meiotin-1 gene expression in normal anthers and in anthers exhibiting prematurely condensed chromosomes

C.A. Hasenkampf, A.A. Taylor, N.U. Siddiqui, and C.D. Riggs

Abstract: We have cloned and sequenced the promoter of a meiotin-1 gene, and have determined the precise temporal and spatial pattern of meiotin-1 gene expression. The expression of the meiotin-1 gene is controlled in two increments. The meiotin-1 gene is not expressed in any of the vegetative tissues examined. Early in microsporogenesis, low levels of meiotin-1 RNA can be detected. At the onset of meiosis, there is a dramatic increase in meiotin-1 RNA levels in both tapetal and meiotic cells. However, while meiotin-1 RNA is observed in both the nucleus and cytoplasm of meiotic cells, it is found only in the nucleus of the tapetal cells. We have also examined the expression of the meiotin-1 gene in aberrant meiotic nuclei that prematurely condense their chromosomes; these nuclei have reduced levels of the meiotin-1 protein. The aberrant nuclei have only the basal level of meiotin-1 RNA; they do not exhibit the transcriptional induction seen for normal cells at the onset of meiosis. Implications for the function of meiotin-1 in regulating chromatin condensation, and in coordinating meiotic and tapetal cell activities are discussed.

Key words: anther development, chromatin, meiosis, meiotin-1, promoter.

Introduction

Meiosis is the specialized nuclear division associated with sexual reproduction. During meiosis the chromosome number is reduced, typically to the haploid level (1n). Subsequent to the reduction in chromosome number, reproductive cells with the reduced chromosome number participate in fertilization to reconstitute the diploid state (2n).

We study meiosis in lily anthers. To successfully produce viable pollen, the events of meiosis must be carefully coordinated with anther development. Early in its development, the anther is an undifferentiated mass of meristematic cells covered by an epidermis. By the time meiosis is initiated, each anther consists of four locules serviced by a central vascular region. Each locule contains a large central chamber containing the microsporocytes that will undergo meiosis and ultimately differentiate into pollen grains. Immediately surrounding the microsporocytes is a single cell layer known as the tapetum. The cell layers that surround the tapetum are collectively known as the anther wall.

Prior to and during meiosis, the tapetal cells serve as nurse cells to the meiotic cells; they synthesize and provide metabolites to the microsporocytes (Gifford and Foster 1989). The tapetal cells and the microsporocytes are connected by plasmodesmata throughout their development until mid-prophase I of meiosis (Spitzer 1970). At approximately the pachytene substage of prophase I, the plasmodesmata are lost as the microsporocytes become surrounded by a callose cell wall (Heslop-Harrison 1966). Even after the direct cytoplasmic connections are lost, the tapetal cells secrete materials onto the microsporocytes. For example, at the end of the second meiotic division, the tapetal cells secrete the enzyme callase to dissolve the callose barrier surrounding the micro-
spores (Stieglitz 1977). The microspores develop into pollen grains that are coated by materials which are synthesized and secreted by the tapetal cells.

Thus, until the second half of meiotic prophase I, the tapetal cells and meiotic cells are in intimate contact, and their behavior appears to be coordinated. While the meiotic cells are synchronized and in prophase I of meiosis, the tapetal cells also appear to be synchronized. As the meiotic cells enter the second half of prophase I, the tapetal cells undergo a synchronized mitosis. Following their mitosis, the tapetal cells undergo endo-replication of the chromosomes (Heslop-Harrison and MacKenzie 1967) and synthesize large amounts of materials needed for maturation of the microspores into pollen grains (Heslop-Harrison 1968). Arrest of tapetal cell development causes failure to produce mature pollen (Kaul 1988).

The extensive cytoplasmic connections between microsporocytes and tapetal cells during prophase I of meiosis, the synchronization of the chromosomal events in the two cell types, and the fact that production of viable pollen requires both cell types all suggest that events in the two cell layers are carefully coordinated. In our labs, we have worked to identify meiosis-specific proteins and their genes. Our purposes have been two-fold: One purpose has been to identify the biomolecules involved in determining meiotic chromosome behavior during prophase I of meiosis. Another goal has been to identify meiosis- and microsporogenesis-specific promoters and transcription factors to understand how the many necessarily parallel events of meiosis might be coordinated with each other and with events occurring in the rest of the anther.

