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Lamin A gene expression is specifically suppressed in v-*src*-transformed cells

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By a differential screening procedure, we have obtained several cDNAs the expression of which was down-regulated in v-*src*-transformed 3Y1 cells compared to parental 3Y1 cells. One of these cDNAs, termed N26, was extensively analyzed. Sequence analysis revealed that N26 cDNA was 3,095 nucleotides in length and contained an open reading frame of 665 amino acid residues. Based on an homology search at the nucleotide level, it was postulated that N26 gene encodes lamin A. The expression of the N26 gene was not suppressed in other types of transformed cells, such as v-*mos*-, v-*ras*- and SV40-transformed 3Y1 cells.

Differential screening; 3Y1 cell; v-*src*; Lamin A; Herbimycin A; Down-regulation

1. INTRODUCTION

The viral oncogene of Rous sarcoma virus, termed v-*src*, encodes a 60,000 Da protein [1,2] with tyrosine-specific protein kinase activity and is necessary for cellular transformation [3]. In spite of a number of studies, the molecular mechanisms by which the v-*src* gene product induces cellular transformation are not well understood. In order to elucidate v-*src*-induced transformation at the molecular level, it is important to investigate the changes in cellular gene expression upon viral transformation [4]. Information regarding the cellular genes, the expression of which is down-regulated in response to v-*src* activity, is limited. The expression of three genes encoding fibronectin, collagen and tropomyosin have been reported to be suppressed in v-*src*-transformed cells [5–7]. Here we show that the amount of lamin A mRNA is decreased specifically in v-*src*-transformed 3Y1 cells.

2. MATERIALS AND METHODS

2.1. *Cells and culture conditions*

Rat fibroblast 3Y1 cells and v-*src*-transformed 3Y1 cells were generously provided by Dr. H. Sakiyama, National Institute of Radiological Science, Japan. The v-*mos*-, SV40- and v-H-*ras*-transformed 3Y1 cells were obtained from the Japanese Cancer Research Resources Bank [8]. All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. Cell lines were grown at 37°C in a 5% CO_2 atmosphere.

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2.2. *Isolation of total RNA and polyadenylated RNA*

Total RNA was prepared by the SDS–phenol method. Poly(A)$^+$ RNA was purified using an oligo(dT)–celiulose column [9].

2.3. *Construction and screening a cDNA library*

3Y1 cDNA liberally was prepared according to the standard method [9] with 1 μg of oligo(dT)-purified RNA using λZAP II digested with *Eco*RI as a vector. For the differential screening, the phage library was plated, transferred onto two sets of nylon membranes and hybridized with one of two different random primer-labeled cDNAs derived from 3Y1 or v-*src*-transformed 3Y1 cells. Plaques showing preferential hybridization signals with 3Y1 cDNAs were picked up and re-screened.

2.4. *Northern analysis*

For Northern blot, 10 μg of total RNA was electrophoresed on a 1% agarose gel containing formaldehyde [9], blotted onto Nylon membrane and cross-linked with UV light. A 0.6 kb *Eco*RI–*Xba*I fragment of N26 cDNA was labeled with [α-^{32}P]dCTP using random primers [10] and used as a probe. The membranes were hybridized in a solution containing 6× SSC, 5× Denhardt's solution [11], 0.1% SDS and 50% formamide at 42°C overnight, and washed sequentially in 2× SSC, 0.1% SDS at room temperature for 10 min and in 0.1× SSC, 0.1% SDS at 50°C for 20 min. Autoradiography was performed at −70°C with an intensifying screen.

2.5. *DNA sequencing*

Recombinant plasmids carrying restriction fragments of the cDNA were prepared according to the standard method [9] and the nucleotide sequences were determined using the dideoxy chain-termination method [12].

