Materials

- freshly prepared CSF extract
- sperm nuclei (3000/ml)
- rhodamine-labelled tubulin (2-3 mg/ml) see Hyman in this issue [Hyman, 1991 #2551; Hyman, 1991 #1915]
- microscope slides
- 22x22 mm coverslips
- fluorescence microscope
- Hoechst dye (bisbenzimide) was purchased from Sigma (Cat. No. H33342)
- corex tubes (15 ml) were equipped with plastic adapters (home-made see [Evans, 1985 #1113]) to support 12 mm round coverslips
- The tubes were centrifuged in an HB-4 rotor.
- pGEX vectors were from Pharmacia.

Solutions

400 mM CaCl2 stock solution

to make 100 ml, add X g CaCl2-2H2O to 100 ml distilled water. Store in aliquots at -20°C.

4.9 M MgCl2 stock solution (Sigma)

Calcium solution

4 mM CaCl2, 100 mM KCl, 1 mM MgCl2. To prepare 100 ml, combine 1 ml 400 mM CaCl2 stock solution, 20.4 µl 4.9 M MgCl2 stock solution and 0.75 g KCl and bring volume to 100 ml with distilled water. Store in aliquots at -20°C.

10 X MMR

see Making Xenopus Extracts

Hoechst dye solution

10 mg/ml bisbenzimide. To prepare 1 ml, add 10 mg to 1 ml distilled water. Store in the dark at 4°C.

Spindle fix

For 1 ml, combine 600 ml of 80% glycerol, 300 µl of 37% formaldehyde, 100 µl of 10X MMR and 0.5 µl of 10 mg/ml Hoechst dye. Always prepare fresh on day of use.

5X BRB80

4 M PIPES, 5 mM MgCl2 and 5 mM EGTA, pH 6.8. To prepare 250 ml, add 302.4 g PIPES, 0.26 ml 4.9 M MgCl2 and 0.48 g EGTA to 200 ml distilled water. While stirring, add KOH pellets until the PIPES dissolves. Adjust final pH to 6.8 with 2 M KOH and bring volume to 250 ml. Sterilize by ultrafiltration and store at 4°C.

Dilution buffer

30% glycerol, 1% triton-X-100, BRB80. For 100 ml, combine 30 ml glycerol, 1 ml triton-X-100, 20 ml 5X BRB80, and 49 ml distilled water. Store at room temperature.

Cushion

40% glycerol, BRB80. For 500 ml, combine 200 ml glycerol, 100 ml 5X BRB80 and 200 ml distilled water. Store at 4°C.

0.2 M Tris pH 8

To prepare 100 ml, dissolve 2.42 g Tris base in 80 ml distilled water. Adjust pH to 8.0 with concentrated HCl, and bring volume to 100 ml. Autoclave and store at room temperature.

Mounting media

90% glycerol, 10 % 0.2 M Tris-HCl, pH 8. For 10 ml, combine 9 ml glycerol and 1 ml 0.2 M Tris-HCl, pH 8.

Steps for sperm DNA spindle assembly

1. On ice, add 0.5 µl rhodamine tubulin and 1.25 µl sperm nuclei to 50 ml CSF extract in a 1.5 ml tube. (about 100 sperm/µl extract)

2. Incubate for 10 minutes at 20°C, then release extract into interphase by addition of 5 µl calcium solution, mix well.

3. Incubate for 1 hour, 15 minutes at 20°C. Check that the extract is in interphase by transferring 1.2 µl to a microscope slide, using a cut-off tip. Carefully place 6 µl of fixation solution on top of the drop of extract, and squash gently by placing a 22x22 mm coverslip on top. If the sample is to be saved, seal the coverslip to the slide with nail polish. By fluorescence microscopy, nuclei should appear large, round and uniform. Microtubules should be long and abundant.

4. At 1 hour, 30 minutes post calcium addition, add 0.5 volumes (25 µl) of fresh CSF extract to the reaction. Continue incubation at 20°C.

5. Take samples at 15, 30, 45, 60 and 90 minutes after addition of fresh extract to assess the spindle assembly reaction.

6. For immunofluorescent analysis of samples, 10-20 µl of spindle assembly reaction is transferred to a 1.5 ml eppendorf tube and 1 ml of dilution buffer is added. The mixture is layered over a 5 ml of cushion in a 15 ml modified corex tube containing a 12 mm coverslip. Tubes are centrifuged for 15 minutes at 10,000 rpm in an HB-4 rotor. Supernatant and cushion are aspirated before removal of the coverslip. Coverslips are post-fixed in -20°C methanol for 5 minutes, then transferred to PBS and stained with primary and secondary antibodies. The DNA is stained with 5 mg/ml hoechst for 2 minutes. After washing, the coverslips are placed upside down on a 4 ml drop of mounting medium and sealed with nail polish.