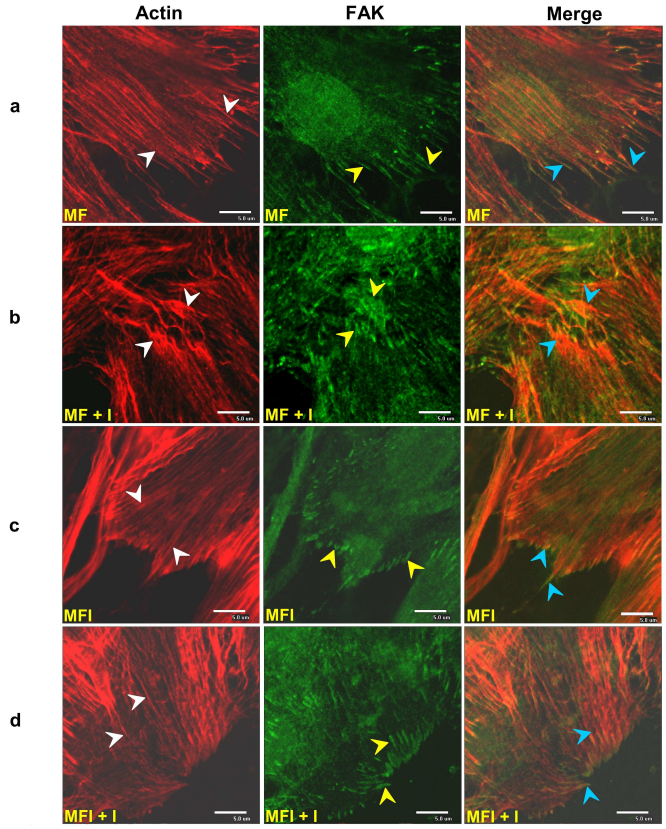
# Šūnu bioloģijas metodes 15.05.2009
# 2. seminārs

**1. Izlasiet tekstu un apskatiet fotogrāfijas.**
**2. Kāds ir eksperimenta mērķis?**
**2. Uzskaitiet sagatavotos preparātus.**
**3. Kādas kontroles tika izmantotas eksperimentā, kuri preparāti atbilst šīm kontrolēm?**

Immunofluorescence studies of C2C12 cells (transfected and untransfected) were carried out as described previously [11,38]. Briefly, myotubes were subjected to starvation for 4 h and treated with 100 nM insulin for 30 min at 37°C. Cells were fixed with 2% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.3 for 30 min. Cells were washed in PBS containing 1% BSA and blocked using blocking buffer (BSA 1%, goat serum 2% in PBS) for 30 min. Cells were permeabilized by incubating with 0.2% Triton X-100 for 10 min, followed by washing with PBS/BSA solution. Cells were incubated with anti-FAK antibody for 2 h at room temperature. Bound antibody was visualized under microscope (Nikon, Tokyo, Japan) by incubating with secondary antibody labeled with FITC. For labeling of actin filaments, fixed and permeabilized cells were incubated for 1 h at room temperature with Phalloidin Texas Red (0.01 U/coverslip). To assess autofluorescence, additional samples were treated for 1 h with PBS without labeled phalloidin. For nuclear staining, cells were labeled with 600 nM DAPI in PBS for 5 min at room temperature. Cells were washed further with PBS and mounted in mowiol on to glass slides.




**Effect of insulin on subcellular distribution of FAK and Actin**. C2C12 cells were differentiated under insulin sensitive (MF) and insulin resistant (MFI) condition and stimulated with or without insulin for 30 min at 37°C after 4 h starvation followed by fixation and permiabilization and probed with anti-FAK antibody. Bar corresponds to 5 μm.
Images were captured from different fields and a representative image of 3 experiments is presented. a: C2C12 cells differentiated under MF condition, b: C2C12 cells differentiated under MF condition, stimulated with insulin, c: C2C12 cells differentiated under MFI condition, d: C2C12 cells differentiated under MFI condition, stimulated with insulin. White arrow head represent actin filaments, Yellow arrow head represent FAK localization and Blue arrow head indicates colocalized FAK and actin.
Bisht and Dey *BMC Cell Biology* 2008 **9**:48
doi:10.1186/1471-2121-9-48

**(2 punkti)** Eksperimenta mērķis:

**(5 punkti)** Sagatavotie preparāti:

**(5 punkti)** Kontroles: