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A meiotic time-course for *Arabidopsis thaliana*

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Abstract A meiotic time-course for *Arabidopsis* pollen mother cells has been established based on BrdU pulse-labelling of nuclear DNA in the meiotic S-phase. Labelled flower buds were sampled at intervals and the progress of labelled cells through meiosis assessed by anti-BrdU antibody detection. The overall duration of meiosis from the end of meiotic S-phase to the tetrad stage, at 18.5°C, was 33 h, which is about three times longer than the mitotic cell cycle in seedlings. The onset of leptotene was defined by reference to the loading of the axis-associated protein Asy1, and this permitted the detection of a definite G2 stage, having a maximum duration of 9 h. It is likely, from two independent sources of evidence, that the meiotic S-phase has a duration similar to that of G2. The durations of leptotene and zygotene/pachytene are 6 h and 15.3 h, respectively, but the remaining meiotic division stages are completed very rapidly, within 3 h. The establishment of a meiotic time-course provides a framework for determining the relative timing and durations of key molecular events of meiosis in *Arabidopsis* in relation to cytologically defined landmarks. In addition, it will be important in a broader developmental context for determining the timing of epigenetic mechanisms that are known or suspected to occur during meiosis.

Keywords Arabidopsis · Meiosis · Time-course · Duration · BrdU

Introduction

Understanding of the meiotic process has been greatly advanced by biochemical, cytological and genetic analyses of the budding yeast *Saccharomyces cerevisiae* (Zickler and Kleckner 1998, 1999), and increasingly in a

number of other model organisms, including *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, *Drosophila*, and mouse. More recently *Zea mays*, *Oryza sativa* and *Arabidopsis thaliana* have been added to this list, as model monocotyledonous and dicotyledonous plants, respectively. *Arabidopsis* is increasingly recognised as a useful system for analysis of plant meiosis, principally because of its simple genome, which has now been sequenced, its short life-cycle and ready access to excellent genetic resources including large pools of knockout mutants (Bhatt et al 2001; Armstrong and Jones 2003; Caryl et al. 2003).

In order to gain a better understanding of the events occurring in *Arabidopsis* meiosis, they need to be placed in a temporal framework of the meiotic pathway. The analysis of yeast meiosis has been greatly facilitated by the ability to generate cultures that are highly synchronised, enabling a meiotic time-course to be established in which the key molecular events of recombination at the DNA level are set in the context of nuclear and chromosomal changes. Thus, programmed double-strand breaks in DNA appear during leptotene and peak at 3.8 h after transfer of cells to sporulation medium, following elaboration of axial elements (AE) but before synaptonemal complex (SC) formation. Single-end invasions (SEI) appear about 1 h later, during zygotene and coincident with SC formation. At 5 h post transfer, SEI disappear, during pachytene, and are replaced by double Holliday junctions; crossovers finally appear at the end of pachytene, at 5.5 h post transfer (Padmore et al. 1991; Hunter and Kleckner 2001).

The duration of meiosis has previously been investigated only in relatively few plant species. An advantage of plant meiosis, in terms of establishing a temporal framework, is that for many species there is a degree of synchrony between pollen mother cells (PMC) within anthers and also between anthers in single flower buds. Early experimental investigations to determine the duration of meiosis in plants relied on direct cytological observations in species with accessible large anthers and several developmentally synchronised anthers per floret,

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such as lily (Erickson 1948) and cereals (Bennett et al. 1971). The technique involved sampling single anthers or anther segments from inflorescences, recording the stage of meiosis in the PMC and subsequently sampling the remaining anthers or anther segments from the same inflorescences after specific time intervals (repeated anther sampling). Other methods based on direct cytological observations involved *in vitro* culturing of anthers or PMC, (Lima-de-Faria 1950; Taylor 1950; Ito and Stern 1967).

Less intrusive techniques for estimating the duration of meiosis in both plant and animal species have involved pulse labelling of DNA with tritiated thymidine during pre-meiotic DNA synthesis (the meiotic S-phase) and determining the minimum times required for labelled cells (detected by autoradiography) to reach cytologically distinct meiotic stages. This technique has been widely applied to animal species, including *Drosophila* (Chandley and Bateman 1962), *Triturus vulgaris* (Callan and Taylor 1968), and mouse (Kofman-Alfaro and Chandley 1970). Some early experimental investigations of meiotic duration in plants also relied on this approach (Lima-de Faria 1965; Bennett et al. 1971). Since meiocytes cannot be successfully cultured *in vitro* from meiotic S-phase through to the completion of meiosis, these labelling experiments must, of necessity, be conducted *in vivo*. In plants, a variety of methods has been used to deliver the label to the meiocytes, through cut flower stems or wicks threaded through stems or, alternatively, by introducing the label directly by injection into flower buds (Bennett 1971 and references therein).

