Nuclei and Chromosomes

1

Sachihiro Matsunaga

The nucleus is a double membrane-enclosed organelle in which the genome is packaged. DNA replication and RNA transcription occur in the nucleus. Franz Bauer first described the nucleus in orchid cells as observed under a microscope in 1802. Robert Brown observed details of the nucleus in orchid cells and named the organelle "nucleus" in 1831.

In this chapter, I. Karahara et al. reveal the ultrastructure of onion nuclei using a high pressure freezing technique. The cross section shows heterochromatic regions of interphase nuclei and mitotic chromosomes as dark staining regions of the chromosomes. The image of interphase nuclei demonstrates that plant nuclei with a large genome have globular domains in the nucleoplasm.

The nucleus contains various subnuclear structures. The most conspicuous structure in the nucleus is the nucleolus. At interphase, the nucleolus is responsible for ribosomal RNA synthesis and ribosome assembly. J. Hasegawa and S. Matsunaga present dynamic changes of nucleoli in tobacco BY-2 cultured cells. Nucleolus morphology changes dynamically through the cell cycle. The outermost structure of the nucleus is the nuclear membrane, which consists of inner and outer membranes. The inner membrane is backed by lamina. Y. Sakamoto and S. Takagi demonstrate the localization of a lamina component LITTLE NUCLEI 1 (CROWDED NUCLEI1). In addition to being present in animal skeletal muscle cells, multinucleated cells are also found in plants. M. Tanaka and K. Hatano present multinucleated cells in a green alga.

In the nucleus, DNA is packaged into chromatin, which is condensed to form chromosomes. Chromosomes were first described in the nucleus by Anton Schneider in 1873, and plant chromosomes were first described by Eduard Strasburger shortly thereafter in 1875. Condensed chromosomes are clearly observed during mitosis and meiosis. Y. Azumi presents the morphology of chromosomes at each stage of meiosis in male gametogenesis. Fluorescent in situ hybridization (FISH) has been a powerful technique for analyses of distribution of DNA sequences and chromosome organization. M. Hizume and F. Shibata demonstrate FISH of pine mitotic chromosomes. The behavior of chromosomes determines heredity, and chromosomal rearrangements are a driving force in evolution. Several sex chromosomes in dioecious plants genetically determine sex. F. Shibata et al. demonstrate the sex chromosomes in *Rumex acetosa* and *Silene latifolia*.

The accurate distribution of the genome during mitosis and meiosis is a direct consequence of chromosome dynamics. Chromosome alignment and segregation is regulated by the interaction of kinetochores and microtubules. D. Kurihara and S. Matsunaga perform live cell imaging to reveal the dynamics of kinetochores and microtubules during mitosis. Recently, chromatin movement was reported in interphase nuclei, suggesting that chromatin is not stable but structurally dynamic. T. Hirakawa and S. Matsunaga visualize chromatin in the root. Condensin is a regulatory protein for chromosome condensation. T. Fujiwara and T. Hirano show the distribution of condensin in primitive red alga.

The development processes of plants are often accompanied by endopolyploidy, which mainly arises from endoreduplication or endomitosis. In endoreduplication, also known as endoreplication or endocycle, DNA replication during the S phase is not followed by subsequent mitosis, leading to a polyploid cell. By contrast, endomitosis lacks sister chromatid segregation and cytokinesis, similarly resulting in a doubling of ploidy. In both cases, the intranuclear DNA content doubles with every cell cycle, giving rise to cells with high DNA content. S. Matsunaga and M. Ito demonstrate that endomitosis and endoreduplication forms gigas and pavement cells on leaf epidermis, respectively.

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T. Noguchi et al. (eds.), *Atlas of Plant Cell Structure*,
DOI 10.1007/978-4-431-54941-3_1,

Plate 1.1

Ultrastructural appearance of nuclei at different cell stages in high pressure frozen onion epidermal cells

The basal region of young onion (*Allium cepa* L. cv. Highgold Nigou) cotyledons is an excellent system for studying plant cell mitosis because ~3 % of the epidermal cells are undergoing mitosis at any time. Such cells were used to study ultrastructural membrane and cytoskeletal changes during formation of the preprophase band, which defines the future site of cell division [1].

Electron micrographs show ultrathin sections of onion epidermal cells exhibiting exceptionally well-preserved nuclei at different stages of mitosis. Cross sections of an interphase (**A**) and a late prophase cell (**B**) (adapted from [1]). Note the differences in staining patterns of the dark, condensed chromosome regions. A longitudinal section of a cluster of epidermal cells at different stages of mitosis after synchronization with the thymine analogue 5-aminouracil (5-AU) (**C**). Inter, interphase; Late pro, late prophase; Prometa, prometaphase; Meta, metaphase; Ana, anaphase.

To avoid chemical fixation artifacts, high-pressure freezing (HPF), by which entire tissues are immobilized in ~1 ms, was employed. 1–2 mm long basal cotyledon segments of 3 day old onion seedlings were cut with a razor blade while submerged in 0.1 M sucrose, and immediately frozen in a BAL-TEC HPM 010 high-pressure freezer (Boeckeler). Frozen samples were freeze-substituted in 2 % (w/v) OsO_4 in anhydrous acetone and then embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and Reynold's lead citrate, and were imaged with a transmission electron microscope. Mitotic synchronization involved treating 2.5 day old onion cotyledons with a 0.6 mg/mL solution of 5-AU for 12 h then transferring the tissue to drug-free medium for 8–9 h followed by high pressure freezing. Scale bars: 5 nm (**A**), 10 nm (**C**).

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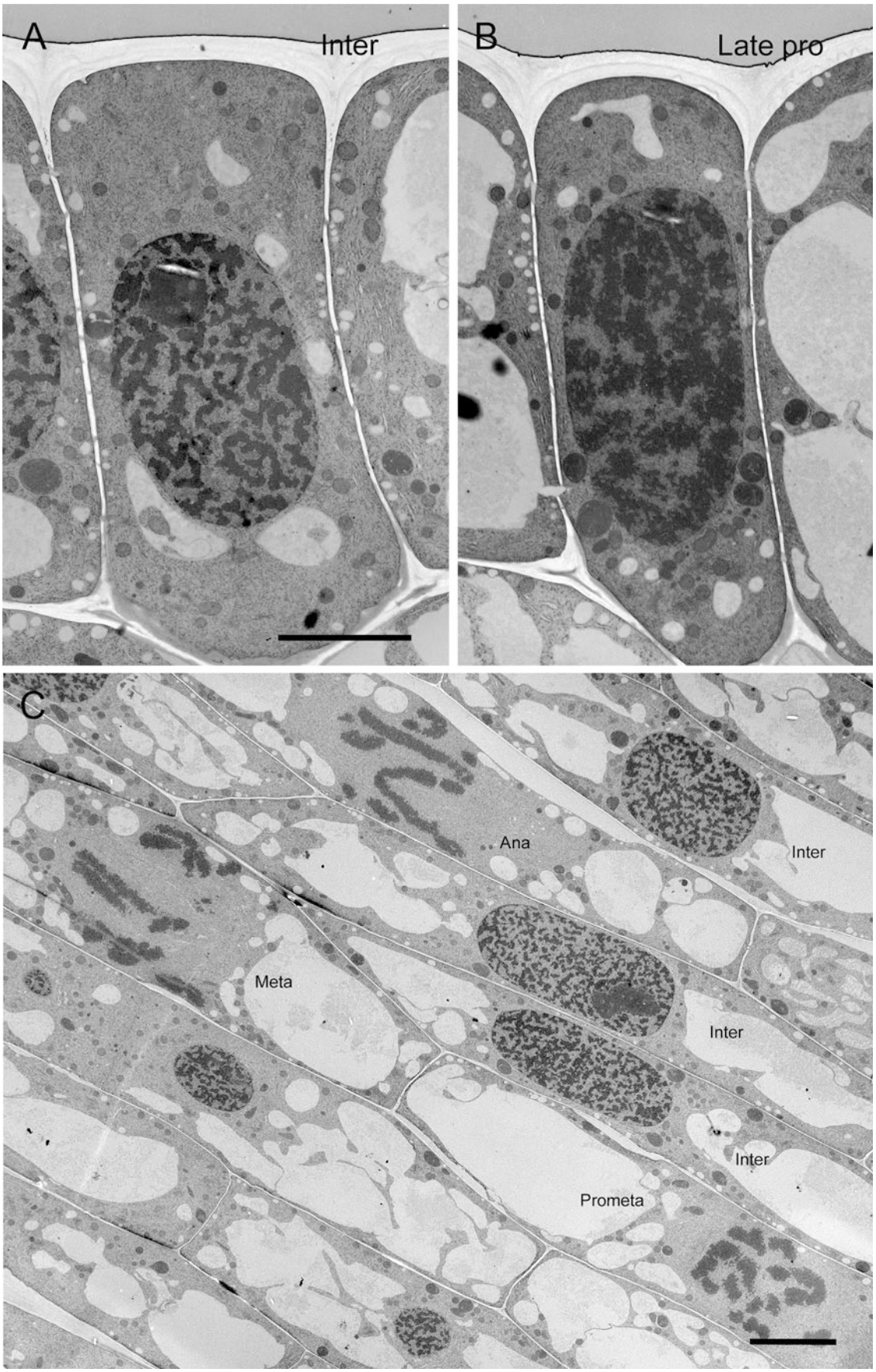
A
Inter
B
Late pro
C
Ana
Inter
Meta
Inter
Inter
Prometa

