Materials and methods

Cell sectioning

Pelleted cells were fixed in Karnosky's fixative (2% Paraformaldehyde, 2.5% Glutaraldehyde) in 0.1 M sodium cacodylate buffer for 3 h at 4 ºC. Cells were washed thrice in 0.1 M sodium cacodylate buffer, 10% w/v sucrose with 30 min intervals at 4 ºC. Secondary fixation was carried out in 2% aqueous osmium tetroxide for 1 h at room temperature. Cells were dehydrated at room temperature in ethanol- 75% and 95% for 15 min each, followed by 100% for 30 min. Cells were dried over anhydrous copper sulphate for 15 min at room temperature. Dehydrated cells were washed twice in propylene oxide for 15 min followed by infiltration in a 50/50 mixture of propylene oxide/Spurr resin overnight at room temperature. Specimens were soaked in full strength Spurr resin for 6-8 h at room temperature and embedded in fresh Spurr resin for 24 h at 60 °C. Ultrathin sections, approximately 70-90nm thick, were cut on a Reichert Ultracut E ultramicrotome and stained for 15 min with 3% uranyl acetate in 50% ethanol followed by staining with Reynolds lead citrate for 2 min. Electron micrographs were recorded at 100 kV on a Philips CM100 microscope fitted with a Gatan Ultrascan 667 CCD camera, at magnifications between 5000X and 9000X.