ISOLATION OF A HUMAN cDNA CODING FOR A NUCLEAR LAMIN

Maribel Guerra

Division of Industrial Biotechnology, Center for Genetic Engineering and Biotechnology, P.O.Box. 6162, La Habana 6, C.P. 10600,Cuba.

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SUMMARY

We have previously described the isolation and cloning of a human DNA fragment (pB48; 1560 bp) that belongs to the fraction of DNA replicated at the onset of S-phase in synchronized HL-60 cells.
Analysis of this fragment indicated that it derives from an expressed region of the human genome which code for several transcripts (Northern experiments). Extended our studies to a chromosomal region of 13.7 kb, named L3OE and encompassing pB48 we have found two closely spaced and non-overlapping genes, one of which corresponded to a B-type nuclear lamin.
Lamin proteins line the inner side of the nuclear envelope with a network of filamentous structures and are thought to play a role in nuclear stability, chromatin structure, and gene expression.
We report here isolation and nucleotide sequences of the coding portion of a B-type human nuclear lamin.

RESUMEN

En trabajos anteriores nuestro grupo reportó el aislamiento y clonaje de un fragmento de ADN humano (pB48; 1560 pb) perteneciente a una fracción de ADN que se replica en los primeros minutos de la fase S del ciclo celular. Análisis posteriores indicaron que esta región es altamente transcripta, demostrado por experimentos de Northern utilizando ARN de células HL-60.
Dos genes separados por aproximadamente 200 pb y pertenecientes a diferentes unidades transcripcionales fueron detectados cuando la región de ADN en estudio fue extendida a 13.7 kb (L3OE) y enteramente secuenciada. Por estudios comparativos a nivel de secuencia nucleotídica y secuencia aminoacídica se pudo comprobar que uno de estos genes codificaba para una lámina humana de tipo-B. Las láminas nucleares son proteínas fibrosas localizadas en la parte interna de la membrana nuclear. Entre sus funciones se encuentra la estabilización del núcleo en desarrollo y juegan un importante papel en la organización y distribución de la cromatina en la interfase celular.
En este artículo se reporta el aislamiento y secuencia nucleotídica de la porción codificante para una lamina humana tipo- B, aislada de una fracción de ADN replicada en los primeros minutos de la fase S en células HL-60.

INTRODUCTION

The nuclear lamina is a fibrous structure interposed between the chromatin and the inner nuclear membrane (Kaufmann, 1988). Because of its strategic location, this structure is thought to be involved in a number of important processes including anchorage of the pore complexes (Newport, 1987), re-establishment of nuclear architecture after meiosis and mitosis (Burke and Gerace, 1986; Gerace and Blobel, 1980) and transduction of chemomechanical information from the cytoplasmic intermediate filament network to the nucleus (Gerace *et al.*, 1978).

The major features of the nuclear envelope are an inner and outer membrane, nuclear pore complexes, and a nuclear lamina. The outer nuclear membrane is continuous with, and form part of, the rough endoplasmic reticulum, while the inner membrane is in close association with the chromatin. Both of these membranes are joined where they are traversed by nuclear pore complexes, the structures forming the channels for nucleocytoplasmic exchange (Feldherr *et al.*, 1984).

In higher eukaryotes the lamina has been found to consist predominantly of one or more related proteins termed lamins (Gerace, 1986). In mammalian somatic cells, where the lamina has been best characterized, three major lamins, A, B, and C (MW, 70, 67, and 60 kd, respectively) are found (Gerace *et al.*, 1978).

Here, it is reported the isolation and nucleotide sequences of specific cDNA (4.3) from screened HL-60 cell cDNA library with a probe (subclone pBE2) that specifically hybridizes only to the 5000 nt RNA and it derives from an expressed region of the human genome (Biamonti *et al.*, 1992).

Alignment of the 4.3 cDNA sequences with that of mouse nuclear lamin B_2 cDNA (Hoger *et al.*, 1990) shows a strinking similarity in the coding region and indicates that clone 4.3 is in effect a partial cDNA that lacks the 5' end, and coding for a B-type human nuclear lamin.

MATERIALS AND METHODS

Isolation of cDNAs: Approximately 8 x 10^5 plaques of a HL-60 cell cDNA library in λgt10 were screened with clone pBE2 as a probe.

