C-terminal end of v-src protein interacts with peptide coded by gadd7/adapt15-like RNA in two-hybrid system

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Received 2 December 1997; revised version received 8 December 1997

Abstract The significant differences in the metastatic properties of hamster fibroblasts transformed by the Rous sarcoma virus (RSV) were associated with mutations in the v-src carboxy-terminal region. To identify the capacity of this region for protein–protein interaction the two-hybrid system was used. The cDNA clone (vseap1), producing the protein specifically bound with the v-src C-terminal part in yeast cells in vivo and in GST-fusion system in vitro was isolated. Vseap1 shared 68% of homology with stressful agents induced RNA-gadd7/adapt15. Two vseap1 specific messenger RNAs were identified: 0.9-kbp RNA expressed in all transformed cells and three times less in embryo fibroblasts; 3.1-kbp transcript was deleted in the cells with suppressed v-src activity and H_2O_2 resistance.

Key words: v-src; Metastasis; vseap1; gadd7; Two-hybrid system; Hamster cell

1. Introduction

V-src oncogene of RSV and its cellular counterpart c-src are membrane-associated phosphoproteins with tyrosine-specific kinase activity. The role of src in cell adhesion, cytoskeletal changes and motility depends upon its cellular localization and state of activity. The src protein is structured in several functional domains [1–3]. The major difference between v-src and c-src resides at the carboxy ends of the proteins. The last 19 aa of c-src contain a tyrosine residue at position 527. This residue, when phosphorylated, down-regulates c-src kinase activity [4]. In v-src the last aa are replaced by a tail of 12 aa generated as a result of recombination events between the 3′ end of c-src and 39-bp sequence from the downstream non-coding region [5]. The changes at the carboxy-terminal aa of v-src do not affect its kinase activity or cell transformation [6]. The function of this region is unclear.

Recently two new variants (srcHM and srcLM) of v-src oncogene from low and highly metastastic hamster cells were isolated [7]. These src versions have significant structural changes that are not observed in other src genes. In the N-terminal unique region of both HM and LM v-src isoforms a GC-rich insert of 60 nucleotides (20 aa) was found. The presence of this insert accounts for the unusual molecular weight of the encoded v-src protein (62 kDa). Both v-src variants have 10 identical amino acid changes compared to the known RSV-derived v-src sequence. The most interesting feature is that v-srcHM and v-srcLM differ one from the other in several amino acids. Most of the changes are localized in the unique v-src domain and in the extreme carboxy-terminal region. Retroviral vectors carrying srcHM and srcLM caused different morphological transformations of cells, and changes of the metastatic activity in vivo. Differences in the C-terminal region of the v-src proteins were shown to be responsible for the differences in the metastatic properties of the corresponding transformed hamster cells [7].

To assess the molecular mechanisms underlying the v-srcHM specific high metastatic activity or the lack of such an activity in v-srcLM, a yeast two-hybrid system was used. To identify cellular genes associated with the unique C-terminal regions of both v-src variants, the cDNA library from RSV-transformed parental hamster fibroblast cell line was constructed in yeast expression vector. By screening the cDNA library with a v-srcHM C-terminal region as bait, several new genes previously not known as v-src protein partners were isolated. Here we describe one of the genes called vseap1 (v-src end associated peptide), highly homologous to gadd7/adapt15 RNA induced by various types of stress factors as well as by DNA damage [8,9]. Two vseap1-specific RNAs (3.1 and 0.9 kb) were found in virus-transformed and spontaneous tumor cell lines.

2. Materials and methods

2.1. Plasmid constructions

pLexA-v-srcHMC and pLexA-v-srcLMC containing the coding sequence of C-terminal amino acids of v-srcHM gene (amino acids 522–546) or v-srcLM gene (amino acids 500–545) respectively inserted in frame with LexA coding sequence were constructed in the bait plasmid pLex11. pLex11 is a derivative of pBTM116 with a modified polylinker (J. Camonis and V. Jung, unpublished). pGEX-v-srcHMC allows the expression of the v-srcHMC fused to GST. The reading frames of fusion proteins in plasmid constructions were verified by Sequenase sequencing kit (Amersham) using LexA-binding domain or GST-specific primers.

