A Comparison of Topoisomerase I Activity in Normal and Transformed Cells

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Many viral oncogenes encode protein–tyrosine kinase activities. However, important \textit{in vivo} substrates of these enzymes have yet to be identified. Recently, type I topoisomerases were shown to be \textit{in vitro} substrates for two tyrosine kinases. Following tyrosine phosphorylation, topoisomerase I activity was reduced 10-fold (Tse-Dinh \textit{et al.} Nature \textbf{312}:785–786, 1984). To determine whether topoisomerase I activity was modulated by tyrosine phosphorylation \textit{in vivo}, we have measured topoisomerase I activity in nuclear lysates prepared from both normal fibroblasts and cells transformed by two different viral oncogenes (\textit{v-ABL, v-SRC}). Under a variety of experimental conditions, we have found no evidence to support the notion that type I topoisomerase activity is modulated by tyrosine phosphorylation \textit{in vivo}.

INTRODUCTION

Nearly half of all known viral oncogenes have been shown to possess an intrinsic protein–tyrosine kinase activity which has been correlated with their transforming potential (reviewed by Hunter and Cooper, 1984). Many workers have tried to identify substrates for these enzymes that may be physiologically important in the transformation process but have met with little success (reviewed by Hunter and Cooper, 1984; Foulkes and Rich-Rosner, 1985).

Eukaryotic topoisomerase type I catalyses the relaxation of supercoiled DNA and has been postulated to have a central role in many aspects of DNA metabolism (reviewed by Gellert, 1981) including DNA transcription (Sanzey, 1979; Gocke \textit{et al.},

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1983), replication (Gellert, 1981), recombination (Kikuchi and Nash, 1978), and repair (Witkin and Wermundsen, 1978). If topoisomerase I activity was modulated by phosphorylation in vivo, this could have a dramatic effect on cell growth, and perhaps contribute to malignant transformation. Using a hepatoma cell line, Durban et al. (1983) have demonstrated that topoisomerase I is phosphorylated in vivo on serine residues. Serine phosphorylation of topoisomerase I correlated with a three-fold increase in enzymatic activity. Recently, Tse-Dinh et al. (1984) reported that in vitro phosphorylation of calf thymus topoisomerase I on tyrosine by either a viral kinase (pp60\(^{v-src}\)) or a cellular protein–tyrosine kinase, resulted in a 10-fold reduction in topoisomerase I activity. It was decided, therefore, to determine if topoisomerase I activity is modulated by tyrosine phosphorylation in vivo. This is particularly important as protein–tyrosine kinases lack specificity and phosphorylate many proteins in vitro which are not phosphorylated in vivo (reviewed by Foulkes and Rich-Rosner, 1985). To approach this question, we determined topoisomerase I activity in nuclear lysates prepared from normal NIH3T3 fibroblasts and NIH3T3 cells transformed by oncogenes encoding two different protein–tyrosine kinases [\(v-abl\) (Foulkes et al., 1985) and \(v-src\) (Hunter and Sefton, 1980)].

**MATERIALS AND METHODS**

**Cell Lines**

Three cell lines were used in the study: NIH3T3, ANN-1, and pV-src. NIH3T3 is an established fibroblast cell line of murine origin (Todd and Green, 1963). ANN-1 was derived from NIH3T3 cells following transformation by the \(v-abl\) oncogene (Scher and Siegler, 1975). The pV-src cell line is a NIH3T3 cell line transformed by \(v-src\) and kindly provided by B. Mathey-Prevot, M.I.T., Cambridge, MA, USA.

**Production of an Enzymatically Active Topoisomerase I Fraction**

1–2 \(\times 10^6\) cells/ml were harvested by trypsinisation and washed twice in ice-cold phosphate buffered saline to remove residual trypsin. The cells were lysed for 30 min on ice in 5 ml of 10 mM Tris–HCl pH 7.9 (2\(^\circ\)C), 10 mM NaCl, 3 mM MgCl\(_2\), 100 \(\mu\)M vanadate, 0.1% Nonidet P-40, and then mixed with an equal volume of the same buffer containing 30% sucrose. The mixture was centrifuged at 4200 g for 15 min to pellet the nuclei. The nuclei were gently resuspended in the above lysis/sucrose buffer and the centrifugation step repeated. The nuclei were resuspended in 0.5 ml of 50 mM Tris–HCl pH 7.9 (2\(^\circ\)C), 50 mM NaF, 30 mM pyrophosphate, 100 \(\mu\)M vanadate, 1 mM DTT, 2 mM EDTA and sonicated for 30 s using a precooled probe.

**Standardisation of Topoisomerase I Activity**

To compare the topoisomerase I activity of each cell line, nuclear lysates were standardised against both the protein concentration and the DNA content of each sample. Cell number does not provide an accurate standard, as the two cell types are of