Double lifts with nylon filters (Amersham) were made from each plate. Filters were prehybridized for 4 h at room temperature

and then hybridized for at least 16 h at 42°C in a solution containing 50% formamide, 4x Denhardt's solution, 5x SSC, 25 mM NaPPi, 200 µg of yeast tRNA per mL, and the ^{32}P-labeled probe at a concentration of 2 x 10^6 cpm/mL. Filters were washed several times at room temperature in 2x SSC, 0.1% SDS, and then for 30 min at 65°C in 0.5x SSC, 0.1% SDS. Isolated clones were purified, and their inserts were subclones in the pUC19 vector and sequenced as described below.

DNA sequencing: Single subclones were sequenced by the dideoxy-chain termination method (Sequenase kit; U.S.Biochemical) utilizing the universal and reverse primers of pUC19. To complete the sequence, several oligonucleotide were produced.

Nucleotide sequence accession number: The 4.3 insert has been assigned the Gene Bank accession number M94362.

Northern hybridizations: RNA preparation and Northern blot analyses was as previously described (Buvoli *et al.*, 1988).

Hybridization conditions were as follows: 5x SSC, 5x Denhardt's solution, 25 mM NaPPi, 0.2% SDS, 200 µg of yeast tRNA per mL, and 50% (vol/vol) formamide at 42°C for 18 h; washing was done in 0.2x SSC, 0.1% SDS at 68°C for 30 min.

Probes were labeled by the random primer technique as specified by the supplier of the kit (Amersham) and used at 2-5 x 10^6 cpm/mL.

RESULTS AND DISCUSSION

Transcription pattern in the L3OE Region

In a previous article, we detailed the isolation and characterization of the genomic clone L3OE (Biamonti *et al.*, 1992). In this report, we extended our studies at the transcription analysis of this genomic region by performing Northern hybridizations on RNA from HL-60 cells.

The results (figure 1) indicate a complex transcription pattern when differents subclones of L3OE are used as probe in Northern hybridization to HL-60 cell poly (A)$^+$ RNA. In addition to the two RNAs previously detected (Perini *et al.*, 1990) another abundant transcript of 1450 nt plus several minor but longer ones (up to 5000 nt) were detected. However, under the same conditions, the pBE2 probe detected only the 5000 nt transcript (figure 1, lane c).

The 5000 nt RNA encodes a B-type human nuclear lamin

To expande the observation refered above, we screened an HL-60 cell cDNA library (see Materials and Methods) with a probe (subclone pBE2) that specifically hybridize only to the 5000 nt.

RNA (figure 1)

All of the inserts, that hybridized positively, were subcloned in pUC19, the longest one (4.3; 4271 bp) was entirely sequenced.

A poly (A) tail is found downstream a canonical polyadenylation site that can be located on L3OE DNA. In fact, alignment of cDNA and genomic sequences allowed determination of the intron-exon structure of this portion of gene.

Three exons are possible, separated by two introns bounded by the canonical AG..GT dinucleotides and with the expected stretch of pyrimidines immediately upstream of the 3' splice site (figure 2).

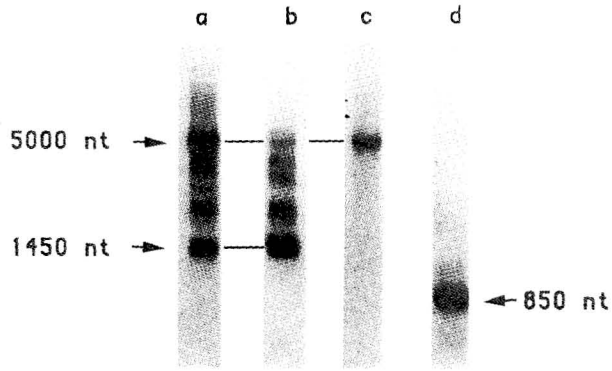

Fig. 1. Northern hybridization with 5 µg of HL-60 cell poly (A)$^+$ RNA, using 4.3 cDNA (see text)(line a) and L3OE subclones pSE10 (lane b), pBE2 (lane c), and pSE17 and pBN1 (lane d) as probe. Positions of the major transcripts are indicated by arrows.