To this end, we have targeted a 43-kDa, DNA-binding protein that was present in preparations of purified microsporocytes only in the developmental interval surrounding meiosis. The protein was purified and an antiserum was generated against it. The antiserum reacted with one protein found within anther extracts during the meiotic interval, and did not react with proteins in any of the vegetative tissues tested. Because of its apparent meiosis specificity the protein was designated as meiotin-1 (Riggs and Hasenkampf 1991).

Meiotin-1 is an abundant, histone H1 - like chromosomal protein that is first obvious in microsporocyte nuclei as they enter the interphase that immediately precedes meiosis. Meiotin-1 does not replace the normal histone H1, but occurs in addition to it (Riggs and Hasenkampf 1991). The structure of the meiotin-1 protein is similar to histone H1 except that it lacks the charged N-terminal region that histone H1 molecules possess (Riggs 1994). Meiotin-1 reaches peak levels at the first stage of prophase I (leptotene), continues to be present through pachytene, then is removed from the chromosomes at, or before, the last stage of prophase I (diakinesis). We have proposed that the role of meiotin-1 is to block the last level of chromosome condensation until after the homologous chromosomes have had the opportunity to become intimately associated and undergo reciprocal genetic exchange (two events that occur in the interval between leptotene and diakinesis) (Qureshi and Hasenkampf 1995; Riggs 1997). This proposal is consistent with the structure of the meiotin-1 protein, its developmental profile in microsporocytes, and with our finding that meiotin-1 is not present in aberrant microsporocytes that undergo precocious chromosome condensation in late interphase and early leptotene (Hasenkampf et al. 1998).

We have cloned the proximal region of a meiotin-1 promoter and here report the DNA sequence and unusual motifs found within it. We also have determined the spatial and temporal pattern of the expression of the meiotin-1 gene in normal anthers and in anthers exhibiting precocious chromosome condensation.

Materials and methods

Cloning of a meiotin-1 promoter

Sequence analysis of the pM1-1 and pM1-3 cDNAs enabled selection of two highly conserved regions in the genes for the design of two gene specific primers for use in suppression PCR (Siebert et al. 1995): GSP1 = 5'-GGATTGGTCTTGTTGCTTGGTGGG-3' and GSP2 = 5'-CTTCCATTGTCGATGCCTCTC-3'. The Universal Genome Walker Kit (Clontech, Palo Alto, Calif.) was employed and used with the two primers as described by the manufacturer. PCR products were purified from agarose gels and used for direct sequencing, and were cloned into pBluescript 2. SURE 2 cells (Stratagene, La Jolla, Calif.) were used to propagate the recombinant plasmids, as recombination proficient strains (e.g., DH5 α) did not give rise to colonies. Sequencing was conducted by Cortec DNA Service Laboratories at Queen’s University (Kingston, Ont.). This sequence has been assigned GenBank accession number AF153203.

Preparation of extracts and gel-shift analysis

Normal anthers from the lily cultivar ‘Enchantment’ were processed to make nuclei as previously described (Riggs and Hasenkampf 1991). The nuclear pellet was homogenized in a small volume of extraction buffer (20 mM N-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) pH 7.9, 0.42 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 1 mM Pefabloc, 3 μM pepstatin A, 0.5 mM dithiothreitol (DTT), and 1 μg/ml E64), and left on ice for 20 min. A second homogenization was then performed and the extract clarified by centrifugation at 25 000 g for 20 min. The supernatant was collected and dialyzed against 20 mM HEPES pH 7.9, 20% glycerol, 0.2 M KCl, 0.2 mM EDTA, then stored in small aliquots at -70°C. For the gel-shift assay, a 268 bp BamHI-XhoI fragment of pM1pro was isolated and radiolabelled with [α-32P]dCTP by a fill-in reaction with the Klenow fragment of DNA polymerase I. Approximately 0.5 ng of the radiolabelled probe was used in 30-μL reactions, which contained 10 mM Tris pH 7.5, 0.5 mM EDTA. The nonspecific competitor, double-stranded poly dI/dC : poly dI/dC (Pharmacia, Que.) was used at 2 μg per reaction. The plasmid pBluescript II SK(−) was restricted with HpaII to generate small fragments for use as nonspecific competitors, and as a specific competitor, unlabelled probe DNA was used at the molar excess ratios indicated in Fig. 2. The volume of extract added was 5 μL, equivalent to 6 μg of nuclear protein. Reactions were conducted at room temperature for 30 min, after which the samples were subjected to electrophoresis on 4% polyacrylamide gels cast and run in 0.2x TBE (Tris, boric acid, EDTA). The gels were fixed and dried, then autoradiography was carried out by standard procedures (Sambrook et al. 1989).