For many purposes tritiated thymidine labelling of replicating DNA has been supplanted by labelling with the thymidine base analogue bromodeoxyuridine (BrdU). This has the advantage that it can be detected immunocytologically by an anti-BrdU antibody carrying a fluorescent tag, thus avoiding the more technically difficult and time-consuming autoradiographic detection and also giving much more precise localisation. BrdU labelling and antibody detection has been successfully applied to mammalian meiosis (Latos-Bielenska and Vogel 1990) and also, more recently, to plant meiosis (Armstrong et al. 2001). In this study BrdU labelling has been used to produce a temporal framework for meiosis in *Arabidopsis* that will form a basis for further analyses of the timing and relationships of events within the meiotic pathway.

Materials and methods

Plant material

Seeds of *A. thaliana* accessions Wassilewskija (Ws) and Columbia (Col0.) were sown on a soil-based (John Innes) compost. Plants were grown in a glasshouse with a 16 h light cycle, with supplementary light when necessary. The temperature was continually monitored and was within the range of 18–23°C.

Marking the meiocyte S-phase

Flowering stems from well-grown plants, with young inflorescences, were cut under water to a length of about 6 cm. The cut ends were quickly transferred to BrdU solution (10^{-2} M), and left for up to 2 h for uptake of BrdU via the transpiration stream and its incorporation into cells in S-phase. After 2 h the stems were removed from the BrdU solution and the ends immersed in tap water. The labelled cut flower stems were maintained in a controlled environment glasshouse chamber at 18.5°C (range 17–20°C).

For analysis of BrdU incorporation, buds were removed from the stems and fixed in 3:1 absolute ethanol:acetic acid at room temperature overnight and, after replenishing the fixative, stored at –20°C. Inflorescences were fixed immediately before the pulse (control), at the end of the 2 h pulse (time point 0 h) and then at 4-hourly intervals up to 46 h. Two inflorescences, each consisting of several flower buds, were fixed at each time point. This procedure was repeated five times, in four cases using the Ws accession and in one case using the Col0 accession.

Preparation of slides for BrdU labelling

The method already described by Armstrong et al. (2001) was used, with some modifications. Single fixed inflorescences were transferred to a black watch-glass containing 3:1 fixative (absolute ethanol:glacial acetic acid) and individual flower buds were separated. Buds between 0.2 mm and 0.4 mm (buds between meiotic interphase and completing meiosis; Armstrong and Jones 2003) were then washed twice in 10 mM citrate buffer pH 4.5 (buffer stock 0.1 M citric acid: 0.1 M sodium citrate, diluted 1:10 for working solution) at room temperature. The buds were then incubated in an enzyme mixture comprising 0.3% (w/w) pectolyase and 0.3% cellulase (both Sigma, St. Louis, Mo.) in citrate buffer for 75 min. Replacing the enzyme mixture with ice-cold citrate buffer stopped the reaction. Single buds, ordered by size, were transferred to slides with a small volume of buffer using a Pasteur pipette. Each bud was tapped out in buffer using a fine needle, before adding 5 µl 60% acetic acid, and placing briefly on a hot block at 45°C. A further 5 µl acetic acid was then added, and material was finally refixed with about 200 µl cold 3:1 fixative, added as a ring around the suspension on the slide. The fixative was drained away and the slide dried with a hair drier. A series of slides was obtained from each inflorescence graded according to increasing bud size and progressively later meiotic stages.

Detection of BrdU

BrdU was detected immunologically using an anti-BrdU (mouse monoclonal antibody) kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. Slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI; 1 µg/ml) in Vectashield antifade mounting medium (Vector Laboratories, Burlingame, Calif.).

Preparation of slides for dual BrdU and ASY1 antibody labelling

Individual flower buds were dissected from inflorescences that had been exposed to a 2 h BrdU pulse and sampled at 2, 4, 8, 12, 16, 20 and 24 h after the start of the pulse, as described above. All anthers from buds less than 0.2 mm were collected from single inflorescences (usually from about five buds), placed directly onto glass slides in 5–10 µl digestion mixture (0.4% cytohelicase, 1% polyvinylpyrrolidone, 1.5% sucrose; Albini 1994) and left in a moisture chamber for 2 min. The anthers were then gently tapped out in the digestion mixture to release PMC. To prepare the spreads, 10 µl Lipsol spreading medium (1% Lipsol detergent in freshly distilled water buffered to pH 9.0 with borate buffer) was added, and left for a further 2 min. Fixation of the cells onto the slides was

accomplished by adding 10 µl fixative (freshly made 4% paraformaldehyde pH 8.0) and allowing the slides to dry in a fume cupboard. When dry, the slides were briefly washed in 0.01% Photoflo (Kodak) in distilled water.

