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**Complete *c-mos* (rat) nucleotide sequence: presence of conserved domains in *c-mos* proteins**

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**ABSTRACT**

Recently we described the isolation of *c-mos* (rat). The gene belongs to the family of oncogenes. Some facts render *c-mos* unique among the oncogenes: a) it does not contain intervening sequences and b) its expression was never detected in a large number of normal mouse tissues examined. We undertook the sequence analysis of *c-mos* (rat) in order to compare it to the nucleotide sequences published for *c-mos* (mouse), *c-mos* (human), *c-src* and bovine protein kinase. *c-mos* (rat) contains an open reading frame of 1017 nucleotides, coding for a polypeptide of 339 amino acids. *c-mos* (rat) makes use of the same ATG that defines the N-terminus of the *c-mos* (human) protein. By comparing all *c-mos* sequences available we found sequences with high mutational rates to be confined to certain domains. This comparison, together with data on the biological activities of the cloned DNA, allowed us to tentatively define regions involved in (a) function(s) of *c-mos* other than transformation.

**INTRODUCTION**

*c-mos*, the cellular homologue of *v-mos*, is a member of the family of oncogenes (1). By molecular hybridization the presence of *c-mos* has been detected in different mammalian species (2, 3). Although in general oncogenes have diverged when compared in different species, they are still homologous enough to be characterized as evolutionary conserved sequences (4-8). *c-mos* from mouse (9, 10) and man (11) have been isolated and sequenced (12). They are 77% homologous on the level of the DNA. The main differences are the start of the open reading frame (translation of the human gene starts at the first internal ATG of the mouse gene) and the lack of transforming activity of *c-mos* (human). Cells transformed by *v-mos* or *c-mos* (mouse) contain extremely small amounts of *mos*-specific RNA and *mos* proteins (13, 14) that are apparently sufficient for transformation. The proteins are detectable with an antiserum raised against a synthetic peptide (15). The *v-mos* protein, p37<sup>MOS</sup>, appeared to be cytoplasmic (15), related to the cAMP-dependent catalytic subunit of bovine protein kinase (BOV-PK) (16) and to the *v-src* protein pp60<sup>src</sup> (17). If expressed to still

very moderate levels the mos gene or its product(s) are lethal to cells (14).

In spite of careful analyses of numerous tissues of adult mice or of mouse embryos (18-20) c-mos expression was never detected. This stands in contrast to the readily observed expression of a large number of other oncogenes in the mouse. Hypermethylation of c-mos might be one of the reasons for this (18) as was suggested by experiments in which in vitro methylation of v-mos greatly reduced its transforming activity (21). The only described case in which c-mos has been implicated as the cause of a mouse plasmacytoma was reported by Rechavi et al. (22). They were able to show that in this case the N-terminal part of c-mos (mouse) was replaced by the long terminal repeat of an intracisternal A-particle-like genome (23). This recombinant mos gene, rc-mos (22), was shown to be able to transform cells in tissue culture.

The c-mos gene can be detected by molecular hybridization in mammals. Although the c-mos gene from mouse is transforming the closely related c-mos gene from human is not. This suggests that the c-mos gene might have (a) function(s) other than transformation.

We recently isolated c-mos (rat) and showed its transient transforming activity in transfection experiments (3). However, we were unable to obtain stably transformed cell cultures : small foci disappeared because of lysis of the transformed cells, suggesting that c-mos (rat) expression is lethal to cells as was reported for v-mos (14), v-src (24), SNV (25) and v-abl (26, 27). Specific mutations in mos might abolish its capacity to transform cells. Therefore we sequenced c-mos (rat) using the Sanger chain termination method (28) and compared it to published sequences of other members of the mos family. It could be shown that c-mos can be divided in well conserved and much less conserved domains.

### METHODS

#### Subcloning of c-mos (rat)

DNA fragments to be subcloned were purified by digestion of the Eco RI insert of bacteriophage lambda D3e DNA (3), followed by electrophoretic separation in 1% low gelling temperature agarose (Sigma, type VII) and elution of the DNA from gel slices as described by Weislander (29). Eco RI plus Hind III digested pBR322 DNA was used directly in ligation reactions, while Hind III digested pBR322 was treated with calf intestinal phosphatase (30) before ligation. Transformation of bacteria, selection and isolation

