

ABSTRACT

Dynamic cellular activity is fundamental to all life. Virtually all life processes, are modulated by the reversible phosphorylation of proteins, mediated by protein kinases and phosphoprotein phosphatases, respectively. This thesis focuses on three enzymes, namely: phosphoprotein phosphatase 1, phosphoprotein phosphatase 2A and protein tyrosine phosphatase-1B. Temporal variations in the expression of the enzyme proteins were examined in the human acute promyelocytic leukaemic cell line, HL-60. The cells were induced to differentiate along the macrophage pathway using phorbol-12-myristate-13-acetate and along the granulocytic pathway using dimethyl sulfoxide, all-trans retinoic acid and 9-cis retinoic acid. Modulation of the rhythmic patterns of protein and messenger RNA was monitored in the absence and presence of inducing agents.

Expression of protein in cell extracts prepared at various time intervals was determined by western immunoblotting, while mRNA expression was assessed by northern blotting and RT-PCR. The probe used for northern blotting was generated during the RT-PCR procedure. In addition, PTP-1B mRNA was cloned into an expression vector to produce recombinant protein.

Results indicate that the expression of phosphoprotein phosphatase 1, phosphoprotein phosphatase 2A and protein tyrosine phosphatase-1B protein is dynamically regulated in proliferating HL-60 cells and modulated after being induced to differentiate along either the macrophage or granulocytic pathway. Similar changes were also noted with PTP-1B mRNA when using northern blot analysis. Using molecular cloning techniques, PTP-1B mRNA was successfully cloned into pGex-4T-1 expression vector to produce recombinant PTP-1B protein, which was checked by sequence and western blot analysis.