, the precursors are polymerized in an oxidative reaction yielding the sporopollenin

(Shaw 1971; Bhandari 1984). Considering the physiological processes taking place in the anther locule concurring with the expression of the e2 gene, we favour the interpretation (Foster *et al.* 1992) that E2, like TA29, TOMA92B9, SATAP35 and SATAP44, is involved in pollen exine wall formation as a carrier of wall precursors.

Oleosins are proteins found in association with membrane-bound oil bodies in seed storage tissues and may help to stabilize these small oil bodies during dehydration and possibly provide binding sites for lipase activity upon germination (Huang 1992; Murphy 1993). The sequences of the tapetum-specific cDNAs sta41-2 and sta41-9 showed homology to sequences encoding oleosins and were found to be present from the free microspore to the bicellular pollen stage in *Brassica* (Robert *et al.* 1933, 1994a,b) (Fig. 1). The expression of these genes coincides with the time of lipid accumulation in the *B. napus* tapetum (Polowick & Sawhney 1990) and with the expression of the e2 gene described above.

GENES THAT MAY ENCODE CALLASE AND INTRALOCULE CALLASE INHIBITORS

One essential step in male gametophyte development is the correct timing and execution of tetrad callose wall dissolution (Frankel *et al.* 1969; Mepham & Lane 1970; Izhar & Frankel 1971; Stieglitz 1977; Warmke & Overman 1972). This suggests that there are multi-level mechanisms to regulate hydrolytic callose breakdown. Such a multiple regulation is indicated by the finding that three tapetal genes specify proteins that could be callase inhibitors. The genes considered below encode callase inhibitors and β-1,3-glucanase (callases) and are coordinately expressed within the tapetum. The excretion of the mixture of proteins into the anther locule allows the accumulation of callases without premature activity and rapid, controlled breakdown of the callose wall in a narrow time span.

A gene with the required local and temporal expression might, in principle, be a candidate for a role in callase breakdown. In tomato, the toma5b transcript accumulates in the tapetal tissue layer at the meiocyte stage, reaches a maximum level at tetrad stage and disappears from the tapetal cells after release of the haploid microspore from the tetrads (Fig. 1) (Smith *et al.* 1990; Aguirre & Smith 1993). The cysteine pattern of the TOMA5b protein is similar to that of members of a seed protein family containing α-amylase and protease inhibitors, and other, bifunctional inhibitors (Kreis *et al.* 1985). The size of this protein (11·1 kDa) and the impermeability of the surrounding callose make it unlikely that the protein will enter the microspores at this stage (Aguirre & Smith 1993).

The cysteine residue pattern in TOMA5B is similar to that of hydrolase inhibitors from other plant species such as α-amylase-, protease- and bifunctional inhibitors (Richardson 1991) which suggests that TOMA5B may be an inhibitor of callase and prevents its premature activity. This idea is supported by several observations, such as: (i) callases are abundantly present in the anther locule during the developmental stages at which toma5b expression occurs; (ii) the TOMA5B protein is very probably secreted into the anther locule at the time of tapetal callase production; (iii) callase activity accumulates in the anther locule prior to callose wall dissolution (Stieglitz 1977); and (iv) hydrolysis of the callose wall is achieved in a very narrow time span (Izhar & Frankel 1971; Worral *et al.* 1992). The callase inhibitor might be inactivated by a signal given by the microspores after they have finished meiosis. The start signal for callose

breakdown most likely originates from the microspores because the information on the progress of meiosis resides in them. Enzyme activity could, alternatively, be regulated by a change in physicochemical conditions; for example, a change in pH. This factor was shown to play an important role in callase activity in *Petunia hybrida*: a strong relationship was observed between locule pH, callase activity and the timing of microspore release. Locule pH and callase activity changed at the same time (Izhar & Frankel 1971).

An mRNA with a similar sequence, toma108z, is present from early meiosis through microspore release in the tapetal cell layer of tomato plants (Fig. 1). The transcript codes for a protein of 102 amino acids with a molecular mass of 10·6 kDa (Smith *et al.* 1990; Chen & Smith 1993). The a9 tapetum-specific mRNA accumulates only during the tetrad stage (Fig. 1) in *Brassica napus* (Scott *et al.* 1991b; Paul *et al.* 1992). The encoded polypeptide is 96 amino acids in length with a molecular mass of 10·3 kDa and a hydrophobic N-terminal signal peptide (Von Heijne 1986). The A9 protein has a pattern of cysteine residues that resembles the distribution of this amino acid in TOMA5B, TOMA108Z and in members of the previously mentioned family of seed proteins containing various enzyme inhibitors (Kreis *et al.* 1985). Because of this similarity, Paul *et al.* (1992) investigated whether a9 was also expressed in developing seeds. No reporter gene activity was detected in transgenic tobacco seed-capsules. The tobacco plants had been transformed with an a9-promoter-glucuronidase (GUS) gene that directed GUS expression to the tapetal cell layer of tobacco plants. The same authors proposed that the protein might play a role in the defence to microbial or insect attack thus attributing to proper pollen development. Recently, it was demonstrated that transgenic *Brassica napus* plants deprived of a9 mRNA by expression of anti-sense a9 mRNA are able to form pollen of normal or almost normal viability (Turgut *et al.* 1994), which means that the gene does not play an essential role in development.