Plate 1.2

Morphology of nucleoli in tobacco BY-2 cultured cells

The nucleolus is a distinct subnuclear structure of eukaryotic cells. The biosynthesis of rRNA and ribosomes occurs in the nucleolus. Molecules required for the synthesis of rRNA and ribosomes such as rDNA, precursor rRNA, mature rRNA, RNA-processing enzymes, snoRNA, ribosomal proteins, and assembly proteins, aggregate at high density in the nucleolus. First, 35S rRNAs are transcribed by RNA polymerase I in the nucleolus. Then, rRNA spacer regions are removed to mature 18S, 5.8S, and 26S rRNA. Each rRNA is modified and subsequently packed into a precursor ribosome. Precursor ribosomes are carried from the nucleolus to the cytoplasm to become mature ribosomes. The size and number of nucleoli depend on the phase of the cell cycle [2]. A nucleolus resides in the nucleus until prophase and disappears from prometaphase to telophase. Many nucleolar proteins are localized on the peripheral region of mitotic chromosomes [3]. Proteins regulating ribosome synthesis are necessary for root growth and epidermal cell patterning in plants.

This figure represents nuclei, chromosomes and cell walls in blue and RNA in green of tobacco BY-2 culture cells (*Nicotiana tabacum* L. cv. Bright Yelleow-2). At prophase, chromatins begin to condensate into chromosomes, but the structure of nucleolus is remained (middle right). At metaphase, the nucleolus disappeared completely (lower left). At telophase (middle left) to early G1 phase (left), two to four nucleoli are formed in a nuclear. At late G1, S and G2 phases, nucleoli reassembled into one nucleolus in accordance with in the progression of G1 phase (center and left side).

A nucleolar-ID™ green detection kit (Cat. # 51009-500, Enzo) was used for visualization of nucleoli. The reagent is a cell-permeable nucleic acid stain that is selective for RNA. The reagent is essentially non-fluorescent in the absence of nucleic acids, but emits green fluorescence when bound to RNA. Because nucleoli contain abundant synthesized rRNA, they stain brightly. The cytoplasmic region is also stained. 1 μL of Nucleolar-IDTM green detection reagent was added to 500 μL of 2 day old BY-2 cells. The cells were incubated in the dark for 30 min at room temperature. After the addition of 1 mL of 1× Assay Buffer diluted with BY-2 medium, those were stained with 50 μg/mL 4′, 6-diamidino-2-phenylindole phenylindole (DAPI). Stained cells were observed under an upright microscope (BX53, Olympus) with a CCD camera (Cool Snap HQ2, Nippon Roper). Scale bar: 30 μm.

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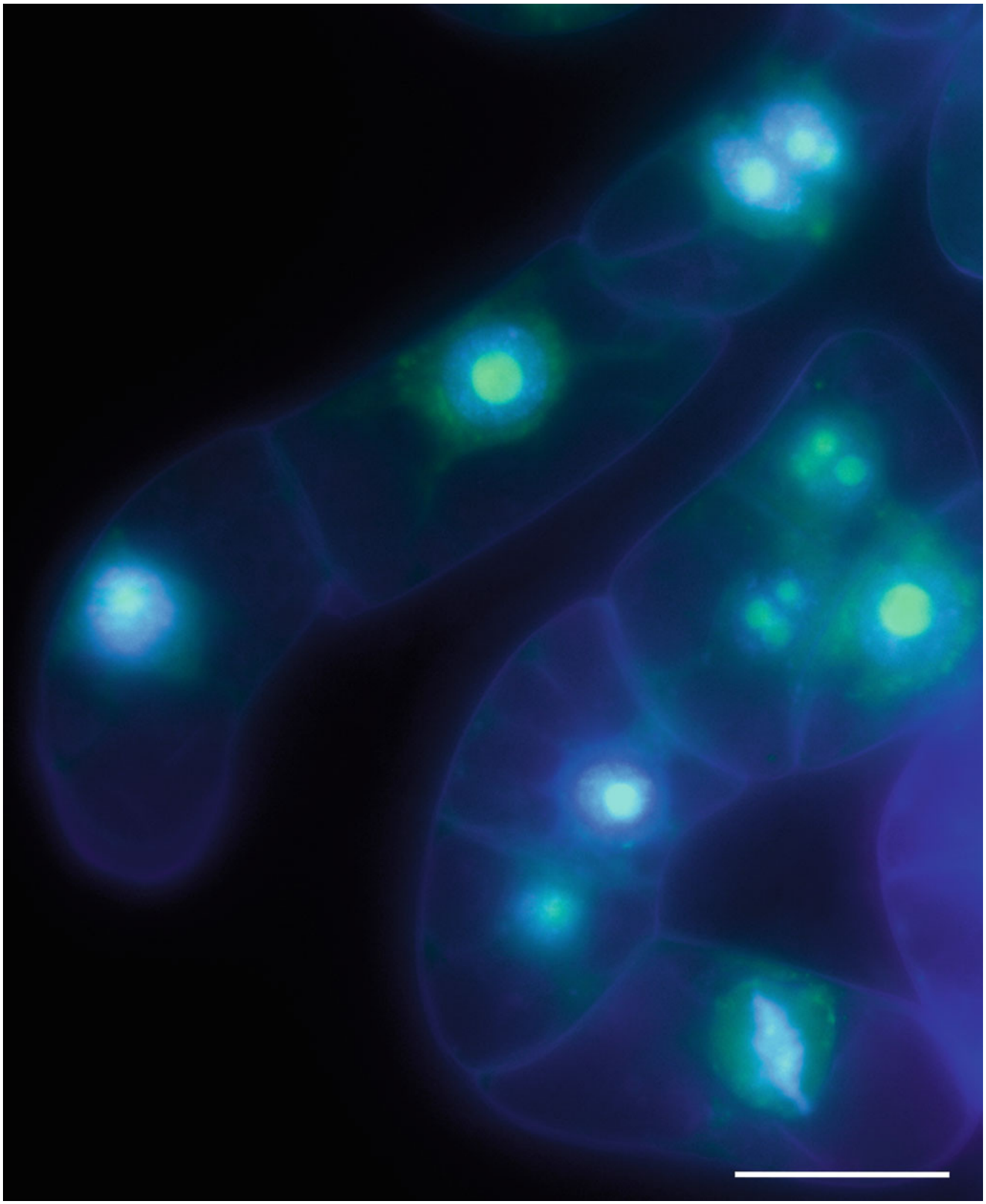

Plate 1.3

Nuclear lamina localized at the nuclear periphery in interphase and at chromosomes in mitotic phase

The nuclear lamina forms a meshwork beneath the inner nuclear envelope. Animal nuclear lamina, constructed of lamins and a variety of inner nuclear envelope proteins, contributes not only to an increase in mechanical toughness but also to regulation of chromatin distribution and gene expression. The components and biological significance of plant nuclear lamina are not nearly as well understood. The *Arabidopsis thaliana* LITTLE NUCLEI (LINC) (also called CROWDED NUCLEI, CRWN) family proteins were discovered based on homology to carrot nuclear matrix constituent protein [4] and detected in the nuclear lamina fraction by liquid chromatography tandem mass spectroscopy. The intracellular localization of LINC1-GFP was investigated in the fixed root apical meristem. The fluorescent images were adapted from [5]. LINC1 was localized mainly to the nuclear periphery in interphase cells (**A**), to the condensing chromosomes after prometaphase and into anaphase (**B**), transferred from decondensing chromatin to the reassembling nuclear envelope in early telophase (**C**), and then again to the nuclear periphery during late telophase (**D**). Hoechst signals are in magenta and GFP signals are in green.

Sample roots were fixed in 4 % (w/v) formaldehyde, freshly prepared from paraformaldehyde, in PIPES buffer (10 mM EGTA, 5 mM $MgSO_4$, and 50 mM PIPES at pH 7.0) for 1 h. Fixed roots were treated with 0.5 % (w/v) Cellulase Onozuka RS and 0.05 % (w/v) Pectolyase Y-23 in PIPES buffer for 45 s at 37 °C. Roots were stained with Hoechst solution (5 μg/mL Hoechst 33342 in PIPES buffer) for 10 min. Samples were visualized using a DeltaVision microscope with Olympus IX70 stand (Personal DV; Applied Precision). Images were deconvoluted using the constrained iterative algorithm implemented in SoftWoRx software (Applied Precision). Scale bar: 5 μm.

