STRATEGIES FOR THE SELECTION OF PKC TARGETS IN MURINE EMBRYONIC STEM CELLS.

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Phorbol esters (general PKC activators) induce ES cell proliferation, however, it is still not clear which specific PKC isozymes and substrates are involved in this process. Elucidating the role of specific PKC isozymes is now possible due to the development of PKC isozyme specific modulators. Our main goal is to determine the role of specific PKC isozymes in ES cell self renewal and proliferation identifying PKC isozyme specific targets.

We used 2-D gel electrophoresis to identify direct and indirect PKC substrates. ES cells were treated with phorbol ester and three strategies were used to identify PKC substrates. 1) Control and phorbol ester treated lysates were labeled with fluorochromes using 2D - difference gel electrophoresis (DIGE). 331 spots were found in control lysates and 595 spots in phorbol ester treated lysates. 2) Gels were stained with a phospho-specific dye, Pro-Q Diamond. 122 spots were identified in control ES cells and 152 spots in phorbol ester treated cells. 3) Using Western blot with phospho specific serine and threonine antibodies we detected 17 spots in control cells as compared to 52 in phorbol ester treated cells.

Proteins that continuously appeared in different experiments and that could also be detected with Coomanssie blue or Silver staining are being identified by mass spectrometry.

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