Dual labelling

The prepared slides were washed twice in PBS +0.1% Triton for 2×5 min. Primary antibody (100 µl; polyclonal anti-ASY1 raised in rabbit), diluted 1:500 in blocking buffer [PBS +0.1% Triton +1% BSA (Sigma)] was applied to the slides, covered with Parafilm and incubated overnight at 4°C in a moist box. The slides were then washed 2×5 min before adding 100 µl secondary antibody (anti-rabbit biotin, 1:50 in blocking buffer; Sigma), covered with Parafilm and incubated for 30 min at 37°C. This step protects the primary antibody against the subsequent BrdU antibody detection step. The slides were next treated with the first step of BrdU detection according to the manufacturer's instructions. After this step, Cy-3 streptavidin (1:400 in blocking buffer) was used to conjugate to biotin. The cells were left for 30 min at 37°C as described above, washed 2×5 min in washing solution and finally anti-BrdU was detected according to the manufacturer's instructions. The slides were mounted in DAPI as already described.

Microscopy and image analysis

Slides were examined by fluorescence microscopy using a Nikon Eclipse T300 microscope. Capture and analysis of images was achieved using an image analysis system (Digital Scientific, Cambridge, UK). The slides were screened for meiotic stages and recorded as BrdU- and or Asy1-positive (labelled) or negative (unlabelled).

Results

Uptake of BrdU via the transpiration stream

Preliminary experiments showed that BrdU reaches the meiocytes in anthers and is incorporated into nuclear DNA during the meiotic S-phase within 1 h of the cut stems being placed in BrdU solution. However, the level

of BrdU incorporation and labelling, though readily detectable, is rather low after 1 h and for this reason the duration of the pulse was extended to 2 h, when the labelling was noticeably heavier. Upon removal of the flower stems from BrdU and transfer to tap water, some free BrdU must obviously still be present in anthers and in the transpiration stream. These experiments gave no indication of the time-span of persistence of BrdU in the internal plant environment, but there is clear evidence from other, related, experiments that it is depleted rapidly (Mercier et al. 2003).

Four separate time-course experiments were conducted using flowering stems from plants of the Ws accession. The time of first appearance of BrdU label at progressively later defined stages was recorded at each sampling time. Detailed observations for one such time course experiment are given in Table 1 to illustrate the form of the raw data. In Table 2 the average times of first appearance of labelled cells at different stages from the four Ws experiments are given together with the estimated average durations of the different phases.

Partitioning meiotic interphase

The interphase preceding meiotic prophase I (meiotic interphase) is relatively prolonged and uniform in appearance, with only small and subtle alterations in nuclear morphology. PMC at this stage of development are large with abundant cytoplasm and large nuclei containing prominent large centrally located nucleoli and with chromocentres marking the pericentromeric heterochromatin and nucleolus organising regions. There is some variation in nuclear size and in the degree of condensation of chromocentres during this interphase, but on their own and without supporting evidence these are insufficient to consistently and reliably identify sub-phases (G1, S, G2) of interphase.

Table 1 Detailed observations from a single time-course experiment using the Ws accession of *Arabidopsis thaliana*. The numbers of BrdU-labelled cells are listed for different meiotic stages at each time point together with the total numbers of cells observed and the

percentages of labelled cells (in parentheses). Time point 0 h is the end of the 2 h BrdU labelling pulse. The control samples were collected and fixed at the start of the 2 h pulse

Meiotic stage	Interphase	Leptotene	Zygotene/Pachytene	Diplotene	Diakinesis/metaphase I	Metaphase II/tetrad
-2(control)	0/20(0)	0/20(0)	0/20(0)	0/20(0)	0/20(0)	0/20(0)
0	25/43(58)	0/10(0)	0/15(0)	0/5(0)	0/10(0)	0/20(0)
2	11/28(39)	0/22(0)	0/19(0)	0/1(0)	0/5(0)	0/20(0)
6	29/43(67)	0/21(0)	0/34(0)	0/26(0)	0/32(0)	0/25(0)
10	15/38(39)	6/13(46)	0/29(0)	0/7(0)	0/4(0)	0/10(0)
14	5/11(45)	7/8(88)	2/35(6)	0/7(0)	0/13(0)	0/21(0)
18	16/24(67)	15/19(79)	2/52(4)	0/6(0)	0/10(0)	0/14(0)
22	10/11(91)	10/12(83)	11/13(85)	0/10(0)	0/20(0)	0/20(0)
26	5/22(23)	5/9(56)	11/30(37)	0/24(0)	0/13(0)	0/19(0)
30	14/30(47)	15/22(68)	11/15(73)	3/8(38)	3/5(60)	0/14(0)
34	7/18(39)	3/7(43)	20/22(91)	6/7(86)	2/2(100)	14/29(48)
38	5/10(50)	3/3(100)	6/12(50)	3/3(100)	6/8(75)	4/10(40)
42	9/24(38)	7/11(64)	22/36(61)	10/10(100)	4/4(100)	16/27(59)
46	0/1(0)	7/11(64)	22/26(85)	8/8(100)	5/5(100)	17/20(85)

Table 2 Average times of first appearance of BrdU labelling at progressively later meiotic stages after a 2 h pulse label; time point 0 h corresponds to the end of the 2 h pulse. The stage durations are derived from the differences between labelling times of consecutive stages (see text for details)