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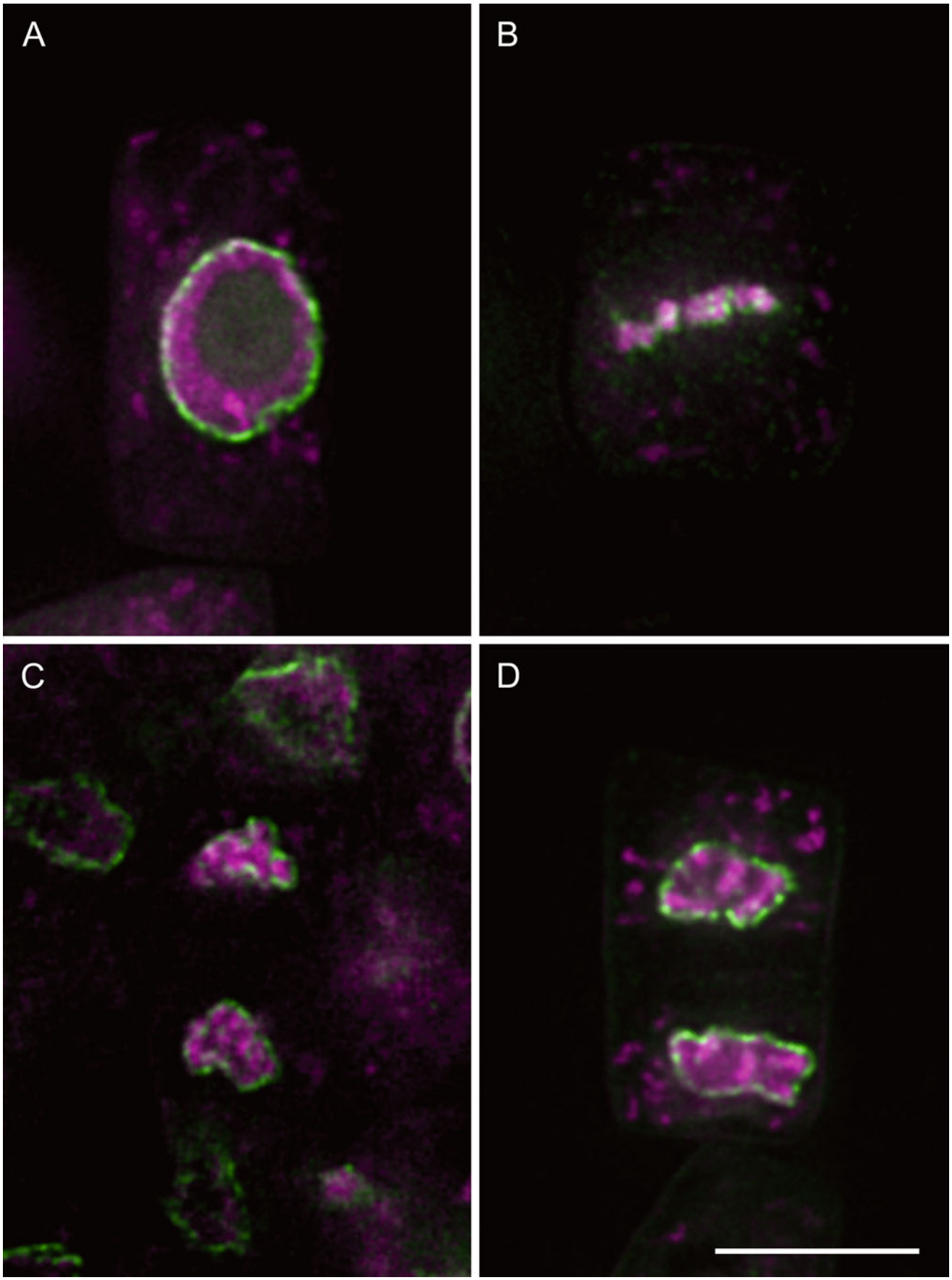
A
B
C
D

Plate 1.4

Nuclei of multinucleate cells in *Hydrodictyon reticulatum*

Hydrodictyon reticulatum (L.) Lagerheim is a free-floating freshwater green alga that forms net-like colonies with a polygonal or hexagonal mesh pattern. During net formation, hundreds of zoospores adhere to one another to form a beautiful network within the parental cell [6]. Recently adhered net-cells, which originated from zoospores, contain one nucleus (blue) (**A**). Each net-cell continues to grow larger, repeating nuclear division without cytokinesis. Large multinucleate net-cells display regularly spaced nuclei (blue) in a stationary cytoplasm (**B**–**D**).

Mitosis in net-cells of *H. reticulatum* is intranuclear. The nuclear envelope remains essentially intact throughout mitosis with polar fenestrae. In smaller net-cells, nuclei divide once in a 24 h period, and all nuclei are in the same phase of mitosis at the same time. In larger net-cells, mitosis occurs synchronously in waves from one end of the cell to the other (**B**). Thus, several stages of mitosis can be observed in a single cell at one time (**C**, **D**).

The arrangement of microtubules, chromatin and chromosomes during mitosis was examined by immunofluorescence microscopy (**C**, **D**) [7]. When mitosis begins, microtubules (red) radiate from each nucleus (blue) in prophase, then converge on opposed perinuclear sites and form mitotic spindles in metaphase. Spindles start to elongate in anaphase, and interzonal spindles thin and elongate as nuclei move farther apart in telophase.

Cells were treated with S-buffer containing 0.25 % glutaraldehyde and 1 μg/mL 4′, 6-diamidino-2-phenylindole (DAPI), and examined under a fluorescence microscope with an ultraviolet excitation filter (**A**, **B**). For microtubule immunolocalization, cells were rapidly frozen in liquid propane and transferred to chilled methanol at −80 °C. After 24 h at −80 °C, they were gradually warmed to room temperature, and incubated in blocking solution for 1 h. Samples were incubated with a monoclonal anti-α-tubulin antibody diluted 1:500 in PBS containing 1 % BSA at room temperature, then washed with PBS. Alexa568-conjugated goat anti-mouse IgG was used as the secondary antibody. After washing with PBS, samples were mounted with a mounting solution containing 1 μg/mL DAPI and examined with a confocal laser scanning microscope (**C**, **D**). Scale bars: 5 μm (**A**), 10 μm (**B**–**D**)

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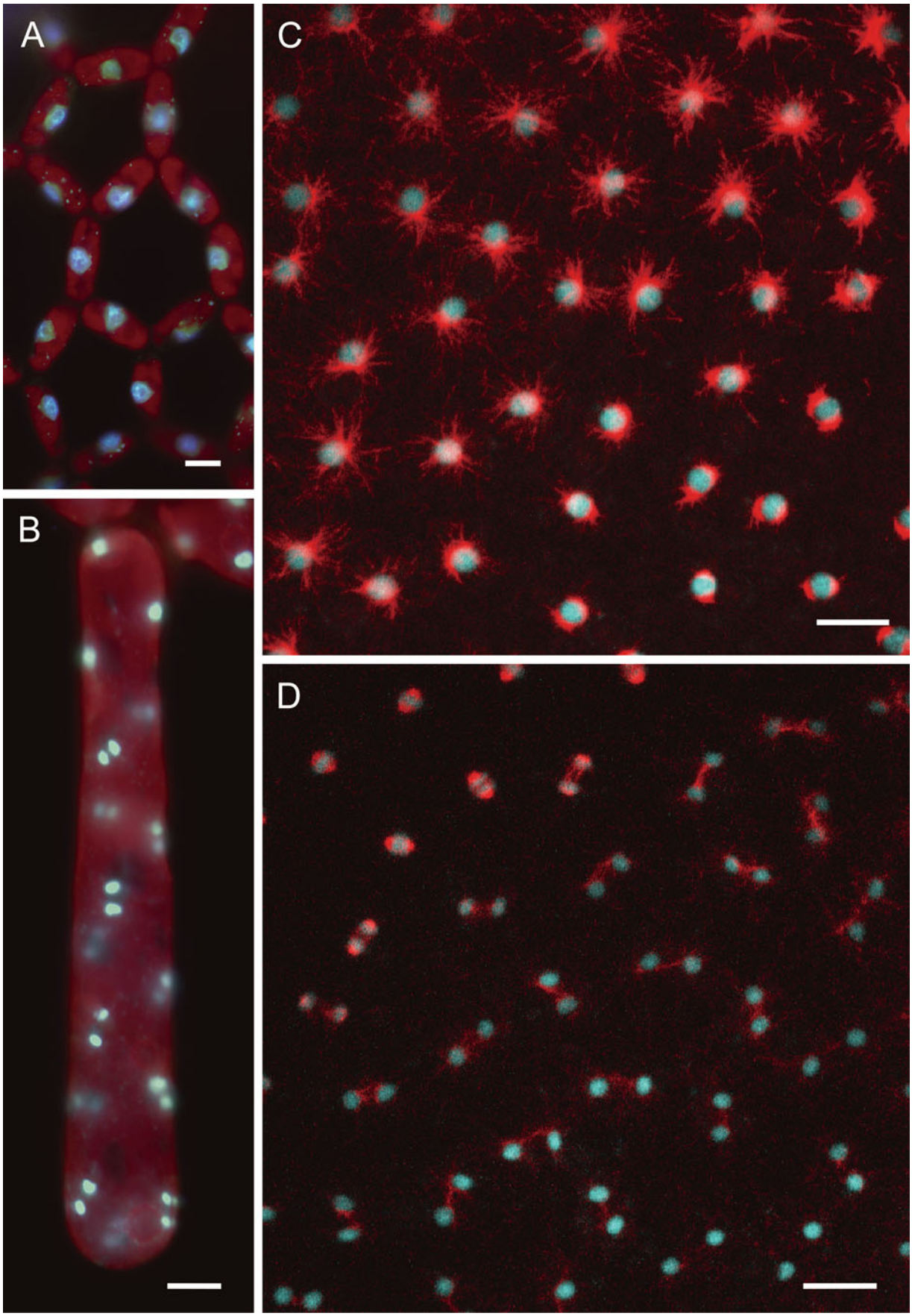
A
B
C
D