2.2. Hamster cDNA fusion library

RNA was isolated from low metastatic cell line of Syrian hamster fibroblasts HET-SR [10] by direct addition of guanidinium thiocyanate and cesium trifluoroacetate (RNA extraction kit, Pharmacia) followed by purification of poly(A)$^+$ RNA on oligo(dT) cellulose column. The cDNA was synthesized using TimeSaver cDNA synthesis kit (Pharmacia) and cloned between *Eco*RI and *Not*I sites of pGAD3S2X, a derivative of pGAD-GH with a modified polylinker

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Abbreviations: ORF, open reading frame; aa, amino acid(s); GST, glutatione S-transferase; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ECL, chemiluminescence detection system; PMSF, phenylmethylsulfonyl fluoride; IPTG, isopropyl-β-D-thiogalactopyranoside; X-GAL, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

PII S0014-5793(97)01568-8

(J. Camonis, unpublished). ElectroMAX DH10B *E. coli* cells (Life Technology) were electroporated; the complexity of the library was 1.3×10^6 with 75% of the clones containing an insert.

2.3. Yeast two-hybrid screening

The *Saccharomyces cerevisiae* L40 reporter strain (genotype: *Mat a his 3D200 trp1-901 leu2-3,112 ade 2 LYS2::(lexAop)4-HIS3 URA3::(lexAop)8-lacZ GAL4*) was used for two-hybrid screening. Yeast growth and transformation were handled according to standard protocols [11–13]. To identify proteins that interact with v-srcHMC, 1.3×10^7 cells containing pLexA-v-src-HMC were transformed with the cDNA library. Double transformants were selected for histidine prototrophy and tested for LacZ expression by a filter X-GAL assay [14]. Total DNA from $His^+/LacZ^+$ colonies was analyzed by PCR with customized primers for cDNA insert. The average length of cDNA inserts was between 700–900 bp. Positive cDNA plasmids were recovered in *E. coli* strain HB 101 (which carries a leuB mutation) and selection for Leu prototrophy on minimal plates [15]. To test for the target specificity, primary positive library DNA was isolated and used for the yeast retransformation with pLexA-v-srcHMC or pLexA-Lamin (negative control). cDNA from 6 clones specific in protein–protein interaction (v-srcHMC versus lamin) was sequenced. Sequences were analyzed against EMBL using the Fasta 3.0t76 search program.

2.4. Cell lines

Spontaneously transformed Syrian hamster fibroblasts (STHF), Syrian hamster embryo fibroblasts (HEF) and its derivatives – HET-SR, HET-SR 8 (HEF, transformed independently in vitro by different stocks of RSV Schmidt-Ruppin D stain) [10]; HET-SR N-ras transfectants (HET-SR supertransfected with an activated N-ras gene mutated in the 12th codon (G/A) [16] and HET-SR N-ras 'antisense' transfectants (HET-SR N-ras cells supertransfected with N-ras 'antisense' construction) [17] were used. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 100 mg/ml gentamycin at 37°C in the presence of 5% CO_2. The transfectants differed in the expression of v-src protein [16,17].

2.5. RNA blot hybridization

RNA was extracted from hamster fibroblast cell lines by the guanidinium thiocyanate/phenol method [18]. Samples were fractionated in 1% agarose/formamide gel and transferred to Hybond N^+ membrane (Amersham). Filters were probed with [α-^{32}P]dATP-labeled vseap1 DNA obtained from pGADvseap1 cut with *Hin*dIII. Hybridization and washings were performed as described [19]. Following autoradiography, blots were stripped and rehybridized with GAPDH (glyceraldehyde-3-phosphate dehydrogenase) probe. Band intensities were quantified by scanning using Image Quant program.

2.6. Immunoblotting of hybrid protein expression

Overnight yeast cells expressing hybrid proteins were grown in SC medium, diluted up to 10^7 cells/ml and grown to an optical density of 0.6 at 600 nm. The cells were pelleted, washed with distilled water and pelleted again. The pellets were frozen in liquid nitrogen and resuspended in SDS-sample buffer. Samples were lyzed by the glass beads method [20]. Proteins were separated by SDS-PAGE (15% slab gel) according to standard technique [21], transferred onto nitrocellulose (BA 83, Schleicher and Schuell) and analyzed with ECL (Amersham). Fusion proteins were detected using anti-LexA polyclonal antibodies (a gift from P. Moreau).

2.7. In vitro translation

For in vitro transcription and translation pGAD3S2X and pGAD3S2X-vseap1 were used as templates for PCR using a forward primer (5′-CGAATTAACCCTCACTAAAGAAGGATGGCCAATTTTAATCAAAGTGG-3′) containing the T3 promoter for T3 RNA polymerase and an ATG initiation codon) and a reverse universal M13–20 primer (5′-GTAAAACGACGGCCAG-3′). The PCR products were purified on agarose gel (Geneclean II) and transcribed/translated in vitro using a coupled transcription/translation mixture (Promega). 40 μCi [^{35}S]methionine (Amersham) for 1 μg of DNA was used to label the in vitro translated proteins.

2.8. GST-fusion proteins

Overnight cultures of *E. coli* BL21, transformed with GST or with GST-v-srcHMC expressing plasmids were diluted in 500 ml of LB with ampicillin (50 μg/ml). After shaking for 90 min at 37°C, protein expression was induced with 0.4 mM IPTG for 3 h. Cells were pelleted and resuspended in 10 ml lysis buffer (PBS, 20 mM EDTA, 2 mg/ml, 50 μg/ml PMSF, 1% aprotinin and 2 μg/ml leupeptin). After 10 min of incubation and three cycles of freezing/thawing (−80°C and 55°C, respectively), 25 mM $MgCl_2$, 500 mM NaCl, 1% Triton X-100 and 5 mM DTT (final concentrations) were added. To reduce DNA viscosity, lysates were sheared with 12–15 passages through a 21-gauge syringe needle and clarified by centrifugation. After sedimentation of proteins on glutathione-Sepharose 4B beads (Pharmacia), beads were washed four times with washing buffer (20 mM Tris-HCl, pH 7.5, 10% glycerol, 1 mM DTT, 5 mM $MgCl_2$, 100 mM NaCl, 1% aprotinin, 50 μg/ml PMSF). Purity and integrity of GST-fusion proteins were assessed by SDS-PAGE and Coomassie Blue R staining of proteins. Protein quantification was done as described [22].

2.9. Detection of GST-v-srcHMC binding with translated proteins

100 μg of GST or of GST-v-srcHMC proteins bound to glutathione beads were incubated with 10 μl of the transcription/translation mixture containing [^{35}S]methionine-labeled protein in binding buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.2% NP-40, 0.2 mM DTT, protease inhibitors) for 3 h at 4°C. The beads were washed five times at 4°C with 1.5 ml of binding buffer, boiled for 10 min in sample buffer and analyzed by SDS-PAGE (17% slab gel). The gel was stained with Coomassie Blue R, dried and exposed to an X-ray film for 1–2 days.

3. Results

3.1. Identification of hamster cDNA encoding proteins interacting with the C-terminal part of v-srcHMC by two-hybrid screening

The two-hybrid system was used to determine cellular genes associated with C-terminal region of v-srcHMC and v-srcLM [23]. The bait hybrid proteins with C-terminal parts of v-srcHM or v-srcLM, fused in frame with the bacterial binding domain LexA were constructed. The second hybrid was a panel of hamster library cDNA fragments ($\sim10^6$) expressed as fusions to the GAL4 transcriptional activation domain (GADad). Around 1.3×10^7 yeast colonies were screened with pLexA-v-srcHMC, only 336 were His^+. Of these 57 were also $LacZ^+$. The majority of the colonies were positive with Lex-Lamin. A specific interaction with v-srcHMC protein was found only in 6 colonies. Negative with lamin and reproducibly positive with v-srcHMC, cDNA clones encoding proteins were sequenced. Two out of six cDNA clones had the same primary structure and were designated as vseap1 gene.

3.2. Structure of vseap1

The cloned cDNA was sequenced in both directions and had 764 bases plus poly(A)$^+$ tail. A search in the GenBank database demonstrated about 68% of homology with recently characterized gadd7/adapt15 gene [8,9]. The homology between these genes was more significant in the 5′ and 3′ regions (Fig. 1A). The presented data strongly suggest that the vseap1 sequence in Syrian hamster cell is homologous to the sequence of the gadd7/adapt15 isolated from Chinese hamster cells. Surprisingly, the ORF in frame with GAL4-fused protein contained only 8 amino acids (Fig. 1B). The longest predicted ORF in any frame was 44 amino acids. A consensus Kozak box-like element at position 70, in contrast to gadd7/adapt15 was not intact: the initiating ATG was mutated (Fig. 1A). No homology with known protein sequences was found in any of predicted ORF of vseap1.