Meiotic stage	Average time to first labelling (h)	Range	Stage duration (h)
G2	0		9 (10–1)
Leptotene	10	0	6
Zygotene/pachytene	16	14–18	15.3
Diplotene	31.3	30–34	0.7
Diakinesis, Metaphase I	32	30–34	2
Metaphase II, tetrad	34	0	

After a short pulse of BrdU only cells in S-phase should be labelled, and therefore this has the potential to identify and characterise the S-phase cells, although it should be borne in mind that under the conditions of this experiment a small proportion of labelled cells will have progressed into G2 during the course of the 2 h pulse. The labelled S-phase cells (Fig. 1a) are relatively large, with large nuclei, prominent nucleoli and chromocentres that are more diffuse and extended than in other interphases. The unlabelled interphases consist of two sub-groups that are morphologically different, principally in size. One sub-group consists of large cells, similar in size to the S-phase cells, with large nuclei and nucleoli and with more condensed chromocentres (Fig. 1b). These were presumed, because of their size, to correspond to meiotic G2 and this is confirmed by the observation that at progressively later sampling times this sub-group of cells appear labeled, as S-phase cells progress into and through G2. The remaining sub-group of small interphase cells (Fig. 1c), by a process of elimination are presumed to be G1 cells. This is confirmed by the observation that they do not appear labelled at early sampling times, although they do so at later sampling times as cells that were labelled in earlier asynchronous (mitotic) cell cycles reach meiotic G1. These cells have small nuclei with diffuse chromatin, small dense chromocentres and again the prominent centrally located nucleoli that characterise interphase meiocytes.

The durations of meiotic G2 and S-phase

In principle the duration of G2 can be determined as the time taken for BrdU labelled cells to progress from the end of S-phase to the onset of prophase I (leptotene). However, estimating G2 duration from these time-course experiments is complicated by the following factors: (1) Defining the onset of leptotene. The transition from G2 to leptotene is gradual as the chromatin undergoes reorganisation and AE appear progressively, at first in short stretches that extend until they run continuously along the entire lengths of all chromosomes. In these experiments early leptotene was defined as the stage at which definite linear structures could be discerned, albeit in short discontinuous stretches. Because the early leptotene AE stretches are indistinct in DAPI-stained nuclei, the time of their first appearance in time-course experiments has been defined by dual-labelling immunofluorescence using the ASY1 antibody (Armstrong et al. 2002), in combination

with the BrdU antibody (Fig. 2). The ASY1 antibody colocalises with AE and SCs during prophase I. This protein is first detected in meiotic interphase as punctate foci, but in early leptotene short linear signals appear, marking the start of AE assembly. (2) Duration of the pulse label. The application of a 2 h BrdU pulse, to increase labelling intensity, will inevitably cause some overestimation of G2 duration, by about 1 h, since at time 0 (the end of the pulse) some labelled cells will have advanced about 1 h into G2. (3) Sampling intervals. In order to cover a long sampling period of 46 h, most sampling intervals were 4 h. This introduces a degree of imprecision in timing the boundaries of key stages, such as G2–leptotene. However, this is partly mitigated by averaging the durations obtained from four time-course experiments.

Labelled early leptotene cells were first observed 10 h after the end of the pulse label, and this was also the average time of label appearance at this stage from four experiments. To allow for the progression of some cells from S-phase into G2 during the pulse, 1 h is deducted, to give a best estimate of the duration of G2, from these experiments, of 9 h. However, since the sampling interval at this part of the time-course experiment was 4 h, the actual duration of G2 could be less than 9 h, but must be at least 5 h since the labelled cells had not reached leptotene at the 6 h time point.

The duration of S-phase cannot be estimated directly from these experiments, but an indirect estimation can be made. S-phase and G2 present a relatively homogeneous population of cells in terms of size and nuclear morphology, with some small differences in chromocentre condensation. At time 0 h, at the end of the 2 h pulse, 55–60% of this population of cells, over the four Ws time-course experiments, were labelled. Allowing for the progression of some labelled cells from S-phase into G2 during the 2 h pulse, it is likely that approximately 50% of the population are in S-phase and 50% in G2. It follows that S-phase and G2 are of roughly equal durations and therefore the duration of S-phase will also lie in the 5 h to 9 h range.

Duration of the meiotic division stages (leptotene to tetrads)

The durations of the remaining meiotic division stages, from leptotene onwards were determined by recording the times of first appearance of BrdU label in consecutive