Total RNA extraction and RNA gel-blot analysis

Total RNA was isolated from whole anthers from L. longiflorum (var. nellore white) buds ranging from 6–13 mm in length. In the lily, bud size is a good indicator of developmental stage, and meiotic cells within the same bud are at the same stage of development. Buds were harvested and measured. Three buds of each size were used for staging as indicated in Hasenkampf et al. (1992),
and the remainder were pooled for RNA purification. Anthers were collected in liquid nitrogen. Total RNA from leaves was extracted from approximately 2 g (fresh weight) of tissue by the SDS (sodium dodecyl sulfate) – Hot Phenol method of Verwoerd et al. (1989). For RNA gel-blot analysis, approximately 10 µg of total RNA was separated on a 1% agarose MOPS [3-(N-morpholino) propane sulfonic acid]–formaldehyde gel and transferred onto a nylon membrane. A 1.4-kb KpnI–SpeI fragment of pM1-1 was used to make a random primer labeled probe. Hybridization, high stringency washing, and autoradiography were carried out by standard techniques (Sambrook et al. 1989).

**In situ hybridization**

For the anthers with normal chromosome condensation, three developmental series, from different positions on the flower buds of *Lilium longiflorum*, were performed. Young flower buds were harvested from greenhouse-grown plants. For each developmental series buds of lengths 8, 9, 10, 11, and 12 mm were used. Anthers were removed from the flower buds and prepared for in situ hybridization (ISH). The bud lengths were selected to achieve a range of developmental stages bracketing the onset of meiosis. The anther sections of flower buds from populations undergoing precocious chromosome condensation were collected from field grown buds from the *Lilium* cultivar ‘Enchantment’.

Anthers were removed carefully from the buds, placed in a freshly prepared solution of 4% paraformaldehyde in phosphate-buffered saline (PBS) pH 7.4, and then processed for wax embedding according to Hasenkampf et al. (1998). Slides with sections were placed on a hotplate at 37°C overnight. The next day, slides were placed in a vertical rack, dewaxed and rehydrated according to the following schedule: 100% histoclear 10 min (two changes), 1:1 Histoclear/ethanol 2 min, 100%, 95%, 80%, 70%, 50%, and 30% ethanol, double distilled water, PBS pH 7.4, each for 2 min. All solutions with more than 5% water were prepared from water treated with diethylpyrocarbonate (DEPC). After rehydration, the sections were post-fixed in 4% paraformaldehyde in PBS for 10 min. Endogenous RNases were neutralized by treating slides in two 15-min incubations in PBS containing 0.1% active DEPC. Slides were then equilibrated in 5x SSC (sodium citrate) for 15 min.

The ISH protocol for the study of gene expression was modeled closely after the protocol of Brassant and Wahl (1998). The 5x SSC was replaced with 150 µL prehybridization mix (50% formamide, 5x SSC, 40 µg/mL salmon sperm DNA without probe RNA). Prehybridization was performed at 38°C for 2 h in a moist chamber. The prehybridization solution was removed and replaced with 45 µL hybridization mix (50% formamide, 5x SSC, 40 µg/mL salmon sperm DNA with probe RNA to a final concentration of 400 ng/mL). The hybridization mix was heated to 80°C for 5 min and then placed in the slides over night. The hybridization mix was then removed and slides were placed in a moist chamber and incubated for 2 h at room temperature, then washed 2× 15 min in the incubation buffer. Slides were rinsed in colourimetric buffer (100 mM Tris, 100 mM NaCl, 50 mM MgCl2, pH 9.5) for 5 min. Colourimetric detection was performed at 30°C in a Coplin jar containing 40 mL colourimetric buffer plus 10% (w/v) polyvinyl alcohol (MW 70 000 – 100 000), and 400 µL of premixed solution of nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indoyl phosphate (Roche). For the meiotin-1 sense and antisense probes, the incubations were done overnight, with slides for all developmental stages done in the same jar. For the rRNA positive control, slide incubations were done for only 20–25 min so that the staining intensity would be comparable to that of the meiotin-1 slides. The colourimetric reaction was stopped by placing the slides in a rack and rinsing with running distilled water for 5 min. Slides were then dehydrated and mounted in permount. Analysis was performed, and photomicrographs generated using a Zeiss axiophot microscope and 40x PlanApo oil immersion lens.

