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Isolation and characterization of a human cDNA clone encoding a novel DNA topoisomerase II homologue from HeLa cells

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We have isolated and sequenced 3 human DNA topoisomerase II (topo II) partial cDNA clones from a HeLa carcinoma cell cDNA library. Two clones were identical to an internal fragment of HeLa topo II cDNA. The third clone, CAA5, had a different and novel sequence which shared significant nucleotide (62%) and predicted peptide (70%) homologies with a region of the HeLa topo II cDNA. Our results suggest that HeLa cells express at least two homologous forms of DNA topoisomerase II. The new HeLa topo II homologue is discussed in relation to topo II isoenzymes recently described in a Burkitt lymphoma and other cell lines.

DNA topoisomerase II; Human cell line; cDNA cloning; Isoenzyme; Protein sequence homology

1. INTRODUCTION

DNA topoisomerase II (EC 5.99.1.3; topo II) is an important nuclear scaffold protein implicated in key biological processes including DNA replication and chromosome segregation [1-3]. The enzyme is a homodimer that acts to pass a duplex DNA segment through a transient enzyme-bridged double strand break in DNA [3,4]. Several clinically useful anticancer agents, e.g. etoposide and doxorubicin, appear to exert their cytotoxic effects by interfering with this enzymatic DNA breakage-reunion reaction [5].

Human topo II was first purified as a 170 kDa polypeptide (p170) from the HeLa cervical carcinoma cell line for which Wang and colleagues reported the cDNA sequence, and we in parallel obtained a homologous partial cDNA clone (CAA1) [6,7]. However, very recent work has demonstrated the presence in the Burkitt lymphoma cell line Raji of two isoenzyme forms of topo II: IIα, analogous to p170 in HeLa cells, and a 180 kDa form, termed IIβ or p180 [8]. Analysis of partial cDNA clones isolated from a Raji library indicated that these proteins derive from two different mRNA species which are differentially expressed in the cell cycle [9].

During the course of cDNA cloning studies using CAA1 as a hybridization probe, we isolated a novel human HeLa cDNA clone that encodes an as yet unreported topo II-related polypeptide. In light of current interest in different forms of topo II and their biological roles, we describe here the initial characterization of the novel cDNA and its relationship to cDNAs for the human p170 and p180 topo II isoenzymes.

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2. EXPERIMENTAL

2.1. *cDNA cloning*

A human HeLa cell λgt10 cDNA library was obtained from Dr D. Gewert. It was constructed by oligo dT priming of mRNA (isolated from interferon-treated HeLa cells) and the resulting cDNAs were cloned into λgt10 using *Eco*RI linkers. The library was screened under conditions of low stringency with a partial cDNA clone (CAA1) encoding part of the C-terminal region of the p170 form of human topoisomerase II [7]. CAA1 was isolated by cross-species expression screening of a HepG2 λgt11 library and spans a 231 base pair (bp) region between nucleotide positions 3495 and 3726 of the 4753bp cDNA sequence [6,7]. Hybridisation was carried out at 42°C in buffer containing 6× SSC, 5× Denhardts, 0.5% sodium dodecyl sulphate, 100 μg/ml denatured salmon sperm DNA and 8% dextran sulphate. Positive clones were plaque purified by at least 3 successive rounds of screening. Phage λDNA was isolated as described [10].

2.2. *DNA sequence analysis*

Insert DNA from λgt10 clones released by digestion with *Eco*RI, was purified by electrophoresis in a 2% low gelling agarose gel (Sigma) and isolated on NA45 paper (Schleicher and Schuell). Insert DNA or fragments arising from digestion with *Hae*III or *Bgl*II were ligated into appropriately cut M13mp10 and mp11 RF DNA and used to transform *E. coli* XL1 *recA*. Single stranded recombinant M13 DNA was purified and sequenced by the Sanger dideoxy method using either Klenow fragment (Amersham) or Sequenase II (United States Biochemical Corp.). Sequence information was analysed using the Genofit/PC Gene Package (Intelligenetics).

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3. RESULTS

3.1. *Isolation of cDNA clones homologous to human p170 DNA topoisomerase II*

Screening of a HeLa λgt10 cDNA library with the partial p170 topo II cDNA CAA1 yielded 3 positive clones, two of which had an identical 1.7 kb *Eco*RI insert (CAA4) while the third contained a 700 bp insert (CAA5) (Fig. 1). The restriction map of CAA4 was identical to part of p170 topo II cDNA (Fig. 1) and this identification was confirmed by DNA sequence analysis. CAA4 spans nucleotide positions 2014–3698 of p170 topo II cDNA encoding amino acid residues 660–1220 of the 1530 residue protein. The probe CAA1 is therefore identical to the 3′ end of clone CAA4 (Fig. 1).