2.6. *In vitro transcription and translation*

N26 cDNA was subcloned into the *Eco*RI site of the pTZ18R expression vector. After complete digestion with *Kpn*I, it was used as the template for in vitro transcription with T7 RNA polymerase. The in vitro-transcribed RNA was translated in a rabbit reticulocyte lysate (NEN Corp.) in the presence of [^{35}S]methionine. Protein products were separated on a 10–20% SDS-polyacrylamide gradient gel.

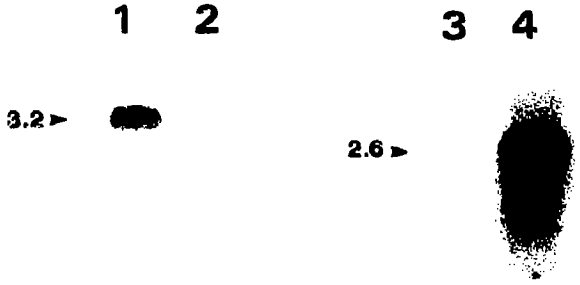

Fig. 1. 3Y1-specific expression of the N26 gene. Total RNA (10 μg) was separated by 1.0% formaldehyde–agarose gel electrophoresis, transferred onto a Nylon membrane and hybridized with a radiolabeled *Eco*RI–*Xba*I fragment of N26 cDNA (lanes 1 and 2) or v-*src* DNA (lanes 3 and 4). Lane 1 and 3, total RNA from 3Y1 cells; lanes 2 and 4, total RNA from v-*src*-transformed 3Y1 cells. Size is shown in kb.

2.7. *Treatment of herbimycin A*

Herbimycin A was generously provided by Dr. Y. Uehara, National Institute of Health, Japan. The drug was added to the culture (0,5 μg/ml) of v-*src*-transformed cells. 24 h after the drug treatment, total RNA was isolated and Northern analysis was performed.

2.8. *Nucleotide sequence accession number*

The sequence of N26 cDNA is deposited in EMBL Data Library under accession number X66870.

3. RESULTS AND DISCUSSION

A cDNA library was made using poly(A)$^+$ RNA from 3Y1 cells and λZAP II as a vector. Approximately 2.5×10^5 phage plaques were differentially screened and several cDNA clones were obtained which hybridized strongly with ^{32}P-labeled cDNAs derived from 3Y1 cells but not with those from v-*src*-transformed 3Y1 cells. One of these clones, N26-1, was picked up further analysis performed.

The mRNA level of the N26 gene was assayed by Northern analysis. As shown in Fig. 1, the amount of N26 mRNA was hardly detectable in v-*src*-transformed 3Y1 cells compared with that in 3Y1 cells.

Because the size of N26-1 cDNA (0.7 kb) was much shorter than that of N26 mRNA (3.2 kb), we tried to isolate the full-length cDNA. By screening the same

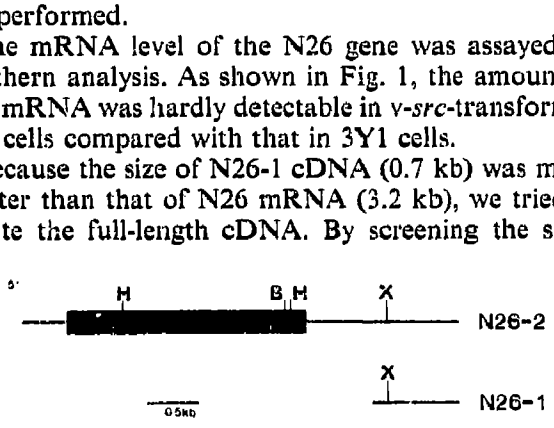

Fig. 2. Restriction map of N26-1 and N26-2 cDNA. Thin line represents the 5'- and 3'-noncoding regions and the filled box shows the coding region. B; BamHI, H; HindIII, X; XbaI.