**Results**

**Cloning and analysis of a meiotin-1 promoter**

The cloning of a meiotin-1 promoter was undertaken to provide a tool for identifying and characterizing meiotic transcription factors. The initial attempts employed standard techniques of plaque filter hybridization, using two meiotin-1 cDNA probes (pM1-1 and pM1-3, Riggs 1994). We failed to identify any genomic clones; affinity methods employing biotinylated primers and inverse PCR also failed. Simultaneously, attempts were made to clone the *Arabidopsis meiotin-1* gene by generating and screening size-selected DNA. Although DNA gel blots indicated that homologous sequences existed in the size-selected DNA, no positive clones were identified in the subgenomic libraries. Failure to generate authentic genomic clones from *lily*, *Arabidopsis*, and tomato (Jayawardene and Riggs 1994) has led us to conclude that the structure of the meiotin-1 gene is not tolerated by commonly used bacterial hosts (Riggs 1997).

Expression PCR (Siebert et al. 1995) was undertaken and did yield amplification products, but these could be clonally propagated only in recombinant–repair deficient strains of *Escherichia coli* (SURE 2 cells). Sequencing of PCR products and the cloned sequences revealed a continuous sequence linking the previously described pM1-1 cDNA sequence (Riggs 1994) to an uncharacterized region. To ensure the authenticity of this region as part of the meiotin-1 promoter, we utilized the sequence information to design new primers for the amplification of genomic DNA.

Products of predicted size were obtained and the sequences of these perfectly matched that of the original suppression PCR product, suggesting that this promoter drives the expression of the pM1-1 gene (data not shown). Moreover, DNA gel-blot analyses demonstrated the presence of hybridizing bands, the sizes of which are consistent with linkage of the promoter clone and the cDNA clone (Riggs 1994, and data not shown). We conclude that the sequence reported here is the proximal region of a meiotin-1 promoter. Analysis of this sequence revealed several unusual features. Figure 1 illustrates the relative positions of these features. Based on the length of the cDNA and conceptualized trans-
lation, the pM1-1 mRNA has an untranslated leader sequence of 261 nucleotides. This untranslated leader contains several copies of an imperfect 49-bp direct repeat.

This same 49-bp motif is repeated four times in the proximal promoter region. Further upstream from the 49-bp repeats there are two tandem repeats of 30 bp, each harbouring 11-bp tandem subrepeats. The region containing the 30-bp repeats exhibits striking strand asymmetry, with the top strand of a 200-bp region being 67% purines (Fig. 1b). This region punctuates a region of 115 bp in which the top strand is about 70% pyrimidines. Last, there is also a sequence of 93 bp that is 90% identical to the untranslated leader region of a second meiotin-1 cDNA: pM1-3 (Riggs 1994).

We believe that the instability of the promoter is related to the 49-bp repeats. Subclones of the promoter consisting almost exclusively of three copies of the repeats could not be propagated in recombination-repair-proficient strains. We did not test other subclones for instability.

A search of the sequence databases revealed few matches to known sequences. The 30-bp repeat region found in the pM1-1 promoter showed some homology to motifs within a number of plant and animal histone genes. However, the regions of homology were within the coding region of these histone genes, and were deemed likely to be coincidental. (For example, histone H1 molecules are rich in lysine and alanine encoded by AAR and GCN codons (see Jayawardene and Riggs 1994), and this type of repeating motif is found in the pM1-1 promoter). Another match was found when we searched a transcription factor - binding sequence database. There are multiple copies of the motif 5'-NGAAN-3' within the meiotin-1 promoter which match the binding site sequence for heat-shock factor (HSF).