Plate 1.5

Meiotic chromosomes of *Arabidopsis thaliana* pollen mother cells

Sexually reproducing *A. thaliana* generates haploid gametes through meiosis. Meiosis is different from mitosis in that it not only do the resulting cells have half the diploid chromosome number, but also in that homologous chromosomes recombine. The meiosis-specific behavior of chromosomes is observed during prophase I and metaphase I. After one round of DNA replication in the premeiotic S phase, pollen mother cells go through G2 phase and enter prophase I. When stained with 4′,6-diamidino-2-phenylindole (DAPI), leptotene *A.thaliana* chromosomes appear as entangled thin thread-like structures (**A**). Through zygotene stages, homologous chromosomes pair and synaptonemal complexes are constructed between them, elongating a thick thread-like synapsed region and leaving a thin unpaired region (**B**). It is during this stage that recombination initiated by double-stranded DNA breaks occurs. The construction of synaptonemal complexes is complete at the pachytene stage. Crossovers become visible on diplotene chromosomes, but at diakinesis chromosomes are so condensed that chromosome structures are no longer distinguishable (**C**). At metaphase I, the five bivalents congress on the metaphase plate, as opposed to the assembly of ten univalents at mitosis (**D**). Coincidental segregation of homologous chromosomes to opposite poles represents the start of anaphase I. These fluorescent images were adapted from [8]. To accomplish meiosis-specific tasks, PMCs require meiosis-specific genes.

Cells for this figure were prepared using the DAPI staining method [8], which is simple and can be easily used in further experiments, such as fluorescent in situ hybridization. Inflorescences were fixed with Farmer's solution for 24 h at room temperature, and digested with 0.3 % cytohelicase, cellulase, pectolyase in 10 mM citrate buffer (pH 4.5) for 3 h at 37 °C. A bud was transferred into a drop of 60 % acetic acid on a slide glass and incubated for 1 m. Chromosomes were the spread on the surface of the slide glass and air dried after washing with Farmer's solution. Chromosomes were stained with 1.5 μg/mL DAPI and observed under fluorescence microscope. Scale bars: 10 μm.

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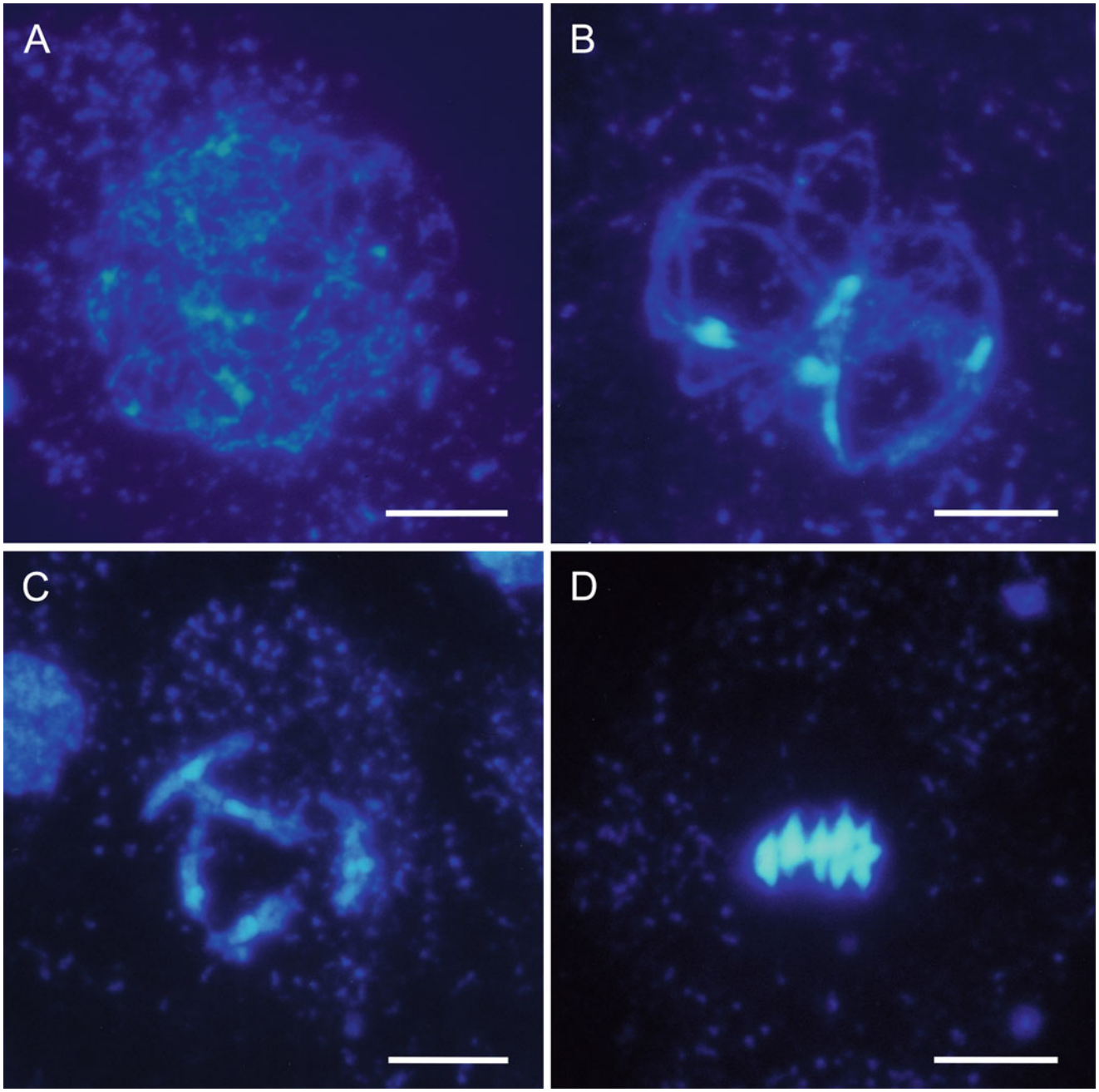
A
B
C
D

Plate 1.6

Multicolor FISH of *Pinus* chromosomes

The genus *Pinus* is a major components of Northern Hemisphere timber forests. The karyotype analyses of *Pinus* species confirm that chromosomes comprising a karyotype exhibit similar morphology. The difficulty in identification of homologous pairs prevents precise comparative karyotype analyses of *Pinus* species. Fluorescence in situ hybridization (FISH) has contributed to the karyotype analysis of *Pinus* species. FISH can identify the homologous chromosome pairs in *Pinus*.

The image adapted from [9] shows FISH of somatic chromosomes of *P. densiflora*, with each chromosome showing unique probe patterns. Color code: magenta, PCSR; green, telomere sequence; red, 18S and 5S rDNA. The *Arabidopsis*-type telomere sequence repeats $(TTTAGGG)_n$ were amplified by PCR with $(TTTAGGG)_5$ and $(CCCTAAA)_5$ primers without template DNA and labeled with biotin. 18S rDNA and 5S rDNA probes were labeled with digoxigenin (DIG). Plasmid DNA containing *PCSR* (Proximal CMA band-Specific Repeat, clone PDCD501; accession no. AB051860) [10] was labeled with FITC. Probes were dissolved in a solution of 2× SSC, 10 % dextran sulfate, and 50 % formamide. Chromosomal DNA was denatured at 80 °C for 1 min in 70 % formamide, 2× SSC. Hybridized probes were detected with Strepetavidin-Cy5 and anti-digoxigenin antibody conjugated with rhodamine and slides were counterstained with DAPI (4, 6-diamino-2-phenylidole). Hybridization signals were visualized and recorded using a chilled CCD camera (Sensys 1400, Photometrics), and pseudcolor images were made using IPLab (Scanalytics). Scale bars: 10 μm.