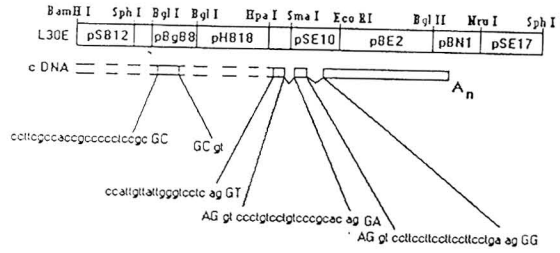

Fig. 2. Schematic representation of 4.3 cDNA belonging to the transcripts of 5000 nt (figure 1). The exon-intron structure and the corresponding junction sequence were determined because L3OE clone was entirely sequenced. The poly (A) tail [$(A)_n$] is indicated

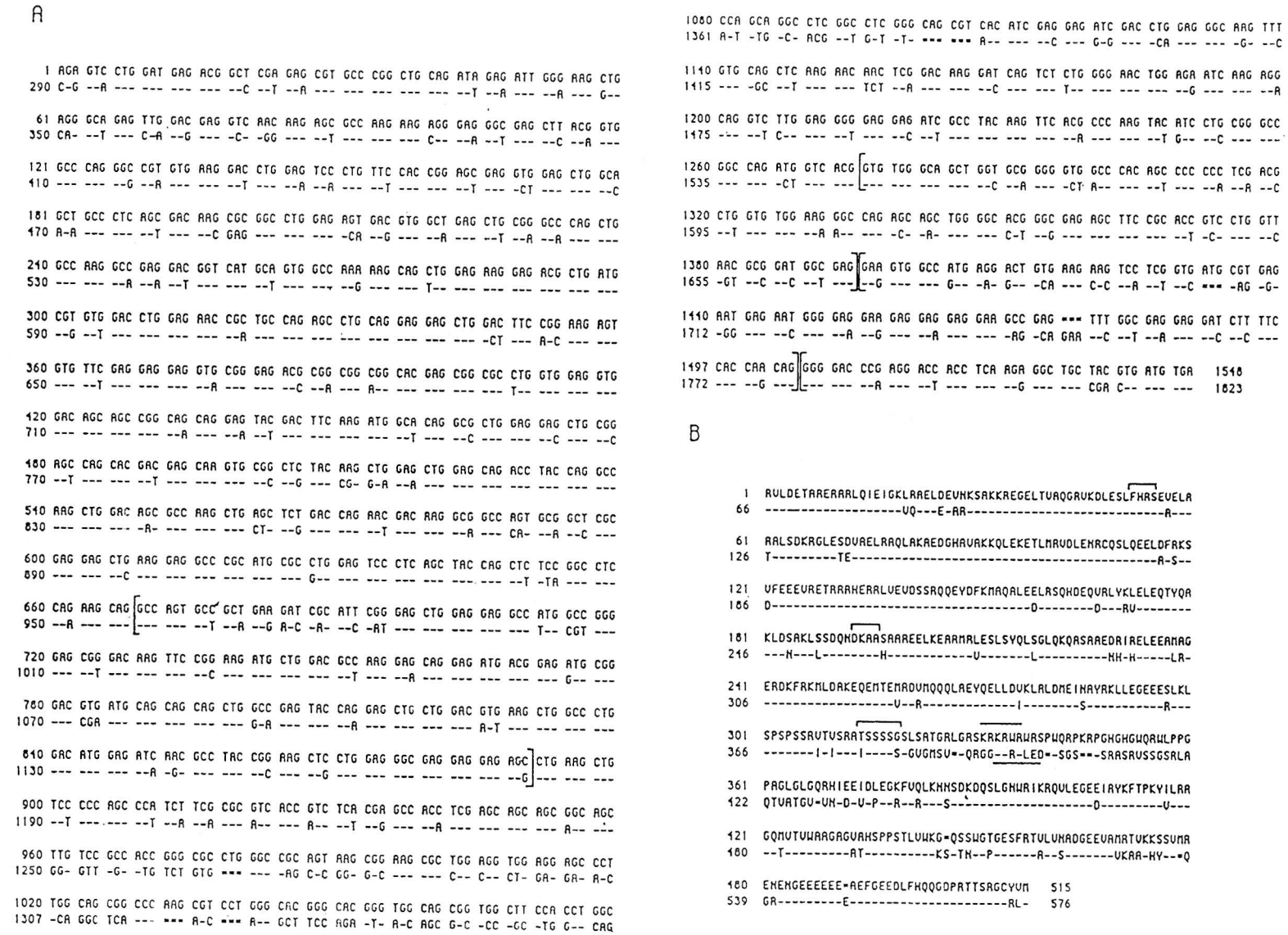

Fig. 3 Nucleotide (A) and deduced amino acid (B) sequences of the coding portion of 4.3 cDNA. The sequence of the mouse lamin B2 cDNA is shown beneath for the sake of comparison. Mismatched nucleotides or aminoacids are indicated. Dotted line, identical sequence. Double-dotted line, missing nucleotides or aminoacids. Brackets in panels A enclse the identified exons. The putative nuclear localization signals(B) are indicated by solid bars. The short lamin B2 diagnosting sequences are bracketed in panel B.