We used two regions of the meiotin-1 promoter in gel mobility shift assays to determine whether meiotic nuclei contain factors that interact with sequences of the promoter. The first probe consisted of three copies of the 49-bp repeats. This fragment gave rise to strong gel-shift patterns, but the bound forms could not be titrated by unlabelled probe (data not shown). It is not clear if this represents authentic binding. The second probe, a 268-bp BamHI–XbaI fragment, contains the region of the 30-bp repeats, the unusual strand asymmetry and the region homologous to the pM1-3 untranslated leader. Figure 2 shows that this fragment binds at least one nuclear factor in a specific fashion, since unlabelled probe competes for binding, but heterologous DNA does not. Preliminary experiments employing nuclear extracts from late meiotic prophase cells did not give rise to this pattern, and we therefore conclude that the factor is specific to early prophase.

meiotin-1 gene expression in normal anthers

We performed RNA gel blotting to examine the levels of meiotin-1 RNA in leaves (Fig. 3, Lf), and in anthers at different stages of anther development (Fig. 3, 6–13 mm). Meiotin-1 RNA was not found in the leaf sample, nor was it seen in any vegetative tissues tested previously (Riggs 1994). The earliest anthers examined contained microsporocytes that were actively dividing by mitosis; they contained a low, basal level of meiotin-1 mRNA (6–8 mm). This basal level continued through early and middle premeiotic interphase (9 mm). Toward the end of premeiotic interphase.
A strong induction occurred, then there was a gradual decline in meiotin-1 RNA levels as prophase I progressed (11–13 mm).

To determine which cell types within the anther were expressing the meiotin-1 gene, we elected to do ISH with sections of anthers. We collected 3 series of flower buds, each from a similar range of developmental stages as performed for the RNA gel blots. All together, we sampled approximately 480 microsporocytes from each anther, for each treatment, for each of the three developmental series. The results are summarized in Table 1.

The results from the third series are typical and are illustrated in Fig. 4. The cell types of interest within each locule are shown in the haematoxylin-stained section of Fig. 4a.

Control sections had the expected results: those hybridized with the positive control (a riboprobe complementary to rRNA) had ISH-staining in all cell types of the anther at all developmental stages examined (e.g., Fig. 4b); sections hybridized with the negative control (sense strand riboprobe for the meiotin-1 gene) had only a low level of background staining (e.g., Fig. 4c).

The signal intensity generated with the riboprobe complementary to the meiotin-1 RNA varied depending on the stage of development and on the cell type within the anther. We found a low basal level of meiotin-1 RNA in the cytoplasm of microsporocytes at the earliest developmental stage we examined. This low level continued into the interphase immediately preceding meiosis (Fig. 4d). A strong increase in meiotin-1 RNA was seen in the microsporocyte cytoplasm near the onset of meiosis (Fig. 4e). This increase disappeared by early zygotene (Fig. 4f). Thus, the profile of meiotin-1 gene expression for microsporocytes examined by ISH fits the pattern seen in the anther RNA gel blots.

A major reason for implementing the ISH analysis was to determine whether the microsporocytes were the only cell type expressing the meiotin-1 gene, or whether the tapetal cells also were expressing it. We found no ISH signal for meiotin-1 RNA in the cytoplasm of the tapetal cells (or another wall cells) at any of the developmental stages examined, despite the fact that the positive controls using rRNA clearly indicate that we can detect RNA in the tapetal cell cyto-

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**Table 1**

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<thead>
<tr>
<th>extract</th>
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<th>+</th>
<th>+</th>
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</thead>
<tbody>
<tr>
<td>p dl/dC</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>268bp</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>pBS2 Hpa II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
</tr>
</tbody>
</table>

(10 mm) a strong induction occurred, then there was a gradual decline in meiotin-1 RNA levels as prophase I progressed (11–13 mm).

Fig. 2. A 268-bp BamHI–XbaI fragment of the meiotin-1 promoter interacts with a nuclear protein. An extract was prepared from early meiotic prophase cells and used in gel-shift experiments with a radiolabelled 268-bp fragment containing the 30-bp repeat region (coordinates 222–480 of Fig. 1). The double-stranded synthetic polymer pdI/dC·pdIdC (p dIdC) was used to abolish nonspecific binding and revealed a bound form (B). The specificity of binding is demonstrated by titration of the bound form to the free form (F) with the unlabelled 268-bp fragment. A nonspecific competitor (pBluescript II cut with HpaII) did not influence binding. The numbers refer to the molar excess ratio of fragments compared to approximately 0.5 ng of labelled probe.

Fig. 3. **Temporal expression of the meiotin-1 gene in lily anthers.** (a) RNA was prepared from a non-expressing tissue, leaves (Lf), and from a series of sized buds representing different stages of meiotic development. The sizes of the buds are expressed in millimeters (6–13 mm). Ten micrograms of total RNA was subjected to RNA gel blotting with a meiotin-1 gene probe. The developmental correlations are: 6–8-mm buds, premeiotic, actively cycling by mitosis; 9-mm buds, interphase; 10-mm buds, premeiotic S phase; 11-mm buds, late S phase to early leptotene; 12-mm buds, leptotene; and 13-mm buds, zygotene. (b) A histogram illustrating the relative amount of meiotin-1 mRNAs found during anther development. The ethidium bromide-stained gel was scanned by densitometry, and the relative amounts of the large rRNA was used to normalize the autoradiographic data in (a). Note that there is an eight-fold induction of meiotin-1 mRNA at the onset of meiosis.
plasm when it is present (Fig. 4b, hollow arrow). Thus, there is no discernible amount of meiotin-1 RNA in the tapetal cell cytoplasm. In contrast to the situation for the cytoplasm, there does appear to be ISH signal for the meiotin-1 RNA in the tapetal nuclei of normal anthers. This is not very obvious when meiotin-1 RNA is at the basal level of expression in the microsporocytes (Fig. 4d and 4f, arrows), but is quite apparent when the meiotin-1 gene is induced to a higher level of expression (Fig. 4e, arrows). A striking observation (apparent in Fig. 4e and 4f) is that the tapetal nuclei with meiotin-1-ISH staining are asymmetrically located in their cytoplasm so as to abut the meiotic cells. The meiotin-1 RNA may be moving directly from the nuclei of the tapetal cells to the meiotic cell cytoplasm.

meiotin-1 gene expression in anthers with prematurely condensed chromosomes

In addition to the normal anthers, we also examined meiotin-1 RNA expression in anthers that were undergoing the phenomenon of precocious chromosome condensation in the late interphase – early leptotene interval (Fig. 4g–i). These aberrant microsporocytes have very reduced levels of the meiotin-1 protein (Hasenkampf et al. 1998). The positive control probe complementary to RNA gave a strong signal in all cell types of these anthers (Fig. 4g), indicating the anthers were appropriately prepared. In the adjacent sections, which were hybridized with the probe complementary to meiotin-1 RNA, we found only a low level of meiotin-1 RNA (Fig. 4h), only moderately higher than the negative control (Fig. 4i). Thus, microsporocytes with precociously condensed chromosomes and reduced levels of meiotin-1 protein have only the basal levels of meiotin-1 RNA (similar to the level seen in Fig. 4d); they fail to undergo the dramatic increase seen in normal cells at the late interphase – early leptotene interval (Fig. 4e).

Discussion

meiotin-1 gene expression in microsporocytes

The results from the RNA gel-blot analysis and from the ISH experiments reveal that meiotin-1 gene expression is regulated in two increments. Meiotin-1 RNA is not expressed in any of the vegetative tissues tested, but was found to be expressed at a low level in anthers well before the onset of meiosis. Near the onset of meiosis, there is a strong induction in normal microsporocytes. High levels of meiotin-1 RNA continue into the leptotene stage of meiosis, but by the zygotene stage meiotin-1 RNA levels have returned to the basal level. Thus, the time of the strong induction immediately precedes the time when we have observed the maximal levels of the meiotin-1 protein (Hasenkampf et al. 1992). Two-stage induction of meiotic RNAs has been previously reported (e.g., Kassir et al. 1988). In plants, the LIM17 and LIM18 genes are transcribed at low levels in young anthers and during premeiotic interphase, and are up-regulated at zygotene (Kobayashi et al. 1994). The LIM17 sequence does not match any known clone in the sequence database, but LIM18 is the lily cognate of HSP70. In maize, members of at least two different heat-shock gene families have been shown to be up-regulated during meiosis in the absence of heat stress (Atkinson et al. 1993; Marrs et al. 1993).