A.

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vseap1   1 CTGAGCCATTACGTTTCTCGGAACAATGACGACATCATTTTCTGGGTCATCGGAGCAGTT
GADD7    8 CTGAGGTTTTTCCTATGTCTGAACAATGACGCCATCGTTTTCTGGGTCTTCGAAGCAGTT
            *****   ** * * * ** *********** **** *********** *** *******

vseap1  61 GCGAAGAGATGCCCAGAGGGAG-AC----GTCTAGACCATCCGAGGGAATTCGTCAAAGG
GADD7   68 GCAAAGAAATGCCCAGATGGAGGACAGAGGGCCAAACCACCCCAGTGAATTCATAACAGG
           ** **** ********* **** **    * * * **** ** ** ****** * * ***

vseap1 116 CAGTAACGTGCTTAAGACCTGACT----TTTAAAGTGAATGCCAGTGCGTGACGATCCCA
GADD7  128 AGATAACTTGCTTAAGACCTGACTGTTTTTAAAAGCGAATGCCAGTGAGTGAA-ATGCCA
              **** ***************    ** **** *********** ****  ** ***

vseap1 172 GCTCCCTGTACAGTTCCTAGAGCTCCTGGGAGTGCATGGCCAGTGACACAATGCTTTACT
GADD7  187 GCTCCCTGTACAGTTACTAGAGCTCCTGGGAATGCATGGCCAGTGACACAA---------
           *************** *************** ******************

vseap1 232 GGTGTGTGAGAATTGGATGTAGTGAACATCCAGAAAAGGCAGACAAAAGAAGAAAATGCA
GADD7  238 -----GTGAGAATTGGATGTTATGACCGTCCAGAAAAG----ACAAGATGAGAAAGTGCA
                ***************  *** * **********    **** *  ***** ****

vseap1 292 GTGCTGGCTAGGTGTTGGAGATGGGTCTTTACATAGCTCTGGCTGTCCTCAAACACATCC
GADD7  289 GTATTGAC-----------------------------------TGTCTTCAAACGCACCT
            **  ** *                                    **** ****** ** *

vseap1 352 ATCTTGGCTCTCGAGTGCTGGGGATTGTGTGTCAACCAGGCCGGCTATTATGAATAGAGC
GADD7  314 GTCCTTGCTCA--AGTGCTGGG-ATTGTGTGCCAACGGCTATGATAGATATGAATAGAGC
            ** * ****   ********* ******** ****      *     ************

vseap1 412 ATGAGATA---------CAGAAAGTTGTTTGAATTGAATCCAATAAATGGAAGCACTCAG
GADD7  371 ACGAGATAATGTGCTTACAGAAAGTTGGTTGAATTGAATGCAATTAATGTTTG----GAG
           * ******         ********** *********** **** ****   *     **

vseap1 463 TCTCTTAATCCTATTGGGNTTAAAATATAGATTGGATTAAGGCTGGGTTTGGAGTGAACC
GADD7  427 GCACTTAGTCTTCTAGTGTTTGAA-TGTGGATGGAATTAAAGCTGGGTTTGTAGTGAGCC
            * **** ** * * * * ** ** * * *** * ***** ********** ***** **

vseap1 523 TTTAGGACTATGGCAA-TGTTTAAGGGA----------TGTATGCTAATGGTTGT-CCTG
GADD7  486 ATTAGGATTATGGCAAATGTCTAGTGTACAGAATTCAGTGTAGGCCGGGAGTTGAGACTG
           ****** ******** *** **  * *          **** **     ****   ***

vseap1 571 TCGC--------------ACATGCTCGACAAAA-CGTAAT----TTGATCCCAAG-CAGT
GADD7  546 GTGTGTGTTTAGTGGATTATCTGATCAGCACATGTGTGACAGTGTTGATCACAAGTCATT
             *               *  ** **  ** *   ** *     ****** **** ** *