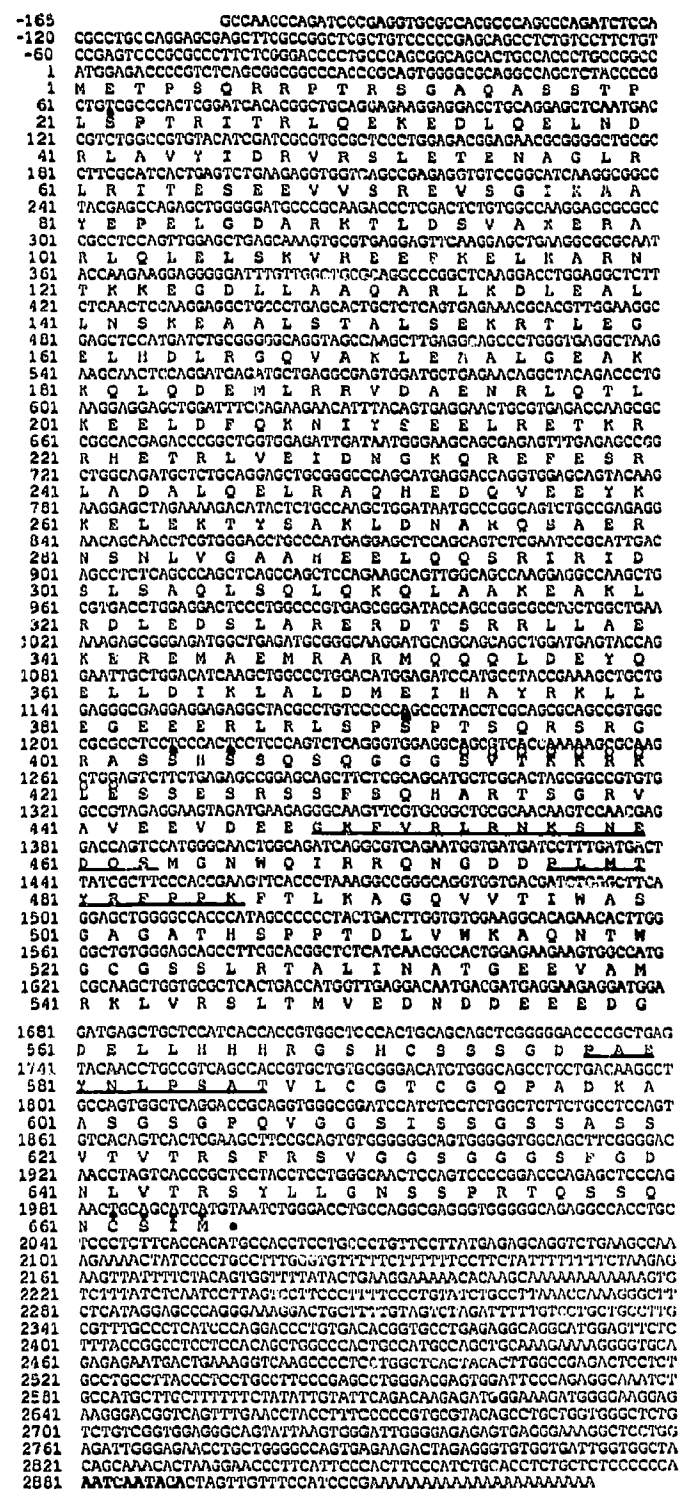

Fig. 3. Nucleotide and deduced amino acid sequence of N26 cDNA. The translation initiation site is referred to as +1. Two 3'-polyadenylation signals are shown by bold letters. The stop codon is represented by an asterisk. Open circles indicate the putative nuclear localization signal and closed circles denote the serine residues which could be phosphorylated (see text). The peptide sequences determined by Fisher et al. [15] are underlined and a CXXM motif is shown by filled triangles (see text).

