DETECTION OF NEW TARGETS OF PKC?? IN EMBRYONIC STEM CELLS USING A PHOSPHOPROTEOMIC APPROACH. Costa-Junior, H.M.[1,2], Glaser, T.[1,2], Andrade, A.[3], Labate, C.A.[3], Sartore, R.[4], Rehen, S.[4], Xavier-Neto J[2], Teixeira da Silva , A.[5], Perales, J.[5], Krieger, J.E.[2], and Schechtman, D.[1,2] – 1. Department of Biochemistry of the Chemistry Institute of the Universidade de São Paulo, Brazil. 2. Laboratório de Genética e Cardiologia Molecular, Instituto do Coração (InCor-HCFMUSP) São Paulo, Brazil; 3. Departamento de Genética, ESALQ, USP,SP, Brasil. 4. Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde,Instituto de Ciências Biomédicas, Departamento de Anatomia, Rio de Janeiro, Brazil; 5. Instituto Oswaldo Cruz, Laboratorio de Toxinologia, Rio de Janeiro, Brazil.

Protein kinase C (PKC) are a serine/ threonine family of 10 kinases extensively expressed in several cell types. PKCs participate in several biological processes of embryonic stem (ES) cells, which include cell proliferation, self-renewal and differentiation. The biological function of each PKC isoenzyme is related to their subcellular location and substrate targets. Identification of PKC isoenzyme specific substrates and determination of isoenzyme localization can lead to a better understanding of the role of these enzymes in ES cell signalling. The murine ES cell line, E14TG2A, expresses mainly PKCs α, βI, βII, δ, ε and ζ. In these cells PKCβI is expressed as a holoenzyme, and as two lower molecular weight forms that are catalytically active. The catalytically active form of PKCβI is localized in the nucleus of E14Tg2A cells. To detect PKCβI specific substrates ES cell lysates were acutely treated with a PKCβI specific peptide inhibitor and resolved in 2D gels, developed with a phospho-specific dye. We identified fifteen proteins whose phosphorylation decrease with the PKCβI specific peptide inhibitor. Six proteins were nuclear proteins. Three proteins were directly related to cell division: retinoblastoma-binding protein4, lamin B1 and α-tubulin. PKCβI co-localized with α-tubulin during mitosis at the mitotic spindle of dividing ES cells. PKCβI directly phosphorylated α-tubulin and inhibition of PKCβI disorganized the mitotic spindle and increased the chromosomal instability, suggesting a role for PKCβI in spindle formation during ES cell division. Importantly, PKCβI co-localized with a-tubulin at the mitotic spindle in mouse blastocysts.