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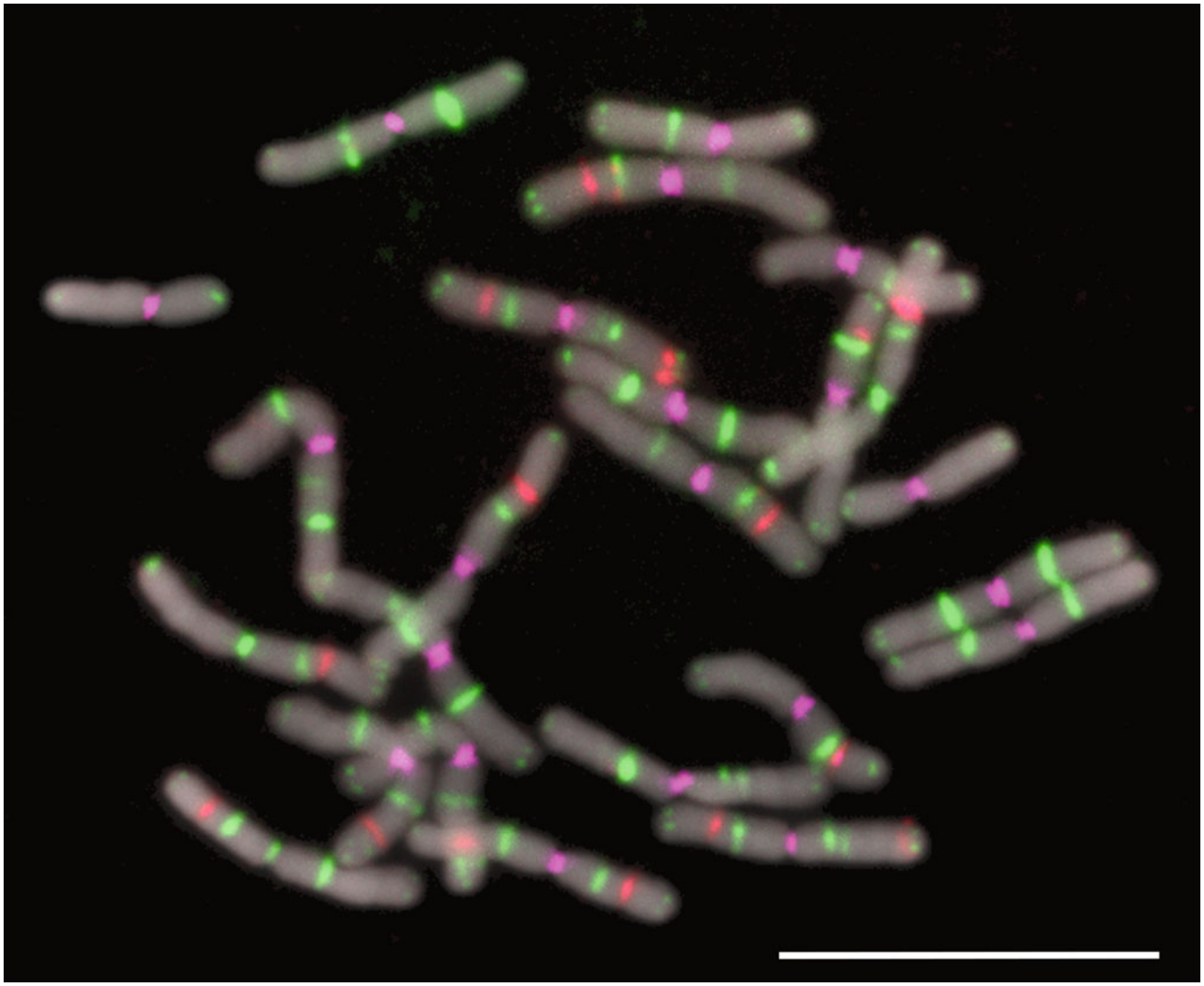

Plate 1.7

Chromosome painting and FISH of distal end satellite DNAs in dioecious plants with sex chromosomes

The dioecious plant *Rumex acetosa* has a multiple sex chromosome system: females are $2n = XX + 12$, males are $2n = XY_1Y_2 + 12$. Two DNA sequences which are abundant on Y chromosomes have been isolated, one is the Y chromosome specific sequence (RAYSI) [11] and the other is located on Y chromosomes and a pair of autosomes (RAE180) [12]. These two repetitive sequences are present in unique, complex patterns on Y_1 and Y_2 [12]. Two bright signals corresponding to these Y abundant repetitive sequences are detected on two Y chromosomes of male *R. acetosa* prometapahase chromosomes probed by fluorescence in situ hybridization (**A**). The image was adapted from [11].

Silene latifolia is also a dioecious plant with heteromorphic X and Y sex chromosomes. The recombining pseudoautosomal regions (PAR) are required for proper sex chromosome division in meiosis. PAR localization on the Y can be detected by FISH with a probe against the *Sac*I satellite subfamily [13]. FISH shows 25 chromosomes in early metaphase of a male *S. latifolia* aneuploidy line (**B**). FISH signals are detected on most of autosomes, both arms of the X, and the non-condensed arm of the Y [14]. Since homologous recombination occurs in PARs, PAR DNA sequences should be similar on both X and Y chromosomes, indicating that the PAR is located on the non-condensed arm of the Y chromosome. The image was adapted from [14].

DOP-PCR amplified Y-DNA of *R. acetosa* was labeled with biotin by nick translation. Labeled DNA was resuspended in hybridization solution (50 % formamide, 10 % dextran sulfate, 0.08 mol/L Na_2HPO_4 in 2× SSC pH 6.5) with unlabeled genome DNA. Chromosomes were denatured in 70 % formamide/2× SSC at 76 °C for 60 s. Hybridized probes were detected using avidin-FITC (EY Laboratories). Slides were counterstained with propidium iodide.

*Sac*I satellite subfamily probe was prepared by direct labeling with Alexa Fluor 546. Chromosomal DNA was denatured at 75 °C for 1 min in 70 % formamide/2× SSC. Chromosome preparations were dehydrated immediately using 5 min treatment with 70 % ethanol at −20 °C followed by 100 % ethanol at room temperature. Preparations were then treated with acetone for 30 min at room temperature. Slides were washed at 42 °C in 50 % formamide/2× SSC and conterstained with DAPI. Scale bars: 5 μm.

