

45. ANALYSIS OF PROTEIN EXPRESSION IN SMALL NUMBERS OF MOUSE OOCYTES AND PREIMPLANTATION EMBRYOS

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Western blotting (WB) is a widely used method for analysis of protein expression. It is generally used with many thousands of cells. The detailed study of protein expression in mouse oocytes and preimplantation embryos has been limited by the low sensitivity of WB and the high cost and logistical difficulty of collecting large numbers of oocytes or preimplantation embryos. This report describes the development of methods that allow quantitative analysis of proteins in small numbers of cells. We used a combination of the Pharmacia PhastSystem for electrophoresis and Pierce Chemiluminescent Substrates for detection. We choose several protein targets that we expected to express at different levels within the embryo: Lis-1 (a structural protein), Bad (a regulatory protein that forms heterodimers) and CREB (a transcription factor). Oocytes were collected in 2 μ L of PBS and proteins extracted in 2 μ L lysis buffer containing (2 $\times$ PBS, 2% Triton X-100, 24 mM deoxycholic acid, 0.4 mM Na vanadate, 1% NP-40, 0.2% sodium dodecyl sulfate, 20 mM NaF, 20 mM $\text{Na}_4\text{P}_2\text{O}_7$, 2 mM PMSF, 3.08 μ M Aprotinin, 42 μ M Leupeptin and 2.91 μ M Pepstatin A). Samples were then boiled for 7 min with 1.5 μ L loading buffer (50 mM Tris-HCl, 5 mM EDTA pH 8.0, 12.5% sodium dodecyl sulfate, 0.05% bromophenol blue and 25% beta-mercaptoethanol). Size separation was performed on 20% homogenous SDS polyacrylamide gels (Pharmacia) on a PhastSystem apparatus (Pharmacia, Sweden). Blotting onto PVDF membranes (Hybond-P, Amersham Pharmacia) was performed overnight by capillary action. Membranes were then incubated with primary antibody overnight at 4°C. Followed by washing and incubation with horseradish peroxidase conjugated secondary antibody for 1 h at RT. Membranes were washed and developed with either 1:2 diluted Pico SuperSignal or 1:4 diluted Femto Maximum Chemiluminescent Substrates (Pierce, Rockford, IL, USA) at room temperature. Using these techniques, Lis-1 was routinely detected in 1 oocyte or embryo, Bad in 5 oocytes and CREB in 15. Furthermore we demonstrated that it was possible to strip and reprobe membranes with different primary antibodies at least 3 times with little loss of sensitivity. The remarkable sensitivity achieved with these methods now allow the power of quantitative WB to be routinely used in the analysis of protein expression and protein interactions in the oocyte and preimplantation embryo.