We will now focus on the target enzymes of these inhibitors: the intra-locule callases. Sequence analysis of the tapetum-specific a6 cDNA from *Brassica napus* revealed an open reading frame encoding a polypeptide of 474 amino acids (Scott *et al.* 1991b; Hird *et al.* 1993) and indicated that the a6 gene encodes a secretory protein of 53 kDa with eight potential N-linked glycosylation sites. A sequence with 86% homology was found in the genome of *Arabidopsis* (Hird *et al.* 1993; Roberts *et al.* 1993). The primary amino acid sequence of the A6 protein of both species is similar to plant β-1,3-glucanases and β-1,3/1,4-glucanases, particularly in the regions that are thought to be responsible for enzymatic activity (Hird *et al.* 1993). The a6 gene is expressed in *Brassica* during meiosis and the tetrad stage (Fig. 1) (Scott *et al.* 1991b). A similar pattern was found in transformed *Brassica* in which the β-glucuronidase reporter gene was fused to the *Arabidopsis* a6 promoter. In these transgenic plants, GUS activity sharply peaked in flower buds before microspore release at the tetrad stage. A polyclonal antibody was raised against a truncated A6 polypeptide to address the question whether the A6 protein undergoes any post-translational modifications and to determine the size of the mature protein in *Brassica* anthers (Hird *et al.* 1993). The antibody reacted with a temporally regulated protein of approximately 60 kDa. At the meiocyte stage, when transcription of a6 is initiated, this 60 kDa protein first appears in the anthers. Its concentration peaks during microspore release and declines rapidly during later developmental stages when a6 mRNA also disappears (Hird *et al.* 1993). The difference in molecular weight (A6 was predicted to have a molecular weight of 53 kDa) might be due to glycosylation at eight sites in the polypeptide chain.

To study the process of tetrad dissolution, Bucciaglia & Smith (1994) prepared primers from conserved regions of β-1,3; 1,4-glucanases to amplify related sequences from a tobacco anther cDNA library by the polymerase chain reaction (PCR). The cDNA library was prepared with mRNA from anthers at meiosis through the free microspore stage. One of the cloned products, tag1, shared 40–50% of its sequence with β-1,3-glucanase genes. The protein encoded by tag1 has a calculated molecular weight of 34·7 kDa after removal of the N-terminal signal peptide. Like A6, the TAG1 polypeptide displays amino acid sequence similarities with callases at sequence positions that are crucial for enzymatic activity. Also like a6, tag1 mRNA appears during the tetrad stage, reaches a maximum level at microspore release stage and disappears rapidly at the free microspore stage (Fig. 1) (Bucciaglia & Smith 1994). The pattern of tag1 expression correlates with callose breakdown in the anther locule of tobacco plants.

The TAG1 and A6 polypeptides exclusively appear in the anther locule at the stage wherein callose is dissolved prior to microspore release from the tetrads, in line with the expression pattern of the tag1 and a6 genes. No β-1,3-glucanase or β-1,3;1,4-glucanase enzyme activity was detected so far.

In lily, the callase enzyme complex responsible for callose breakdown consists of two subunits, a 32 kDa endoglucanase and a 62 kDa exoglucanase (Stieglitz 1977). Digestion of tetrads with the endoglucanase activity alone resulted in significant reduction in the thickness of the callose wall, whereas exoglucanase activity had no discernible effect in the absence of the other enzyme. The molecular weights of the A6 and TAG1 proteins are of the same order of magnitude as those of the proteins of the callase complex in lily (Stieglitz 1977). If these intralocule β-1,3-glucanase enzymes are conserved among the angiosperms, the 32 kDa TAG1 protein would exhibit endoglucanase activity whereas the A6 protein would display exoglucanase activity. The studies of Hird *et al.* (1993) and Bucciaglia & Smith (1994) thus support the hypothesis that the tetrad callose wall is dissolved by β-1,3-glucanases synthesized and secreted by the tapetum (Frankel *et al.* 1969; Izhar & Frankel 1971; Stieglitz & Stern 1973).