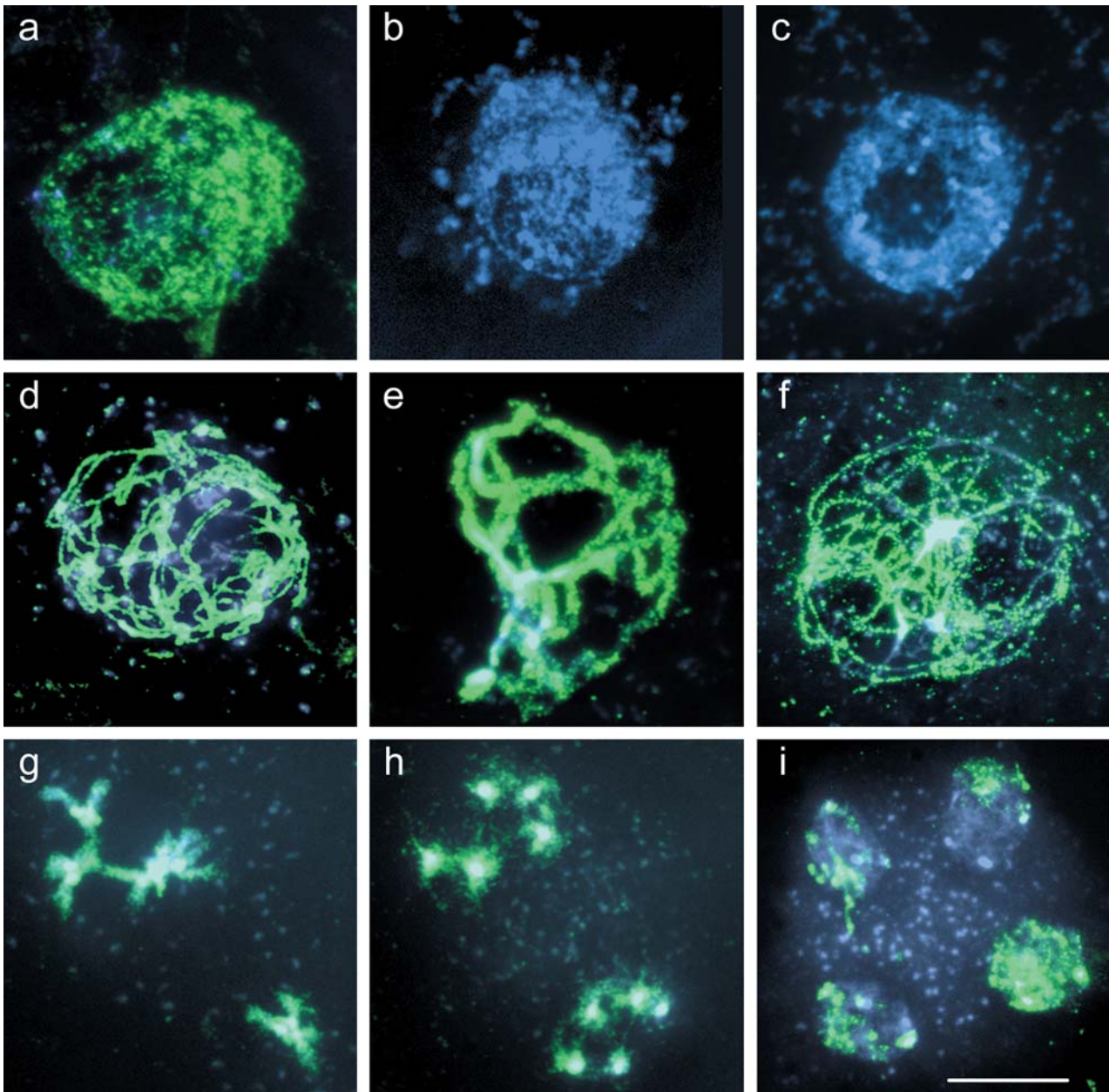


Fig. 1a–i Detection of BrdU labelling of *Arabidopsis* pollen mother cells (PMC) by fluorescein isothiocyanate (FITC)-conjugated anti-BrdU antibody (*green*), counterstained with 4',6-diamidino-2-phenylindole (DAPI). **a–c** Cells sampled at time-point 0 h showing FITC-labelled nucleus of an S-phase cell (**a**) and

unlabelled DAPI-stained nuclei of G2 (**b**) and G1 (**c**) cells. **d–f** Appearance of BrdU labelling (*green*) at successively later meiotic stages of leptotene (**d**), pachytene (**e**), early-diplotene (**f**), late-diplotene/diakinesis (**g**) metaphase II (**h**) and tetrads (**i**). Bar 10 μm

stages. There should be less ambiguity in these determinations, compared to G2, because the stages involved are better defined and more readily recognised. Thus the average time to first appearance of BrdU label at leptotene and zygotene/pachytene are 10 h and 16 h, respectively, from which the average duration of leptotene is 6 h. Similarly, the average duration of zygotene/pachytene is 15.3 h, while diplotene is much shorter at 0.7 h. The remaining first and second division stages (diakinesis, metaphase I, interkinesis, and prophase II,

metaphase II, anaphase and telophase II) are completed very rapidly, within 2 h. The durations of all the meiotic stages from the end of the S-phase to the appearance of tetrads are summarised diagrammatically in Fig. 3.