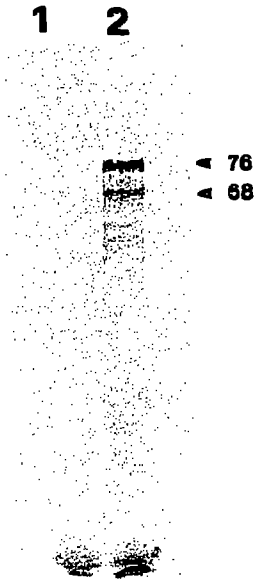

Fig. 4. In vitro transcription and translation of N26 cDNA. Cloning vector alone or N26 cDNA was transcribed in vitro and the synthesized RNA was translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Protein products were separated by 10–20% SDS polyacrylamide gel electrophoresis. Lane 1, pTZ 18R; lane 2, N26 cDNA. Size is represented in kDa.

cDNA library, we obtained an N26-2 cDNA clone that contained, approximately, a 3 kb insert (Fig. 2). From a comparison of the estimated sizes between N26-2 cDNA and mRNA, we tentatively concluded that N26-2 cDNA was nearly full-length.

The nucleotide sequence of the cDNA insert was determined by the dideoxy chain-termination technique [12]. The sequence data of N26-2 cDNA is shown in Fig. 3. N26 cDNA is 3,095 nucleotides in length and the first AUG codon at position 1 opens a reading frame that encodes a 665 amino acid polypeptide with a predicted molecular weight of 74,244.84 Da. The sequence near the initiation site (CCGGCCATGG) matched the consensus sequence for the initiation of translation proposed by Kozak (GCCA/GCCATGG) [13]. In order to confirm that this open reading frame (ORF) truly encodes a protein, we carried out in vitro transcription and translation experiments (Fig. 4). As a result, two specific bands were observed, the estimated molecular weights of which were about 76 and 68 kDa, respectively. As the size of the slower migrating band was in close agreement with that predicted from the ORF, the faster migrating band might have been generated by degradation of the 76 kDa product. Therefore, we concluded that the ORF does encode the protein product. This cloned cDNA included 165 and 935 nucleotides in the 5′- and 3′-untranslated regions, respectively (Fig. 3).

The nucleotide sequence of N26 cDNA was compared with those filed in the data base (EMBL, release 25.0). A significant similarity was detected between N26 cDNA and lamin A cDNA of human, chicken and *Xenopus*. The nucleotide sequence homology in the coding region of N26 cDNA for human [15], chicken [16] and *Xenopus* lamin A cDNA [17] was 92.6, 73.7 and 69.8%, respectively. In addition, the amino acid sequence homology in N26 peptide for human, chicken and *Xenopus* lamin A was 96.2, 75.9 and 71.5%, respectively. Rat lamin A was previously purified from liver and its partial amino acid sequences were dtermined [15]. These amino acid sequences corrspond to residues 449–463, 477–486 and 578–587 in our sequence data (Fig. 3). Comparing these peptide sequences with that of the N26 product, one mismatch was observed: arginine in the third peptide was replaced with Pro-584. We do not know whether this was due to a cloning artifact or the different source of materials. Lamin A and C have been known to share the same sequence except at the C-terminal region [15]. In our experiments, we used a 0.6 kb *Eco*RI–*Xba*I fragment of N26 cDNA which is specific to lamin A as a hybridization probe, so we could distinguish both types of mRNA. This is the first observation that the amount of lamin A mRNA is extremely decreased in v-*src*-transformed cells.

N26 protein includes several structural features which are also found in lamin A of other species. It contains a central α-helical rod domain extending from residue 31 to 390. A putative nuclear localization signal can be found between amino acid positions 414 and 422 (SVTKKRKLE). In addition, the CXXM motif is located at the C-terminal sequence. The potential phosphorylation sites (Ser-22, Ser-392, Ser-404 and Ser-406) are present on the N26 sequence [18].