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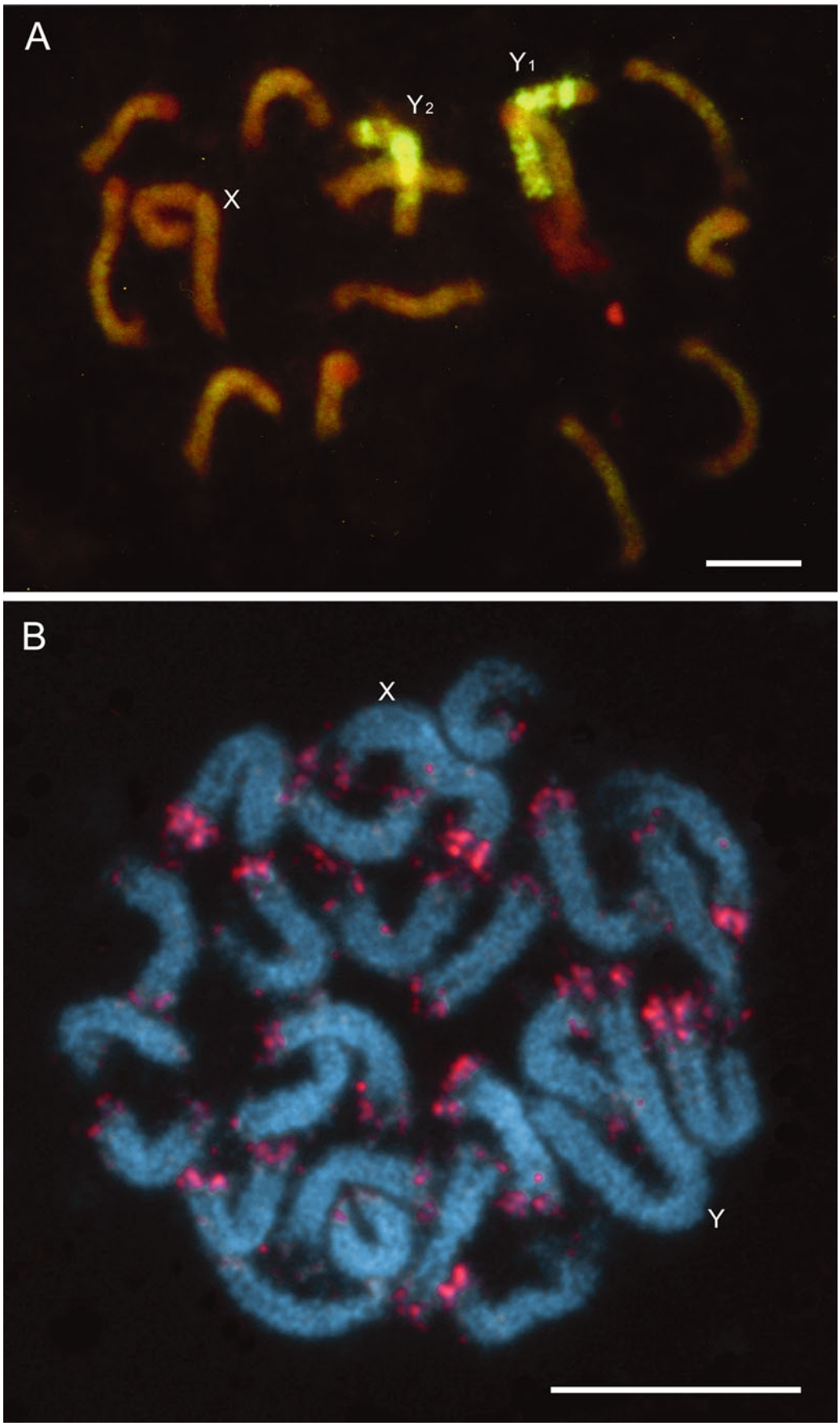
A
Y_1
Y_2
X
B
X
Y

Plate 1.8

Kinetochore and microtubule dynamics during cell division of tobacco BY-2 cells visualized by live-cell imaging

Mitosis and cytokinesis are crucial for the equal separation of genetic information to both daughter cells [2]. A series of highly coordinated events such as bipolar spindle formation, and chromosome alignment, and chromosome segregation occur in mitosis. The accuracy of cytokinesis in plant cells is also ensured by phragmoplast formation.

Kinetochore and microtubule dynamics were visualized in living BY-2 cells expressing green fluorescent protein (GFP)-fused α -tubulin and tdTomato (a tandem dimer variant of red fluorescent protein [RFP])-fused CenH3 (centromeric histone H3: *Arabidopsis thaliana* HTR12) [15]. The centromeric region contains histone H3 variant CenH3. The kinetochore is established by the attachment of spindle microtubules to the centromere. Before nuclear envelope breakdown at prophase (Pro), cortical microtubules are replaced by the densely packed preprophase band (PPB). Microtubules begin organizing at prometaphase (Prometa) and kinetochores move to the spindle equator. As cells reach metaphase (Meta), CenH3s oscillate and align on the spindle equator. After complete CenH3 alignment, CenH3s segregate equally to the opposing sides. The phragmoplast emerges at the midzone at late anaphase. Lateral expansion of the phragmoplast is observed during telophase (Telo). The cell wall is formed after disappearance of the phragmoplast. After cytokinesis (Cyt) cell walls are synthesized and new cell nuclei are visible following nuclear envelope reconstruction in the two daughter cells.

Tobacco BY-2 cells on coverslips were transferred to petri dishes (Matsunami Glass Ind., Ltd.). Dishes were placed on the inverted platform of a fluorescence microscope (IX-81; Olympus) equipped with an electron multiplying cooled charged-coupled device (EMCCD) camera (Evolve 512; Photometrics), a Piezo focus drive (P-721; Physik Instrumente), and 488 nm and 561 nm lasers (Sapphire; Coherent). Images were acquired every 1 min with a 60× objective lens (UPLSAPO 60XS, Silicone Oil; Olympus). Green and magenta fluorescence represent α-tubulin and CenH3, respectively. Scale bar: 10 μm.

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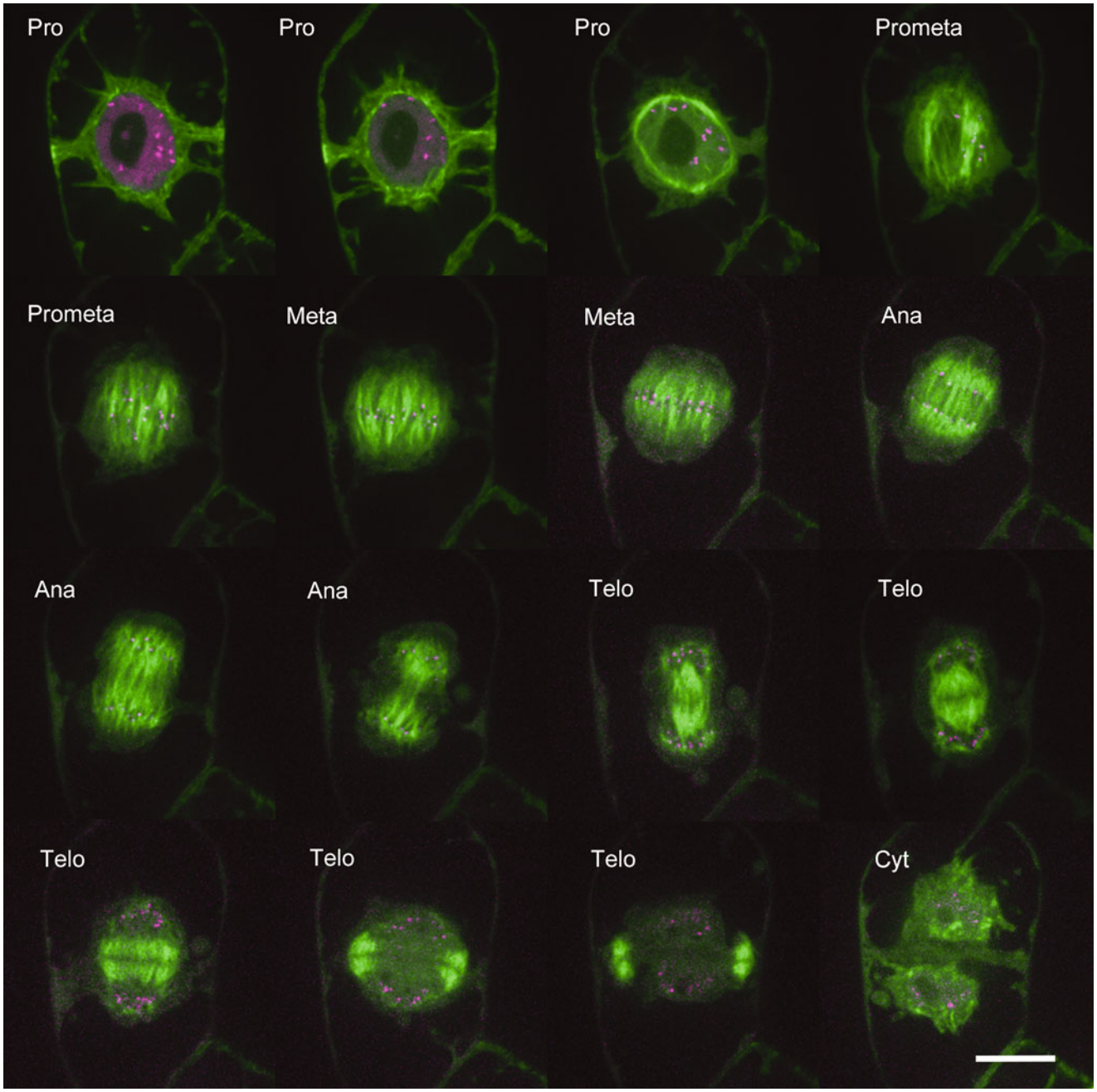
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Plate 1.9

Visualization of chromatin dynamics in the root of *Arabidopsis thaliana*

Chromatin and nuclear dynamics are closely related to many biological processes in eukaryotes. Chromatin movement is associated with subnuclear events such as DNA replication, transcription, and repair. The chromatin fluorescence tagging system is a technique using the bacterial operator/repressor system combined with a fluorescence protein, for example, *lacO*/LacI-EGFP. This system can visualize specific loci where the operator tandem array is inserted [16]. FISH and immunofluorescence experiments require the fixation of cells but the system can analyze mitotic and interphase chromatin dynamics in living cells.