The average overall duration of meiosis in *A. thaliana*, from the end of the meiotic S-phase to the appearance of tetrads is therefore 33 h under the conditions of these experiments. In addition to the four Ws time-course experiments, a single experiment was conducted using the Col0 accession of *Arabidopsis*. The timings and durations

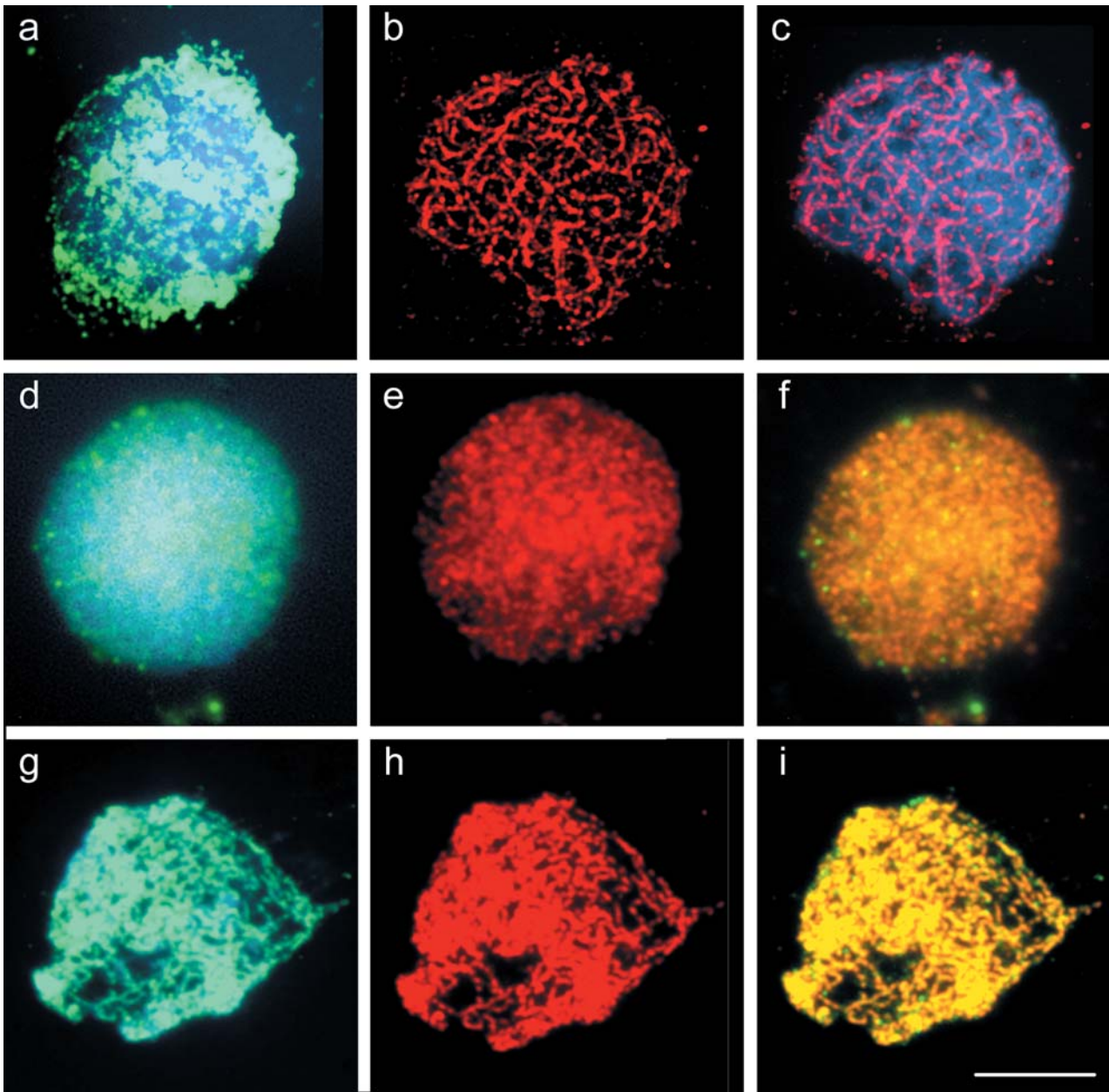


Fig. 2a-i Simultaneous detection of incorporated BrdU and expressed ASY1 protein in PMC of *Arabidopsis* by means of anti-BrdU (*green*) and anti-ASY1 (*CY3 red*) antibodies. **a-c** Cells sampled at time-point 0 h showing BrdU labelled S-phase nucleus (**a**). At this sampling time leptotene nuclei are not labelled with BrdU but are expressing ASY1 protein (**b, c**). Counterstaining of **a**

and **c** with DAPI. **d-i** Cells at very early leptotene, sampled at the 10 h time point (**d-f**) and late-zygotene/early pachytene, sampled at 22 h (**g-i**); **d, g** FITC labelling, counterstained with DAPI, showing that these cells are BrdU-labelled; **e, h** CY3 labelling to show that ASY1 protein is expressed in these cells; **f-i** merged images (FITC + CY3). *Bar* 10 μ m