The second type of cDNA clone, CAA5, isolated at the same time under the same conditions as CAA4, had a different restriction map to those of CAA4 and p170 cDNA (Fig. 1) and suggested it may encode a homologous but novel form of DNA topoisomerase II.

3.2. *Protein encoded by the novel human cDNA CAA5 aligns and shares substantial homology with human p170 topo II*

The 702 bp nucleotide sequence of CAA5 was determined and found to exhibit 62% homology with a segment of the p170 topo II cDNA – nucleotide positions 3062–3764 (Figs 1, 2). CAA5 has a single uninterrupted open reading frame encoding a 234 amino acid residue polypeptide (Fig. 2B). The CAA5 protein sequence aligns with residues 1021–1253 of p170 topo II displaying a 70% overall homology (58% identical residues and 12% conserved residues) (Fig. 3). Homology with

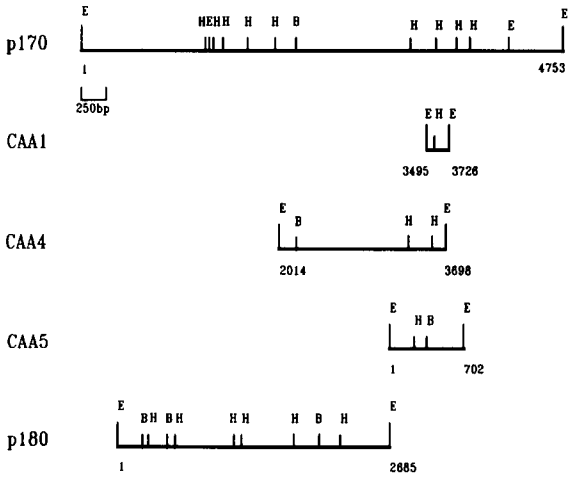

Fig. 1. Restriction maps and alignment of topo II cDNA clones. p170 and p180 isoenzyme maps were generated from DNA sequence in references [6] and [9]. E, H and B denote restriction sites for *Eco*RI, *Hae*III and *Bgl*II. CAA1 and CAA4 are identical with p170 cDNA between the indicated nucleotide positions. CAA5 and p180 are aligned by their respective homology with the p170 cDNA sequence.

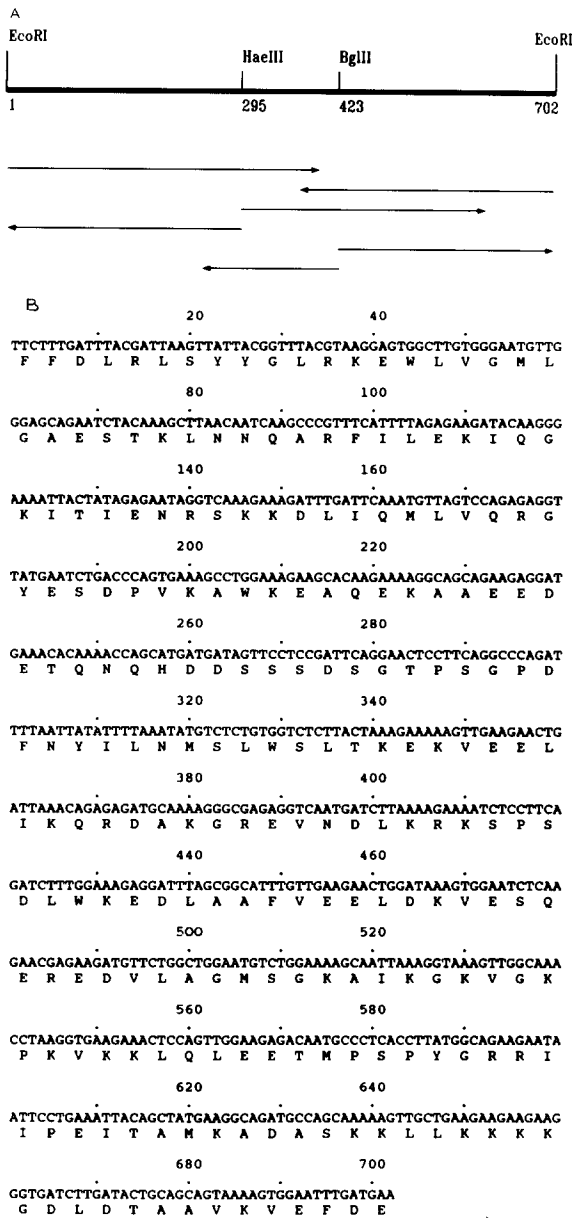

Fig. 2. DNA sequence analysis of CAA5, a topo II-related HeLa cDNA. (A) DNA sequencing strategy. (B) Nucleotide sequence of CAA5 and the derived amino acid sequence of its single open reading frame.