We have cloned a meiotin-1 gene promoter and have used subcloned fragments in gel-shift assays. In these assays, we have observed that the region of the promoter that contains the 30-bp repeat, the unusual strand asymmetry, and the region of homology with the pM1-3 untranslated leader, interacts specifically with at least one nuclear protein. Within this 268-bp fragment there are also multiple copies of the motif 5'-NGAAN-3', which is the consensus sequence for the binding of heat-shock factor (HSP). However, the arrangement of this motif in the meiotin-1 promoter is not the same as that described for optimal HSF binding (Fernandes et al. 1994), in response to heat shock. It is possible that the arrangement of heat-shock motifs in the meiotin-1 promoter may be customized to produce a meiosis-specific pattern of gene expression. It has been observed that some heat-shock-protein genes are induced during meiosis in the absence of heat shock. Once conditions for stronger binding can be optimized, we will undertake DNA footprint analysis to deter-

Table 1. Meiotin-1 RNA levels in microsporocytes of lily anthers.

<table>
<thead>
<tr>
<th>Series</th>
<th>Bud length</th>
<th>Stage of microsporocyte</th>
<th>Level of expression</th>
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<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Actively cycling</td>
<td>Basal level</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>Actively cycling</td>
<td>Basal level</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Premiotic interphase</td>
<td>Basal level</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>Late premeiotic interphase–leptotene</td>
<td>Induced level</td>
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<tr>
<td>12</td>
<td>8</td>
<td>Leptotene</td>
<td>Intermediate level</td>
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<td>2</td>
<td>8</td>
<td>Actively cycling</td>
<td>Basal level</td>
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<tr>
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<td>Premiotic interphase</td>
<td>Basal level</td>
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<td>10</td>
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<td>Late premeiotic interphase–leptotene</td>
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<tr>
<td>11</td>
<td>8</td>
<td>Leptotene</td>
<td>Intermediate level</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>Leptotene–early zygotene</td>
<td>Intermediate level</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>Actively cycling</td>
<td>Basal level</td>
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<td>10</td>
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<td>Early leptotene</td>
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<td>Leptotene–early zygotene</td>
<td>Intermediate level</td>
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</table>

Note: Each value represents results from approximately 480 microsporocytes.
mine which of the motifs within the 268-bp fragment are responsible for the observed binding.

The strong induction of the meiotin-1 gene at the onset of meiosis, appears necessary for normal meiotic chromosome behavior. In this study we have shown that the cells with precociously condensed chromosomes fail to undergo this strong induction of the meiotin-1 gene at the onset of meiosis. They have only the basal level of meiotin-1 RNA. The failure of this induction is the likely reason why these nuclei have reduced levels of meiotin-1 protein. The reduced levels of both meiotin-1 RNA and meiotin-1 protein in microsporocytes with precociously condensed chromosomes are consistent with our hypothesis that threshold levels of meiotin-1 in microsporocytes are needed in a normal meiosis, to block chromosome condensation during prophase I until after reciprocal genetic exchange.

meiotin-1 gene expression in tapetal cells

In a previous study (Qureshi and Hasenkampf 1995), we found that meiotin-1 protein was present in the tapetal nuclei as well as the microsporocyte nuclei. We have proposed that meiotin-1 blocks tapetal cell chromosome condensation, and hence blocks the completion of mitosis. In both microsporocytes and tapetal cells, meiotin-1 accumulates in the nuclei prior to the onset of meiosis, and reaches the peak level by the time the microsporocytes are at the leptotene stage of meiosis. Beyond this developmental interval, the relative amounts of meiotin-1 in the two cell types changes. The meiotic cells maintain their peak level of meiotin-1 protein until after the pachytene stage of their development. While the meiotic cells are entering pachytene, meiotin-1 protein levels within the tapetal nuclei are declining, and this is temporally correlated with a synchronized mitotic division of these tapetal cells. After this, their last mitosis, the tapetal cells endo-reduplicate their chromosomes and begin a phase of intense metabolic activity that is essential for development of the microspores into mature pollen. Thus, the presence of meiotin-1 in both cell types may keep the last

