PROTOCOL FOR PREPARING SURFACE-SPREAD SYNAPTONEMAL COMPLEXES OF ARABIDOPSIS THALIANA

Sue M. Albini
(School of Biological Sciences, University of Birmingham, P.O.Box 363, Birmingham, B15 2TT, U.K.)

1. SOLUTIONS & MATERIALS
   (i) DIGESTION MEDIUM
   0.05 g Cytohelicase (Reactifs IBF)
   0.125 g polyvinylpyrrolidone (mw 40,000)
   0.188 g sucrose
   12.5 ml sterile distilled water
   Mix together, on ice, then aliquot into 4 or 5 sterile bijous. This solution remains active for about 14 d, but after 10 d, activity is considerably reduced, so it is best to only keep it for a week.

   (ii) SPREADING MEDIUM
   2 ml Lipsol
   98 ml pH 9 water
   * pH 9 water: bring distilled water to pH 9 using borate buffer. Make fresh as required.
   Mix together and store in fridge, keeps for several months

   (iii) SODIUM HYDROXIDE-BORATE BUFFER
   3.1 g Boric acid
   250 ml distilled water
   ~30 ml 1M NaOH
   Dissolve the boric acid in the water and adjust the pH of the solution to 9.5 using approximately 30ml 1M NaOH. Keeps in the fridge for a month or so.

   (iv) FIXATIVE (4% paraformaldehyde)
   100 ml distilled water brought to pH 8.5-9 using borate buffer
   4 g paraformaldehyde
   1.7 g sucrose
   Add the paraformaldehyde to the water and mix. Heat to not more than 65oC and perform the whole process in a fume cupboard. When the paraformaldehyde has dissolved, cool the solution and add the sucrose. This solution is then filtered through Whatman grade three filter paper, buffered to pH 8.5 and stored in the fridge. It will keep for several weeks, but is usually made up fresh every 1-2 weeks. Check the pH before use and readjust to pH 8.5 if necessary.

   (v) PLASTIC COATED SLIDES
   Wash twin frosted slides in acid-alcohol, dry and polish with velin tissue.
   Dip the slide into a 0.75% solution of plastic petri dish (Falcon: Optilux) in chloroform. Remove the slide. As the chloroform evaporates a plastic film coats the slide. The plastic coated slides are made hydrophillic by glow discharging in a coating unit.

   (vi) 50% SIVER NITRATE
   For every 10 slides stained:
   1 g AgNO3
   1 ml distilled water
   Dissolve the silver nitrate in the water. Make fresh as required.
(vii) NYLON GAUZE
Nybolt 3xx-300 Swiss silk bolting cloth

2. PLANT MATERIAL
* The late flowering types of Arabidopsis thaliana are the best to use because they produce large inflorescences with many buds. This makes finding enough buds, at a suitable stage of meiosis, much easier than in a type which flowers quickly and only produces a few buds in any one inflorescence.

3. PROCEDURE
(i) SELECTING POLLEN MOTHER CELLS AT PHASE I OF MEIOSIS
* Select anthers which contain pollen mother cells (PMCs) at prophase I of meiosis:

Remove one anther from a bud estimated to be at the right stage (~0.3mm) place on a slide and add one drop of acetic-orcein. Tap out the anther using a brass rod and place a small cover slip over the top. Place the slide in folded filter paper, blot the excess stain, and squash gently. View the slide under a light microscope and determine the stage of the PMCs. The other five anthers in the bud will be approximately at the same stage.

(ii) RELEASING PMCs AND DIGESTING THE POLLEN MOTHER CELL WALL
Accumulate about 15 or 20 anthers containing PMCs at prophase I and place in 40 ul of digestion medium in a cavity slide. Tap out the anthers using a brass rod to release the PMCs into the digestion medium. Progress in breaking up the anthers should be monitored under a dissecting microscope. This procedure is timed for four minutes from the onset of tapping.

(iii) SPREADING THE NUCLEI
Place 40 ul of the detergent solution on a plastic coated slide. The cell suspension is transferred from the cavity slide and added to the detergent solution on the coated slide. This slide is on a hotplate running at about 30-35oC under a fume hood.

(iv) FIXING THE NUCLEI
Three min after the cell suspension has been added to the detergent solution 60 ul of fixative are added to the slide. Once the slide is dry, remove it from the hotplate to a flat surface at r.t.

(v) WASHING
The slides are left to dry down o/n. Following this they are washed in distilled water to remove any traces of the solutions applied during spreading. After air drying they may then be stained or stored.

(vi) STAINING
Two drops of silver nitrate are added to a slide and then a piece of nylon gauze, the size of a large coverslip (50x22mm), is pre-soaked in the silver nitrate solution and placed on the slide. The slides are then incubated in a moisture chamber at 65oC for about 2 h. After staining, the gauze is floated off in tap water and the slides are rinsed and washed in fresh tap water. The slides are air dried and can be scanned by light microscopy.
(vii) SCANNING AND TRANSFER TO GRIDS
The slides are scanned by light microscopy. Suitable surface-spread nuclei can be identified using the x25 and x40 objectives. When located the nuclei are marked by making a dot with an indelible felt tip pen, on the slide, just above the nucleus. The plastic film is scored around the area of the marked spread nuclei and floated off onto a clean water surface. Electron microscope (EM) grids are then placed on the film at the sites of marked nuclei and the film and grids are picked up on a small piece of plastic-backed filter-paper (Benchkote). When the film and grids are dry, the grids are removed and are scanned by light microscopy. The locations of surface spread nuclei are then noted.

(viii) VIEWING
The surface-spread nuclei are viewed in a transmission electron microscope at 80kv. The preparations can then be photographed and analysis of the synaptonemal complex complements is undertaken.