vseap1 611 GCCATCTCTGTTCATTTTCTTCCCTGGTCTAAAT-GGTAATTCCCATCCTGAAAATGGTG
GADD7  606 GCCAGCTCTGTTCATTTTCTTCCCTGGTCTAACTCAATAATTGCCATCATGAAAATTCTG
           **** ************************** *   ***** ***** *******  **

vseap1 670 GGTGACATTTTGTGGCAGTGGAGTTCCATTATAATCCACATTTCCTTGTCCAGCAGTGCT
GADD7  666 GGTGAC-TTTTGTGAC--TAAAGTCCCATTGTAAGCCACATT-CTTTGTCCAGCAGTGCT
           ****** ******* *  *  *** ***** *** ******* * ***************

vseap1 730 CTCTGTAAATGCTGAAATAAAGGTTCAGTTGCAAC
GADD7  722 CACTG--ACTGCTGAAATAAATGTTCATTTGCAAC
           * ***  * ************ ***** *******
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B.

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E P L R F S E Q Stop R H H F L G H R S S C E E Met P R G R R L D H P R E F V K G S N V L K T
Stop L L K Stop Met P V R D D P S S L Y S S Stop S S W E C K R R K C S A G Stop V L E Met G L
Y I A L A V L K H I H L G S R V L G I V C Q P G R L L Stop I E H E I Q K V V Stop I E S N K W K
H S V S Stop S Y W X Stop N I D W I K A G F G V N L Stop D Y G N V Stop G Met Y A N G C P V A H
A R Q N V I Stop S Q A V P S L F I F F P G L N G N S H P E N G G Stop H F V A V E F H Y N P H F
L V Q Q C S L Stop Met L K Stop R F S C N
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Fig. 1. Structure of vseap1. A: The nucleotide sequence of the hamster gene vseap1 obtained by two-hybrid cDNA library screening is shown in the first line (EMBL Nucleotide Sequence Database accession number AJ003192). The second line is the sequence of the gene gadd7 from EMBL. Nucleotide similarities between vseap1 and gadd7 are indicated by stars, gaps indicated by a dash. Stop codons in frame of fusion protein are underlined. B: Inferred amino acid sequence. Stop codons and methionine residues are indicated.

3.3. Comparison of v-srcHMC and v-srcLMC interaction with vseap1 gene encoding peptide

The ability of vseap1 peptide to interact with v-srcHMC and v-srcLMC was tested. The specific pGADvseap1 plasmid was introduced into yeast cells in the presence of pLexA-v-srcHMC or pLexA-v-srcLMC. The expression of fusion proteins LexA-v-srcHMC and LexA-v-srcLMC, verified with LexA polyclonal antibodies, was practically at the same level. Vseap1 protein interacted with both v-srcHMC and v-srcLMC (Table 1). However, we found that this interaction was weaker with the C-terminal part of v-srcLM protein. Probably, the relatively decreased level of interaction with v-

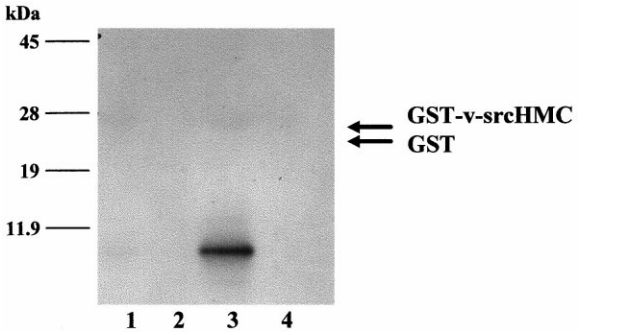

Fig. 2. Protein binding of C-terminal region of v-srcHM to vseap1 in vitro. Purified GST or GST-v-srcHMC (approximately 100 μg) proteins were precipitated with ^{35}S-labeled GAL4ad or GAL4ad-vseap1 translated proteins as described. Precipitates were separated on 17% SDS-PAGE. Autoradiograph of the dry gel is presented. Lane 1: GAL4ad-vseap1+GST; lane 2: GAL4ad+GST-v-srcHMC; lane 3: GAL4ad-vseap1+GST-v-srcHMC; lane 4: GAL4ad+GST. The positions of GST and GST-fusion protein are indicated by arrows.