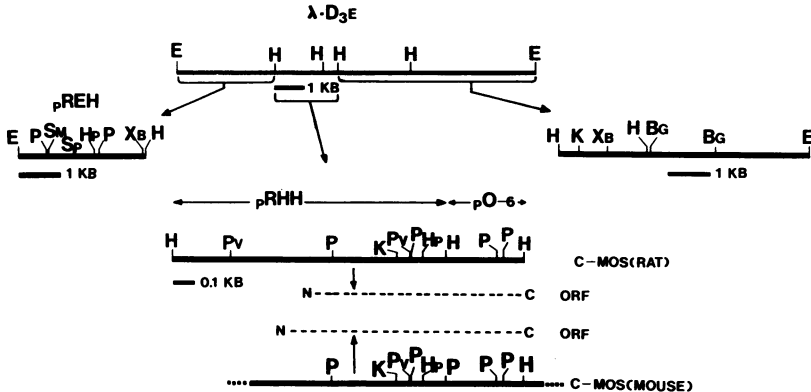


Figure 1. Restriction map of parts of  $\lambda$ -D<sub>3</sub>E. Using subclones pREH, pRHH and pO-6 derived from  $\lambda$ -D<sub>3</sub>E (3) for detailed restriction enzyme analysis as described in methods, we constructed the map of *c-mos* (rat) and its flanking sequences. It is compared to the map of *c-mos* (mouse) (9, 17). E = Eco RI, P = Pst I, Sm = Sma I, Sp = Sph I, Hp = Hpa I, Xb = Xba I, H = Hind III, Pv = Pvu II, K = Kpn I and Bg = Bgl II. Also indicated are the two open reading frames (ORF). N = NH<sub>2</sub>-terminus and C = COOH-terminus.

of plasmid DNA were as described before (9). Subclones were analyzed by restriction enzyme analyses and hybridization to *mos* specific sequences present in pMSV-31 (9) according to published procedures (3).

#### DNA sequence analysis

Several DNA fragments were isolated from the subclones pRHH and pO-6 (see Fig. 1) and cloned in M13mp8, M13mp9 or mWB328 (31). The chain termination method of Sanger and gel electrophoresis of DNA fragments were as described (28).

## RESULTS

### Restriction mapping of *c-mos* (rat)

A detailed restriction enzyme analysis of the subclones pREH, pRHH and pO-6, together with hybridization data of transferred DNA fragments to pMSV-31 (9), allowed us to construct the restriction map shown in Figure 1. It appeared that *c-mos* (rat) sequences, homologous to the *v-mos* sequences present in pMSV-31, were localized on clone pO-6 and on clone pRHH. Furthermore, comparison of the sites for the enzymes Pst I, Kpn I, Pvu II and Hpa I on *c-mos* (mouse) (17) and *c-mos* (rat) indicated the close relationship between these two genes. The major difference was the disap-

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Rat      1  AGCAGCAAGACGTGAGACACAGTTGTGCTGGTTTTGAGAATACAAGAAAAAGGAAA-----TGG
Mouse    CTTGTGG TA T A CA G G GAACTG