As the expression of the N26 gene was down-regulated in v-*src*-transformed 3Y1 cells, it seemed important to investigate whether or not N26 gene expression was also suppressed in other types of transformed cells. Total RNA was isolated from the following cell lines,

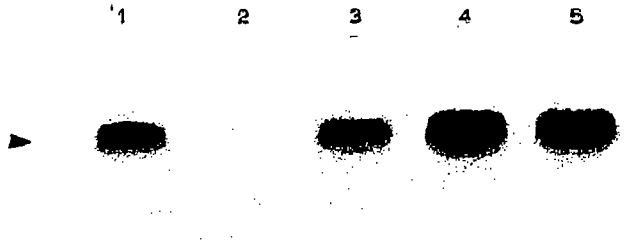

Fig. 5. N26 gene expression in other types of transformed cells. Total RNA (10 μg) derived from 3Y1 (lane 1), v-*src*-3Y1 (lane 2), v-*mos*-3Y1 (lane 3), SV40-3Y1 (lane 4) and v-H-*ras*-3Y1 cells (lane 5) was electrophoresed on a 1.0% formaldehyde–agarose gel, blotted onto a Nylon membrane and probed with a ^{32}P-labeled *Eco*RI–*Xba*I fragment of N26 cDNA.

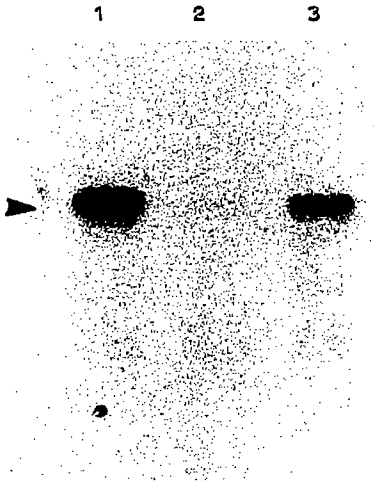

Fig. 6. Effect of herbimycin A on N26 gene expression in v-*src*-transformed 3Y1 cells. Cells were treated with 0.5 μg of herbimycin A per ml for 24 h. Total RNA was isolated and Northern analysis was carried out using ^{32}P-labeled cDNA as a probe (lane 3), along with RNA from 3Y1 cells (lane 1) and v-*src*-transformed 3Y1 cells (lane 2).

v-*mos*-, SV40- and v-H-*ras*-transformed 3Y1 cells, and the N26 mRNA level of each cell line was assayed by Northern analysis. As shown in Fig. 5, the amount of N26 mRNA was not decreased in these transformed cells. From these observations, down-regulation of N26 (lamin A) mRNA level seems to be a specific event in v-*src*-transformed 3Y1 cells.

As previously described, herbimycin A (one of the benzenoid ansamycin antibiotics) can induce the loss of pp60$^{v\text{-}src}$ tyrosine kinase activity and conversion to normal phenotypes in RSV-transformed rat kidney cells [20]. In order to investigate the relationship between tyrosine kinase activity and N26 gene expression, the effect of herbimycin A was tested in the present study. As shown in Fig.6, the treatment of herbimycin A can increase the amount of N26 mRNA. This result suggests that the expression of the N26 gene depends upon the tyrosine-specific protein kinase activity encoded by v-*src* and also supports the idea that the down-regulation of N26 mRNA levels is a specific phenomenon in v-*src*-transformed 3Y1 cells.

Microinjection of purified pp60$^{v\text{-}src}$ into frog oocytes results in a large amount of protein phosphorylation, not only on tyrosine residues but also on serine/threonine residues [21]. This result indicates that pp60$^{v\text{-}src}$ can interact with cellular serine/threonine kinases. Recently, it has been proposed that several transcription factors could be phosphorylated by cellular kinases, suggesting that activities of transcription factors might be modulated by protein phosphorylation [22–24]. In v-*src*-transformed cells, some cellular factor(s) which could stimulate the expression of the lamin A gene might be inactivated by v-*src*-induced protein phosphorylation. Elucidation of the molecular mechanism regarding the suppression of lamin A gene expression could lead to a more precise understanding of the cellular transformation induced by v-*src*.

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