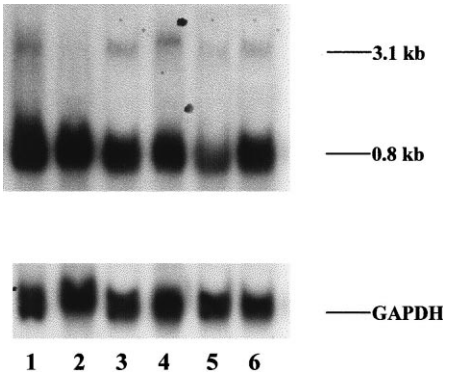

Fig. 3. Northern blot analysis of vseap1 RNA from different cell lines of hamster fibroblasts. Total RNA samples were fractionated in 1% agarose/formamide gel and transferred to Hybond N$^+$ membrane. The blot was hybridized with vseap1 specific probe and rehybridized with the GAPDH probe. Lane 1: HET-SR line (low metastatic); lane 2: HET-SR-N-ras transfectant; lane 3: HET-SR N-ras 'antisense' transfectant; lane 4: HET-SR-8 line (highly metastatic); lane 5: normal hamster fibroblasts; lane 6: cell line obtained from spontaneous hamster tumor.

srcLMC is connected with unique amino acid changes in this region of the v-srcLM oncoprotein [7].

3.4. Protein binding of C-terminal region of v-srcHM to vseap1 in vitro

To verify whether the vseap1 binding to v-srcHMC obtained through two-hybrid screening was specific, we tested the protein interaction in vitro. GAL4ad-vseap1 fusion protein was synthesized using corresponding pGADvseap1 fragment for transcription and translation in vitro in rabbit reticulocytes lysate. GAL4ad protein was also translated as a control. V-srcHMC protein was expressed as GST-fusion protein. The purified GST-protein (as a control) or GST-v-srcHMC fusion protein bound to the glutathione-Sepharose beads were incubated with GAL4ad-vseap1 or GAL4ad [^{35}S]methionine-labeled proteins and analyzed by SDS-PAGE (Fig. 2). Staining the gel confirmed that equal amounts of GST-v-srcHMC and GST were taken for the reaction. GAL4ad-vseap1 was not able to associate directly with pure GST (lane 1) and neither was GST-v-srcHMC able to interact with pure GAL4ad (lane 2). Only the C-terminal region of v-srcHM could bind specifically to the vseap1 protein (lane 3).

3.5. Vseap1 RNA analysis

Gadd7/adapt15 gene was isolated from Chinese hamster cells on the basis of specific RNA expression after UV-radiation [24] or hydrogen peroxide treatment [8]. This gene was also induced by growth arrest signals and DNA-damaging agents [25]. The observed high steady state level of gadd7/adapt15 mRNA was due to its increased stability in treated cells [26]. Previously RSV-transformed Syrian hamster cells were demonstrated to be highly resistant to H_2O_2 damage [10]. This phenotype directly correlated with an increased catalase activity in the cells [27]. Transfection of the cells by N-ras oncogene was shown to induce the suppression of v-src activity, coinciding with the decrease of H_2O_2 resistance and catalase activity [16,27,28]. The steady state level of vseap1-specific RNA was analyzed in the panel of v-src transformed hamster cells, as well as in spontaneously transformed and normal hamster fibroblasts (Fig. 3, Table 2). Vseap1 probe hybridized with the main 0.9-kbp transcript in all transformed cell lines including spontaneous tumor cells. In normal fibroblasts vseap1-RNA was less expressed (Fig. 3, lane 5). Gadd7/adapt15-specific messenger of the same size was identified in normal as well as stress agents-treated Chinese hamster cells [8,9]. However, vseap1 also hybridized with the additional 3.1-kbp transcript which was not described in gadd7/adapt15 analyses. Interestingly, this RNA was reduced in cell lines with suppressed H_2O_2 resistant phenotype and low catalase activity (Fig. 3, Table 2).