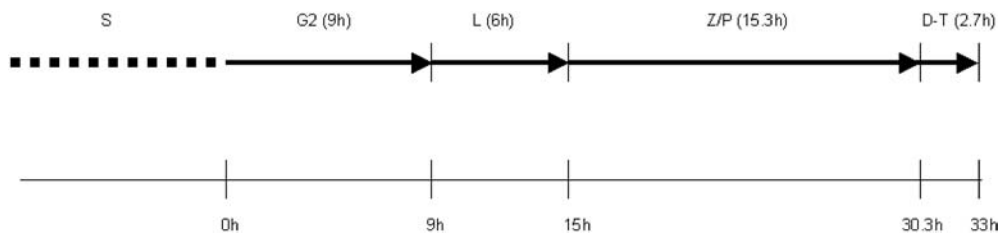


Fig. 3 Diagram to summarise the durations of the meiotic stages in *Arabidopsis* PMC set in the context of a time-course for the progression of PMC through meiosis. *S* Meiotic S-phase, *L* leptotene, *Z/P* zygotene/pachytene, *D-T* diplotene to tetrads

obtained for this accession, albeit from just one experiment, were broadly similar to those for Ws (data not shown).

Discussion

In vivo labelling of cells in meiotic S-phase with BrdU together, followed by immunocytological detection of BrdU in meiocytes at successive sampling times, provides a convenient and reliable method to establish a meiotic time-course for *Arabidopsis* that could also be applied to other plant species. Information thus gained on meiotic duration and the timing of key meiotic events extends our knowledge base of wild-type meiosis in this important model plant species.

Comparative studies of meiotic duration in plants are limited because relatively few species have been adequately investigated. Furthermore, a variety of different methods and conditions of growth have been employed to determine meiotic durations, and different criteria have been applied for judging when meiosis commences. Bennett et al. (1971) estimated the duration of meiosis for several plant species principally by means of "repeated anther sampling", but also some tritiated thymidine labelling. For the purposes of these experiments they considered that meiosis commenced at the onset of leptotene, which they defined as the point at which linear continuity of the entire chromosome complement could first be resolved in normal preparations. They determined a meiotic duration of 51 h for rye but considerably shorter durations for the polyploids wheat (24 h) and Triticale (21 h). Bennett (1971) drew a relationship between duration of meiosis and DNA content per cell in diploid cells, finding a highly significant correlation between the duration of meiosis and the DNA amount per cell in nine diploid plant species. Polyploid plants appear to diverge from this rule and have a shorter duration of meiosis compared to that of their constituent diploid parents. Using the same criteria as Bennett et al. (1971) for estimating the start of meiosis, the duration from leptotene to tetrad stage in *Arabidopsis* by our technique was 24 h. This is very similar to the meiotic duration for *Antirrhinum majus* (also 24 h) reported by Bennett (1971) for the same interval. However, the relationship between duration of meiosis and genome size does not appear to hold for *Arabidopsis* since it has a much smaller DNA content per cell ($3C=0.54$ pg) (Bennett et al. 2000) than *Antirrhinum majus* ($3C=1.5$ pg) (Bennett and Leitch 1995) despite their similar meiotic durations. It is probable that the genome size/meiotic duration relationship breaks down for very small genomes, indicating a minimum time requirement for meiosis in plants regardless of genome size.

Generally, the duration of meiosis is very variable among different eukaryotes (Bennett 1971; Callan and Perry 1977). Male animals lack the meiotic developmental checkpoints that characterise female meiosis, but

nevertheless, meiosis takes significantly longer than in plants, ranging from 4 days in *Drosophila melanogaster* (Chandley and Bateman 1962), to 11.5 days in the mouse (Oakberg 1956) and 24.3 days in humans (Heller and Clermont 1963). On the other hand, meiosis in yeast has been found to occur very rapidly. Generally the yeast *S. cerevisiae* reproduces by asexual budding and only enters a meiotic pathway when genetically competent cells are exposed to nutritionally deficient growth media. Under these circumstances meiosis is initiated and is completed within 9 h (Padmore et al. 1991).

It is not surprising, given the complexity of meiosis, that a relatively long period is required for completion of the pathway compared to the mitotic cycle. Bennett (1971) collected data for both the duration of the mitotic cycle and the duration of meiosis for seven plant species. In each case the reported duration of the mitotic cycle was less than that of the meiotic pathway. The length of meiosis for these species ranged from $\times 2$ compared to the mitotic cycle (*Haplopappus gracilis* and *Triticum aestivum*) up to $\times 9$ for *Trillium erectum*. *Arabidopsis* also fits this relationship. Seedling cells of *Arabidopsis* complete the mitotic cell cycle in 8.5 h at 22°C (Van't Hof et al. 1978) and it would appear therefore that the meiotic pathway is at least three times longer than the mitotic cycle in this species.