The left image represents chromatin dynamics in a root of *Arabidopsis thaliana* expressing H2B-tdTomato, a histone H2B protein, fused with fluorescent protein, tdTomato. The two green dots in a nucleus are *lacO*/LacI-EGFP signals which show specific loci on two homologous chromosomes. An epidermal cell at metaphase is located in the upper left region of the image. Mitotic chromosomes align at the metaphase plate, as shown by the alignment of *lacO*/LacI-EGFP signals.

A. thaliana roots consist of two regions: a meristematic region of mitotic cells and an elongation region of rapidly expanding cells [2]. In the elongation region, repeated DNA replication without cell division, endoreduplication, coupled with rapid cell expansion [17]. Nuclei are larger in the elongation region than in the meristematic region. The *lacO*/LacI-EGFP signals also enlarge in the elongation region. The distance between the two dots, the inter-allelic distance, is longer in the elongation region than in the meristematic region.

Seeds of *A. thaliana* expressing *lacO*/LacI-EGFP and H2B-tdTomato were germinated on 1/2MS medium (0.1 % sucrose, 0.6 % gellan gum) in a glass bottomed dish. The dish was placed at 4 °C for 1 day, then moved to incubator and grown at 22 °C in 16 h light/8 h night cycle. Seedling roots were observed 5 day after germination under an inverted fluorescent microscope (IX-81, Olympus) equipped with a confocal scanning unit (CSUX-1, Yokogawa) and a sCMOS camera (Neo 5.5 sCMOS ANDOR). One stack consists of 40 images at 0.5 μm z-axis steps collected for 90 min (time interval: 5 min). Images were analyzed with ImageJ software. Scale bar: 50 μm.

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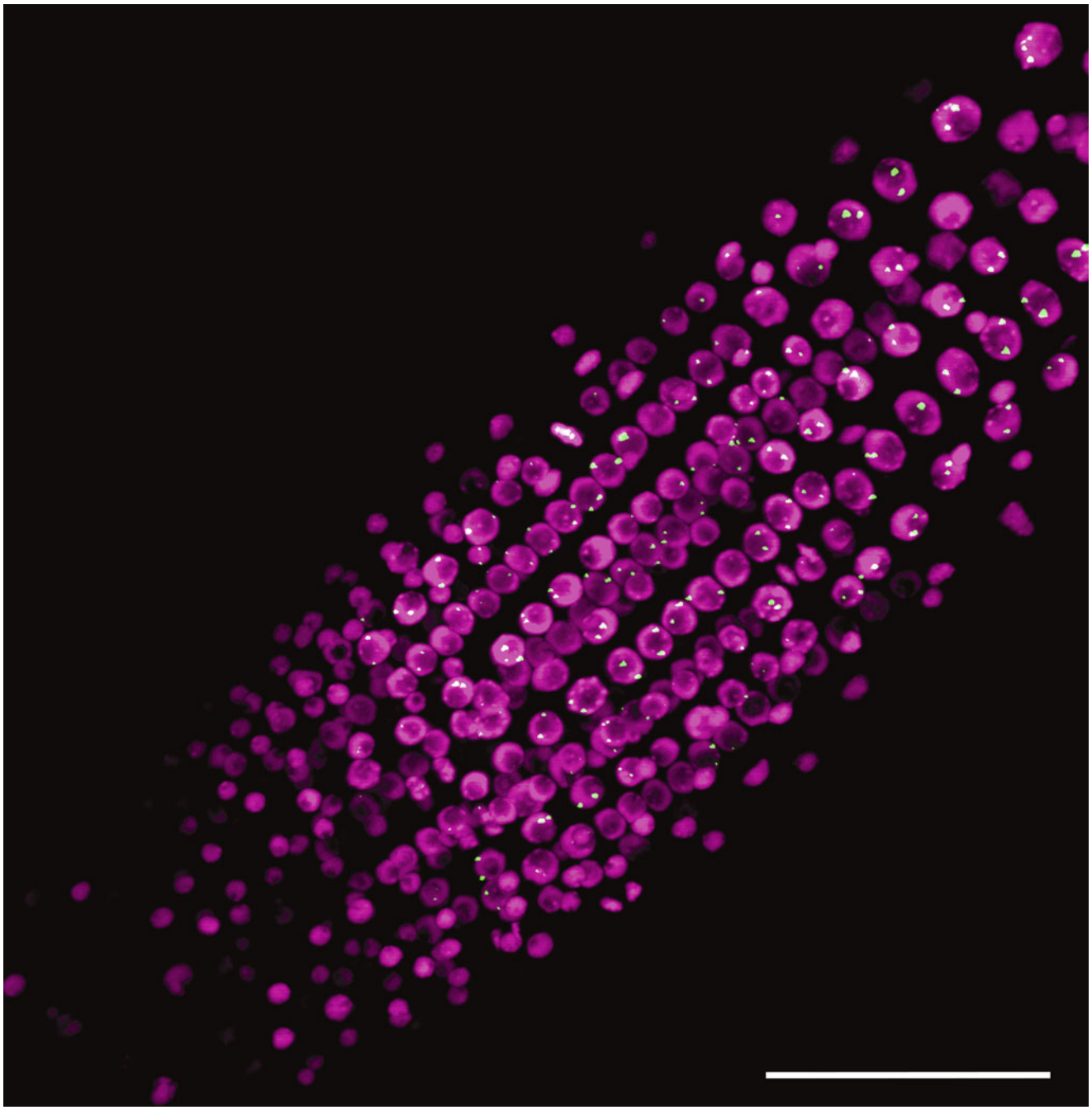

Plate 1.10

Specific contribution of condensin II to sister centromere resolution in *Cyanidioschyzon merolae*

Condensins are multisubunit complexes that play central roles in chromosome organization and segregation in eukaryotes. Many eukaryotic species, including humans and flowering plants, have two different condensin complexes (condensins I and II), which leads to many interesting questions. Why do the two types of condensins exist? Do they have unique functions? To address these questions, the red alga *Cyanidioschyzon merola*e was used because it represents the smallest and simplest organism that is known to possess condensins I and II. Despite the great evolutionary distance, spatiotemporal dynamics of condensins in *C. merolae* and mammalian cells are strikingly similar: condensin II localizes to the nucleus throughout the cell cycle, whereas condensin I becomes visible on chromosomes only after the nuclear envelope partially dissolves at prometaphase. Unlike in mammalian cells, however, condensin II is concentrated at centromeres in metaphase, whereas condensin I is distributed more broadly along chromosome arms. A targeted gene disruption technique was established in *C. merolae* [18], and this technique demonstrated that condensin II is not essential for mitosis under laboratory growth conditions. Condensin II does, however, play a crucial role in facilitating sister centromere resolution in the presence of a microtubule drug. These results offer an excellent example of how the combination of genetics and cell biology in *C. merolae* can provide fundamental insights into basic cellular processes such as chromosome architecture and dynamics [19].

Shown here is a phase-contrast image of *C. merolae* metaphase cells immunolabeled with an antibody against centromere-specific histone H3 (CenH3) (light blue) adapted from [19]. The contour of a wild-type cell is pseudo-colored blue; the contours of condensin II–knockout cells are pseudo-colored red. In the wild-type cell, two CenH3 clusters are well resolved at metaphase in the presence or absence of functional microtubules. In condensin II–knockout cells, however, sister centromere clusters fail to resolve in the absence of functional microtubules. Scale bar: 1 μm. This image was chosen as the cover of *Molecular Biology of the Cell* (vol 24, no. 16).