OTHER TAPETUM-SPECIFIC GENES

A considerable number of genes have been isolated that are exclusively expressed in the tapetum during well-defined stages of development, but the encoded amino acid sequences do not provide information on a possible function. These genes may or may not play a role in development and fall outside the scope of this review. The tapetum-specific cDNAs that are considered in this section were isolated in an attempt to unravel early stamen development and encode secreted proteins.

One of the cDNA clones, tap1, encodes a small polypeptide of 107 amino acids including an N-terminal signal peptide. The secreted polypeptide consists for about 14% of cysteine residues which are arranged in two pairs of two particular motifs, CX_3CX_3C and CX_3CX_4C (X may represent any amino acid). On the basis of the cysteine content, a relationship of the TAP1 protein with plant thionins was proposed (Nacken *et al.* 1991a). The secreted character of the TAP proteins led to the hypothesis that they may be directly involved in the support of microspore development by the tapetum as described in the Introduction.

Several transcripts of which tap-1 is a representative, reach their highest concentrations early in anther development shortly after the onset of tapetum differentiation but the various mRNAs disappear at different moments (Fig. 1) (Nacken *et al.* 1991a,b).

Recently, two anther-specific cDNAs, designated osc4 and osc6, were isolated from monocotelydonous crop plants, maize and rice. The temporal expression of the osc6 gene was measured in a transformant with the β-glucuronidase reporter gene. The transcript is accumulated in tapetal cells from pollen mother cell meiosis to the bicellular pollen stage.

FINAL REMARKS

An important question concerning anther development is the communication between the developing male gametophytes and the sporophytic tapetum. Overall synchronization of tapetal activity with the developmental progress of the male gametophytes is essential to guarantee an optimal development and therewith male fertility. An example of such a synchronization is the timing of callase secretion and intralocule activity which is crucial for male fertility. In mutants of *Petunia* and *Sorghum*, premature (Frankel *et al.* 1969; Warmke & Overman 1972) or delayed (Izhar & Frankel 1971) callase activity results in microspore abortion and male sterility. The timing of callase secretion and intralocule callase activity certainly requires some sort of communication between the tapetum and the developing microspores. In many species, meiosis is initiated synchronously in the meiocytes whereas there is no synchrony in the premeiotic mitoses (Bennet *et al.* 1973; Scott *et al.* 1991a). This synchronization might be entirely controlled by the meiocytes themselves, but the possibility cannot be excluded that the sporophytic tissues, especially the tapetum, are involved in the induction of meiosis in the pollen mother cells. Coordination of development in the gametophyte and the tapetum is also indicated by the increase in the metabolic and secretory activity of the tapetum, which precedes the time at which the cytoplasmic connections between meiocytes and tapetum disappear. A second example of coordinate development in the anther is the maturation of the pollen grains which precedes anther dehiscence. Preliminary results (Ruiter *et al.* 1996) suggest a function of the tapetal cells in desiccation of the maturing pollen at anthesis.

Expression of the tapetum-specific genes that are most probably involved in male gametophyte development, is stage-specific (Fig. 1). It may be assumed that aberrant timing of gene expression leads to developmental failure and male sterility. This would be particularly true for tapetal genes such as the a6 and tag1 genes, which are active during a very narrow time span. Unfortunately, there is no evidence for a specific communication system between gametophytic and sporophytic cells that would be responsible for correct timing. Changes in development induced by transformation of plants with temporally expressed tapetal genes may make visible the communication between tapetal cells and developing pollen.

Despite the availability of recombinant proteins from some of the cloned genes, little attention has been paid to biochemical characterization or immunolocalization. Speculations on the possible functions of the proteins are based on the primary amino acid structure and correlation of stage-specific processes with expression patterns of the corresponding genes. Comparison of amino acid sequences can only serve as a first indication of the activity of a specific gene product. Presence of a specific mRNA is not conclusive evidence for the presence of a functioning protein: translation might be inhibited or the protein might be stored in the cell in an inactive form. Only in a few cases has the presence of the protein been demonstrated and related to the time of gene expression.

In the context of gene manipulation, it is an advantage that genomic sequences conferring temporal and tapetum-specific expression seem to be conserved among distantly related plant species. After the discovery that the regulatory genomic DNA sequence of the *Nicotiana tabacum* ta29 gene confers tapetum-specific expression in a heterologous host (Mariani *et al.* 1990), regulatory sequences of *Brassica*, the monocot *Oryza sativa*, and *Antirrhinum majus* have been shown to direct temporal and tapetum-specific expression in *Nicotiana*. This conservation of regulatory sequences makes it possible to achieve spatially and temporally restricted expression of genes of choice in a variety of species by fusing them with regulatory DNA fragments. This will lead to sophisticated methods of interfering with pollen development at distinct stages.

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