The durations of individual meiotic stages in *Arabidopsis* are very variable, presumably reflecting their molecular and cellular complexity. Meiotic S-phase and G2 each occupy 5–9 h and prophase I (leptotene-pachytene) lasts 21.3 h. Most of the critical molecular events of meiosis, including DNA and chromosome replication, the establishment of sister-chromatid cohesion and the complex events of chromosome pairing, synapsis and recombination take place during these prolonged stages. The remaining meiotic stages, concerned with segregation of the chromosomes into dyads and then into tetrads, occur relatively quickly (2.7 h). The same general pattern of extended S-phase, G2 (if recognised, see below) and prophase I, with brief division stages, is a common feature of eukaryotes (Bennett 1971).

The reality of meiotic G2 requires some attention since in at least two plant species its existence has been questioned. Bennett (1971) stated that, in wheat, meiotic G2 is either very short or completely absent, while Holm (1977) indicated that in *Lilium longiflorum* meiocytes proceed directly from late meiotic S-phase into early leptotene. However, the real issue appears to be how best to define the onset of leptotene since, as noted earlier, the transition from G2 to leptotene is gradual and difficult to define precisely based simply on the appearance of chromatin in stained preparations. This difficulty was avoided in the present study by relying on the appearance of continuous Asy1 antibody signals to mark early leptotene. By this criterion *Arabidopsis* has a clear and well-defined meiotic G2 phase.

Extended duration of the meiotic S-phase, compared to the S-phases of mitotically cycling cells, has been demonstrated in yeast (Williamson et al. 1983), plants

(Bennett et al. 1971; Holm 1977), and vertebrates (Crone et al. 1965; Callan and Taylor 1968). In *Triturus* this has been attributed to the activation of fewer but longer replicons in meiotic S-phase (Callan 1972). In mouse however, meiocytes and somatic cells have similar sized replicons but replication rates differ by a factor of 2.6 and there is a 2-fold difference in the duration of the S-phase (Van't Hof 1988 and references therein). In yeast, on the other hand, neither of these situations is found and it has been suggested that S-phase extension is due to the staggering of replication initiations (Williamson et al. 1983). The underlying reasons for S-phase extension are likely due to a requirement for chromatin reorganisation at this time that is essential for subsequent meiotic events. For example, it is known that the cohesin complex, a key element in ensuring accurate chromosome segregation, is established during meiotic S phase, when the two sister chromatids are synthesised (reviewed by Naysmyth 2001).

In the present study the duration of meiotic S-phase was estimated indirectly as occupying 5–9 h, which again exceeds the estimated duration of S-phase in mitotically cycling cells (2.8 h at 22°C, Van't Hof et al. 1978). The meiotic estimate receives support from the observations of Mercier et al. (2003) based on dual-immunolabelling of BrdU and SWI protein, that is required for sister chromatid cohesion in *Arabidopsis* though not itself a component of the cohesin complex. Expression of the *SWI1* gene has previously been described as being associated with early meiotic cells (Mercier et al. 2001). In dual-labelling experiments following a 2 h pulse label of BrdU, SWI protein was found to be present in meiotic S-phase and late G1 nuclei, but not in G2. The time taken for all BrdU labelled cells (i.e. those in S-phase at the time of the pulse) to enter G2 (no longer SWI labelled) was between 4 h and 8 h.

Establishment of a meiotic time-course provides an essential framework for determining the relative timing and durations of key molecular events in relation to cytologically defined landmarks of meiotic progression. This approach has been invaluable in developing a comprehensive understanding of the chronology of meiosis in yeast (Zickler and Kleckner 1998, 1999). In *Arabidopsis*, the development of a reliable meiotic time-course based on BrdU labelling of meiotic S-phase enables the expression patterns of meiotic proteins, in relation to meiotic events, to be determined. Some progress has already been achieved in this respect. So, for example, the *ASY1* protein that is required for chromosome synapsis in *Arabidopsis* (Caryl et al. 2000; Armstrong et al. 2002) is first detected in mid-to-late G2 nuclei from 5–9 h post-S and persists until the diplotene stage of prophase I some 24 h later. The *SWI1* protein, on the other hand, is expressed much earlier and is detected in late G1 of meiotic interphase, persisting throughout S-phase (Mercier et al. 2003). This approach has therefore already yielded valuable information and will become increasingly important in future as antibodies to more meiotic proteins become available.

Establishing a time-course for meiosis may also have significance in a broader developmental context. There is increasing realisation that regulation of higher-order chromatin structures and gene expression by DNA methylation and histone modification are components of complex epigenetic mechanisms that are integral parts of normal development. Many of these events are known to occur during meiosis or closely associated stages of gametogenesis in mammals, although the precise timings of these events have not been defined (Li 2002). Plants differ fundamentally from animals in the ontogeny of meiocytes and there is no evidence of complete genome-wide demethylation in plant “germ lines”. Nevertheless, imprinting effects are well documented in plants and methylation levels have been shown to be much reduced during pollen formation (reviewed by Goodrich and Tweedie 2002). It is therefore conceivable that comparable regulatory processes occur in plants, giving added value to the determination of a reliable meiotic time-course for the model plant *Arabidopsis*.

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