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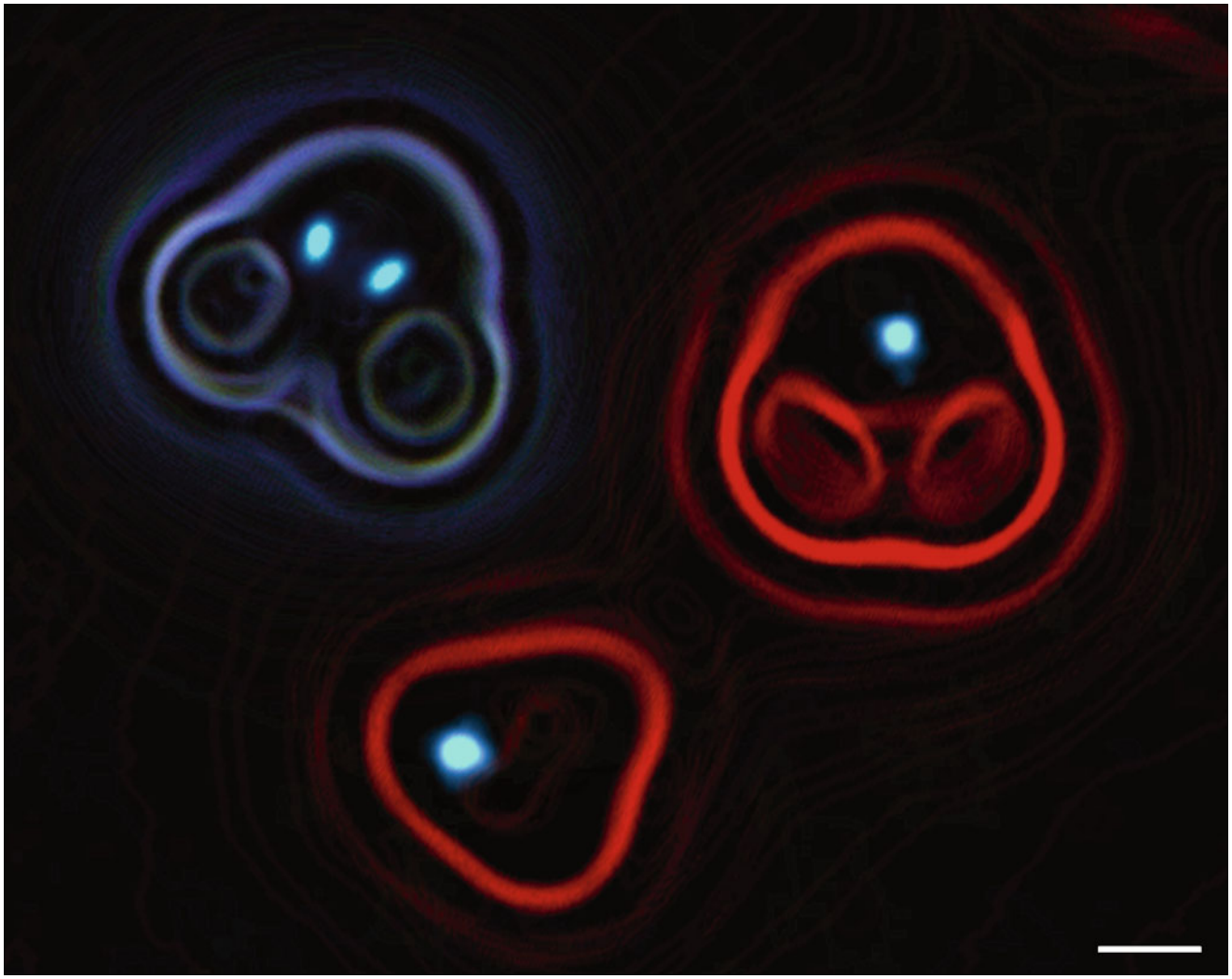

Plate 1.11

Endomitosis induces a giant polyploid cell on the leaf epidermis

An increase in ploidy can be caused by one of two events, endoreduplication or endomitosis, whereby chromosomes replicate but cells do not divide. Endoreduplication, in which cells skip the entire processes of mitosis, does not increase the number of chromosomes but generates polytene chromosomes. Cells in endomitosis enter but do not finish mitosis, proceeding through anaphase without nuclear division and cytokinesis. In contrast to endoreduplication, endomitosis causes doubling of the chromosome number [2].

GIGAS CELL1 (GIG1) encodes a plant-specific inhibitor of anaphase-promoting complex/cyclosome (APC/C) in *Arabidopsis thaliana* [20]. APC/C is responsible for the transition between mitotic processes by degradation of cell cycle proteins. GIG1 prevents the ectopic occurrence of endomitosis by inhibiting APC/C. The recessive *gig* mutat was identified as an enhancer of the *myb3r4* mutant phenotype. MYB3R4 is a member of the Myb family of transcriptional regulators that positively regulate mitotic progression in *A. thaliana*. Giant cells, called *gigas* cells, are observed in the epidermis of *gig1* cotyledons. The frequency of *gigas* cells is increased when MYB3R4 and GIG1 are simultaneously mutated.

The left figure shows a fluorescent image of the epidermis of cotyledons in *gig1/myb3r4* double mutants expressing TOO MANY MOUTHS (TMM)-GFP (green) and tdTomato-CENH3 (red). TMM is a marker for stomatal precursor cells and is essential for stomatal development. The *gigas* cell in the center of the image strongly expresses TMM, suggesting that *gigas* cells may have a guard cell–like identity that arises from a developmental pathway similar to the one that generates stomata. The nuclei of *gigas* cells are larger than those of normal guard cells, and their precursors and are equivalent in size to endoreduplicated nuclei in pavement cells (jigsaw puzzle–shaped cells). Ploidy level can be estimated by the number of chromosomes in each nucleus using a kinetochore-specific marker, tdTomato-CENH3. Guard cells show 10 tdTomato-CENH3 signals, which is equivalent to the diploid chromosome number ($2n = 10$) of *A. thaliana*. In contrast, the nuclei of *gigas* cells contain 20 signals. This suggests that *gigas* cells have 20 chromosomes which are generated by endomitosis.

Imaging was performed using an inverted fluorescence microscope (IX-81; Olympus) equipped with a confocal laser scanner unit CSUX-1 (Yokogawa Electronic) and a charge-coupled device camera (CoolSNAP HQ2; Roper Scientific). Scale bar: 50 μm.

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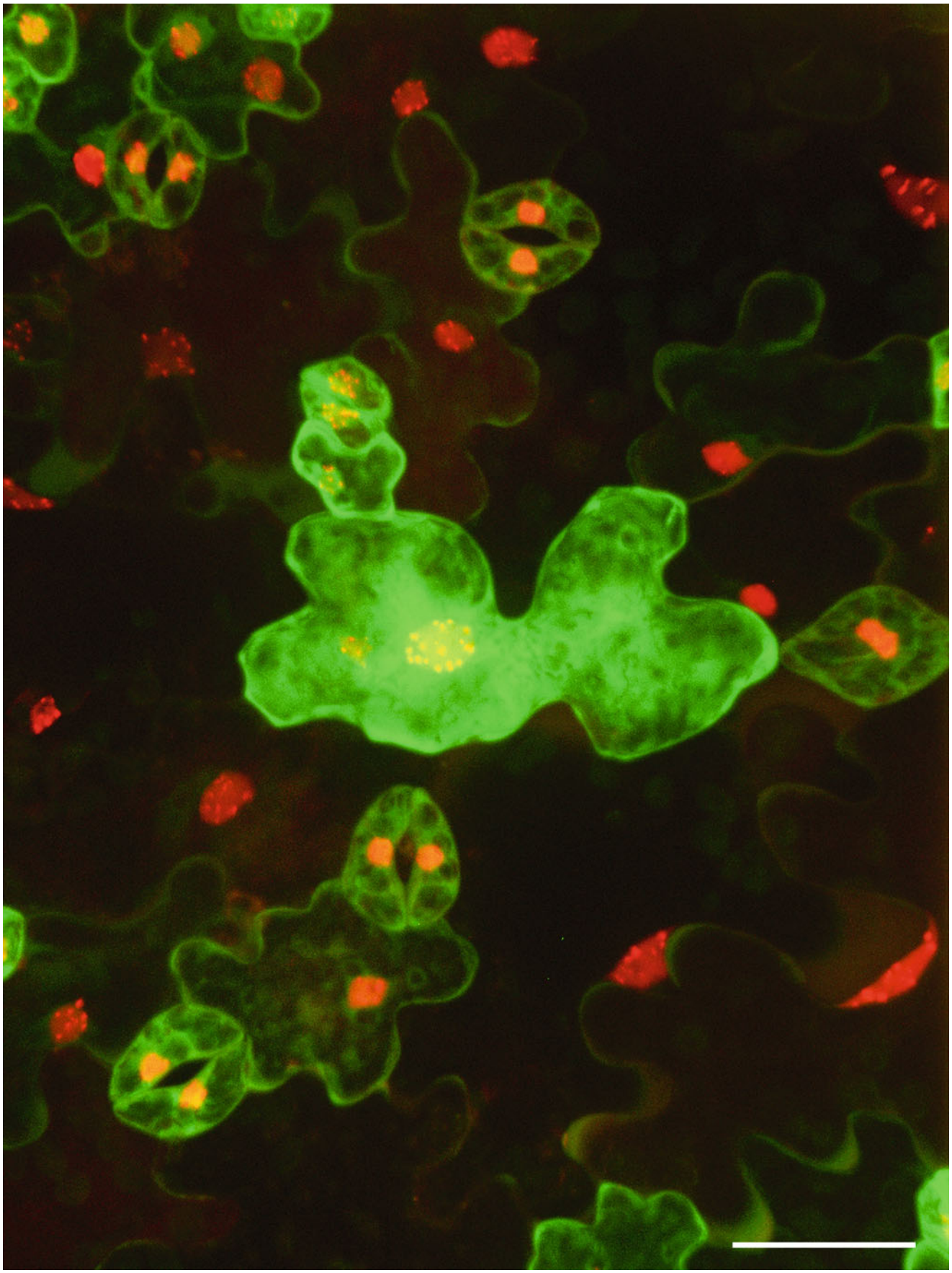

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