Fig. 4. Meiotin-1 and rRNA in situ hybridization on sections of lily anthers. Parts (a–f) are from plants with normal meiosis. Parts (g–i) are from a plant with precociously condensed chromosomes. In (a), one anther locale stained with Erhlich’s haematoxylin is given to illustrate the three cell types. The microsporocytes (M) occupy the central region of each locale, and are surrounded by a single layer of tapetal cells (T). Peripheral to the tapetum is the anther wall (AW). (b) Illustrates results with the positive control, a riboprobe complementary to the rRNA. The hollow arrow draws attention to an example of cytoplasmic hybridization signal. (c) Illustrates the negative control, a riboprobe identical to a portion of the meiotin-1 RNA. Parts (d–f) are from sections hybridized with the riboprobe complementary to a portion of the meiotin-1 RNA and illustrate the developmentally regulated expression of the meiotin-1 gene. Arrows point to some examples of tapetal nuclei. (d) Is from an anther in premeiotic interphase; (e) is of an anther at early leptotene; and (f) is from an anther at the transition between leptotene and zygotene. The positive and negative control shown are both from the same anther as (e). Parts (g–i) are all from one lily anther with precociously condensed chromosomes. (g) Illustrates results with the positive control, a riboprobe complementary to the rRNA. (h) Shows the level of hybridization found with the riboprobe complementary to a portion of the meiotin-1 RNA. (i) Illustrates the negative control, a riboprobe identical to a portion of the meiotin-1 RNA. Bar = 50 μm.
chromosome division of the microsporocytes and the last chromosome division of the tapetal cells in sync, so that the tapetal cells will undergo their burst of metabolic activity at the time appropriate for the production of viable pollen.

In the work reported here, we have seen a dramatic increase in the meiotin-1 RNA levels in normal anthers, in both tapetal and microsporocyte nuclei in the developmental interval corresponding to the onset of meiosis. Both cell types seem to be responding to a signal to increase the level of meiotin-1 RNA. The fate of the RNA in the two cell types is different. In the meiotic cells, the meiotin-1 RNA is found in both the nucleus and the cytoplasm. The increased levels of meiotin-1 RNA in the meiotic cytoplasm contributes to the dramatic increase in meiotin-1 protein seen between premeiotic interphase and leptotene. In the tapetal cells, even though the level of meiotin-1 RNA increases in the tapetal nuclei, there is no visible accumulation in its cytoplasm. Therefore, the difference between the meiotic and tapetal cells, in meiotin-1 protein levels seen at the pachytene developmental interval is likely due to the different fates of the meiotin-1 RNA in the two cell types in the second induction interval.

At this point, we can only speculate why meiotin-1 RNA does not accumulate in the tapetal cell cytoplasm. The meiotin-1 RNA may not be processed and shipped from the nucleus at all, or it might be shipped from the nucleus only to be rapidly degraded in the cytoplasm. Another intriguing possibility is that meiotin-1 RNA is being shipped directly from the tapetal nuclei to the meiotic cytoplasm via the plasmodesmata that exist between the meiotic and tapetal cells. Several recent studies have demonstrated that plants possess a macromolecular trafficking system that directs the transport of specific RNA molecules through plasmodesmata. One interesting example is that of the KNOTTED-I homebox factor from maize, which influences shoot apical meristem development. Lucas et al. (1995) have shown that sense RNA, but not antisense RNA for the knotted-I gene is transported to neighboring, non-expressing cells. Kuhn et al. (1997) used in situ hybridization, combined with transmission electron microscopy, to detect movement of specific mRNAs from the companion cells to the sieve elements via plasmodesmata. A similar approach could be used in our system to determine if meiotin-1 RNA is moved via plasmodesmata from the tapetal to the meiotic cells. The possibility of transport from tapetal cells to the microsporocytes is supported by the observation that the tapetal nuclei at this stage are asymmetrically located in their own cytoplasm, so as to be positioned very close to the microsporocytes. By such a transport mechanism, the tapetal cells might contribute to the observed, rapid accumulation of the meiotin-1 RNA (and meiotin-1 protein) in the microsporocytes without increasing the amount of meiotin-1 protein in their own cells.

Acknowledgements

CAH and CDR acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) for grants supporting this project. The authors wish to thank Dr. François Belzile (Laval University, Que.) for his assistance in providing evidence of meiotin-1 cognates in Arabidopsis and for making and screening size-selected libraries.

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