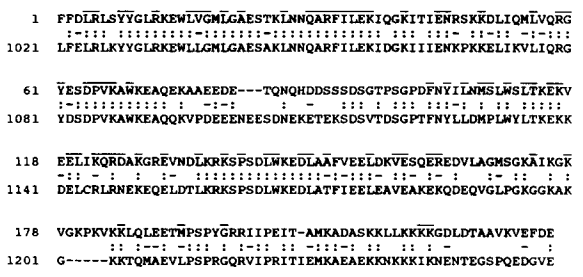

Fig. 3. Alignment of the predicted amino acid sequence of CAA5 (top line) with that of p170 topo II [6] showing identical (:) and conserved residues (-). Amino acid residues conserved between CAA5 and *Drosophila* topo II [11] are overlined.

p170 topo II is especially marked at the N-terminal end of the CAA5 polypeptide: of the first 81 residues, 62 are identical and a further 10 are conserved substitutions. Significant homology at the protein level is also observed between CAA5 and the appropriate region of topo II from other species including *Drosophila* (Fig. 3) and yeast (not shown). Comparison of CAA5 with the recently published partial cDNA and protein sequence of the human p180 topo II isoenzyme [9] (Fig. 1) did not reveal significant homology (not shown).

4. DISCUSSION

Topo II from HeLa cells has previously been isolated only as a 170 kDa polypeptide form [4]. The corresponding cDNA has been described and the suggestion made that HeLa topo II is encoded by a single copy gene, mapped to chromosome 17 [6]. Our cDNA cloning studies reported here indicate that HeLa cells also express another protein, partially encoded by CAA5, that exhibits remarkable sequence conservation with p170 topo II and other eukaryotic topo II enzymes and indeed may represent a distinct human isoenzyme form.

At present, we do not know whether the CAA5-encoded protein is the same as or different from the p180 topo II isoenzyme described in Raji cells [8]. The longest partial cDNA clone (SP11) for p180 isolated from a nitrogen mustard resistant Raji line, appears to encode the N-terminal region of the protein, judged by its homology to nucleotide region 379-3065 of the p170 topo II cDNA (Fig. 1) [5,9]. In contrast, CAA5 encodes protein sequence homologous to the C-terminal end of p170 topo II and does not overlap that determined for p180 (Fig. 1).

It is interesting, however, that the p180 and CAA5 sequences are homologous to directly *adjacent* regions of p170 topo II, each sequence terminating at an *Eco*RI site in the respective cDNA (Fig. 1). The derived amino acid sequence for p180 topo II reported by Chung et al. [9] finishes at a position equivalent to residue 1021 of the p170 form (terminating in the cDNA at a natural *Eco*RI site) while that from CAA5 is homologous to p170 residues 1021–1253 (Fig. 1). Thus, the p180 and CAA5 partial cDNA clones could correspond to contiguous *Eco*RI cDNA fragments. Conceivably, CAA5 encodes a C-terminal segment of the p180 topo II isoenzyme.

Chung et al. isolated their Raji p180 topo II partial cDNA clone by screening with a probe encoding *Drosophila* topo II [9]. In contrast, CAA5 was obtained using a C-terminal *human* HeLa topo II probe (Fig. 1). It is known that HeLa, *Drosophila* and other eukaryotic topo II cDNA sequences are closely homologous in the N-terminal two thirds of the coding sequence whereas nucleotide sequences for the C-terminal regions are more divergent [11]. These considerations may explain why the different screening approaches yielded topo II-related cDNA clones that align with different ends of the HeLa p170 cDNA sequence.

Topo II p170 and p180 isoenzymes have been detected in cell extracts from a number of sources in addition to Raji cells including human U937 monocyte and COLO 201 colon carcinoma cell lines and in murine P388 leukaemia and NIH 3T3 fibroblast cell lines [9]. HeLa p170 topo II was isolated before the existence of isoenzyme forms was generally recognized. It will now be important to examine HeLa cell extracts for the presence of other topo II proteins, e.g. p180.

The physiological and chemotherapeutic roles of topo II isoenzymes are of great interest and remain to be elucidated. Further work is in progress to characterise fully the structure, expression and biological function of the novel CAA5 encoded HeLa protein.

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