Table 1
V-srcHMC and v-srcLMC interactions with vseap1 gene encoding protein in the two-hybrid system

Binding domain	Activation domain pGAD vseap1	Reporter genes expression
pLexA-Lamin	−	His
	−	LacZ
pLexA-v-srcHMC	++++	His
	++++	LacZ
pLexA-v-srcLMC	+	His
	+	LacZ

C-terminal parts of v-srcHM and v-srcLM proteins were expressed as fusion proteins with LexA domain. The *His3* (His) expression and β-galactosidase activity (LacZ) are indicated. In the case of *HIS3* expression (+) indicates His$^+$ colonies, compared to the total quantity of yeast transformants and (−) means no growth in the absence of histidine. In the case of LacZ expression (+) indicates the blue colored colonies compared with the quantity of His$^+$ colonies, (−) means no blue color development. His−/LacZ− means absence of interaction.

Table 2
Characteristics of analysed cell lines

Culture	SMA [10]	Relative expression of v-src protein [27]	Catalase activity (Ug/mg protein) [27]	$H_2O_2^{CA}$ [27]	vseap1-specific RNA	
					0.9 kbp	3.1 kbp
HET-SR	−	1	17.7	10–20 s	3.15	2.6
HET-SR N-ras	−	0.22	0.67	6.5 min	2.6	0.7
HET-SR N-*ras* 'antisense'	NT	0.6	6.0	NT	1.9	1.2
HET-SR-8	++	1.37	16.3	10–20 s	2.1	2.9
STHE	−	−	8.6	3 min	2.2	1.4
HEF	−	−	4.0	10 min	1.0	1.0

SMA, spontaneous metastasizing activity; +, highly metastatic cell line; −, low metastatic cell line; NT, not tested; $H_2O_2^{CA}$, time rate of H_2O_2-catabolising activities. Time rates (seconds, minutes) necessary for inactivation of 95% of H_2O_2 (10 mM), added to cell extracts.

4. Discussion

The function of C-terminal region of v-src oncoprotein was analyzed by studying the possibility for this region to interact specifically with the other proteins. The study is based on two premises: (a) this region is very conserved in all known v-src genes [3]; (b) the observation that the mutations in this area are associated with the differences in the metastatic potential of the transformed hamster cells [7].

Using a two-hybrid screening of hamster cDNA library and a GST-fusion protein system the C-terminal aa of v-src (at least in vitro) were found to have the capacity to interact directly with other proteins. Several genes coding such proteins were isolated. One of the identified genes, called vseap1, is related to a member of growth arrest and DNA-damage inducible genes gadd7/adapt15 [8,9,24]. These genes are rapidly induced in hamster cells by UV-radiation, by H_2O_2 treatment or after DNA-damaging agents and certain growth arrest treatment [8,25]. Modulation of gadd genes expression specifically protects the cells against different stressful signals and is involved in adaptative response. We also observed the activation of vseap1 in tumor cells, in comparison with normal cells: whether being virus-transformed or spontaneously transformed. In contrast to gadd7/adapt15, two RNAs specific for vseap1 (3.1 kbp minor and 0.9 kbp major) were found. The expression of long 3.1-kbp transcript correlates with the level of H_2O_2 resistance and catalase activity of the cells. Probably, vseap1 is involved in the mechanisms of cell antioxidant defense. Moreover the expression of this gene is stimulated in the hamster cells resistant to γ-radiation (Musatkina, Tirsvina, personal communication).

The main enigma of vseap1 and gadd7/adapt15 is the contradiction between the biological properties exerted by these genes and the lack of genuine protein products. No significant ORF was shown both for vseap1 and gadd7/adapt15. The interaction with v-src was due to 8 amino acids coded by 5′ nucleotides of cloned sequence. We suggest that this region represents only one part of a longer gene. According to the DNA analyses, vseap1-specific sequences are distributed in the area of 12 kbp, at least. First 120 nucleotides of the cloned fragment are separated from the downstream sequences by a region of about 9.5 kbp (data not shown). Probably, the complete vseap1 gene could be expressed as much longer messenger RNA. The possible candidate for such a messenger is the 3.1-kbp hybridizing with vseap1 probe. Sequencing of the 3.1-kbp RNA, currently in progress, should clarify the structure of this novel gene. It also might help to understand the role of vseap1-v-src protein interaction.

Acknowledgements: We thank Jean de Gunzburg for fruitful discussion and Galina Ershova for helpful advice. The research was supported by French INSERM PECO grant, the Russian National Foundation for Basic Research 96.04.4926J. O.M. was supported by fellowships from French Foundation for Medical Research and UICC.

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