63  GATGAAGCGGAAACCTTCAGCCATGCTTCCAAACTCCCTGGGTGTTCTCGTTCATTTCTCTAGCG
   AT A C T C C AC C T

132  TCTCTTGTACTGTCCCATCTGAGGGTGA ATG CCT TCG CCT CTC ATC CTG TGT CGC TAC
   A A G

192  CTC CCT CGC GAG CTG TCG CCA ACG GTG GAC TCG AGG TCC TGC AGC AGC CCC
   T T C T C C T T T

243  TTG GTG GCC TCG AGG --- GCG GGG AAG --- TTC CTG GGG GCC ACT CCT CCT
   C AAG A CTC

288  CGG GCC CCG CGG CTG TCA CGC CGG CTG GCC TGG TGC TTC ATA GAC TGG GGA
   T C G A C C T C

339  CAG GTA TGC CTG CTG CAT AGG CTG GGT TCT GGA GGG TTT GGC TCG GTG TAC
   T A C

390  AAA GCC ACT TAC CAC GGT GTT CCT GTG GCC ATC AAG CAA GTG AAC AAG TGC
   A

441  ACC AGA ACC CTA CGT GCA TCC CAA CGG AAT TTC TGG GCT GAA CTG AAC ATT
   AG GA G G

492  GCA AGG CTG CAC CAC GAC AAC ATA ATC CGG GTT GTG GCT GCC AGC ACG CGC
   A A G G T

543  ACG CCG GAA GGT TCC AAC AGC CTT GGT ACC ATA ATC ATG GAG TTT GGG GGC
   T AC A

594  AAT GTG ACT CTA CAC CAA GTC ATC TAC GGT GCC ACC CGC TCC CCA GAG CCT
   C A G

645  CTC AGC TGC AGA GAG CAA CTG AGT TTG GGA AAG TGC CTC AAG TAT TCC CTA
   A G

696  GAT ATT GTT AAC GGC CTG CTT TTT CTC CAC TCA CAA AGC ATT TTG CAC TTG
   G

747  GAC CTG AAG CCA GCG AAC ATT TTG ATC AGT GAG AAG GAC GTT TGT AAG ATA
   C A C

798  AGT GAC TTC GGC TGC TCC CAG AAG CTT CAG GAT CTG CCG TGC CGG --- CCG
   G CAG G

846  TCC CTT CAC CAC ATC GGG GGC ACG TAC ACG CAC CAA GCT CCG GAG CTC CTG
   C C A

897  AAA GGA GAG ATC GCC ACG CCC AAA GCC GAC ATC TAC TCT TTT GGC ATC ACC
   T T

948  CTG TGG CAG ATG ACC ACC AGG GAG GTG CCT TAC TCC GGC GAG CCT CAG TAC
   A

999  GTG CAG TAC GCA GTG GTA GCC TAC AAT CTG CGC -CCT CAC TGG CAG-- GAG
   T T C GA

1047  GTG TTC ACC GCC TCC CTG ACT GGG AAG ACG CTG CAG AAC AAT GTC CAG AGC
   C A A

1098  TGC TGG GAG GCC CGC GCC CTG CAG AGG CCG GGT GCA GAA CTG CTC CAG AAG
   A G

1149  GAC CTG AAG GCT TTC CGA GGG GCA CTG GGC TGA CTCCATCGAGCCGATGTGCAGATAAGCTT
   C A AG

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Figure 2. Nucleotide sequence of c-mos (rat)

The sequence of c-mos (rat) was established by the chain termination method of Sanger (28) and compared to the c-mos (mouse) sequence published by Van Beveren et al. (12, 17). The boxes denote the start of the c-mos (rat) open reading frame and the TGA opal stop codon used. Only differences in the sequences are indicated for c-mos (mouse). Several of the restriction sites shown in Figure 1 were included in this figure. Bars denote deletions.

pearance of one c-mos (mouse) Pst I site and the acquisition of a Hind III site in c-mos (rat).

#### Sequence of c-mos (rat)

Figure 2 shows the complete nucleotide sequence of c-mos (rat), compared to that of c-mos (mouse). The nucleotide sequences that constitute the open reading frames start at nucleotide 162 and end at nucleotide 1178 (see fig. 2). The DNA sequences in these regions are 93% homologous. Three-c-mos (mouse) base triplets are deleted in c-mos (rat) at nucleotide positions 257, 266 and 842. Furthermore we observed the deletion of one base at position 1031 and of two bases at position 1043, that leave the open reading frame intact. The c-mos (rat) protein product consists of 339 amino acid residues encoded by an uninterrupted stretch of 1017 nucleotides. From the sequence it is clear that the first ATG of c-mos (mouse) has been mutated to TTG in c-mos (rat). The region in c-mos (mouse) covering amino acids -48 to -1 has undergone 14 mutations and one 6 bp deletion to generate the c-mos (rat) region. It appears that the first ATG used in c-mos (rat) is the first internal ATG of c-mos (mouse). Also the 2 genes, as well as c-mos (human), make use of the same TGA opal stop codon. The restriction sites shown in Figure 1 are indicated in Figure 2. From this sequence it can be seen that the conversion of the c-mos (mouse) Pst I site to the c-mos (rat) Hind III site was the result of a T G point mutation.

#### c-mos proteins of mouse, rat and human origin

Human and mouse are evolutionary more distantly related than rat and mouse. The last two species are close enough to define those regions of the mos protein, which allow a higher mutational rate than other domains, by comparing their nucleotide sequences. Figure 3 shows the amino acid sequences of the mos proteins of the three species mentioned. Only differences in these sequences were indicated for the rat and human proteins. We defined as number 1 the first internal methionine residue of c-mos (mouse), which acts as the NH<sub>2</sub>-terminus for the other two mos-proteins. The arrows indicate the boundaries of the sequences present in rc-mos, the biologically active recombinant form of c-mos (mouse) (22) as well as the region of c-mos (mouse) that was shown to share 25% homology with BOV-PK (16).

Based on the number of replacement site substitutions occurring in certain regions of c-mos when going from mouse, to rat and to human species, it is clear from Figure 3 that some domains are very well conserved, others less so. Thus the evolutionary best conserved domain extends approximately from amino acid residue 170 to 220. Slightly less conserved

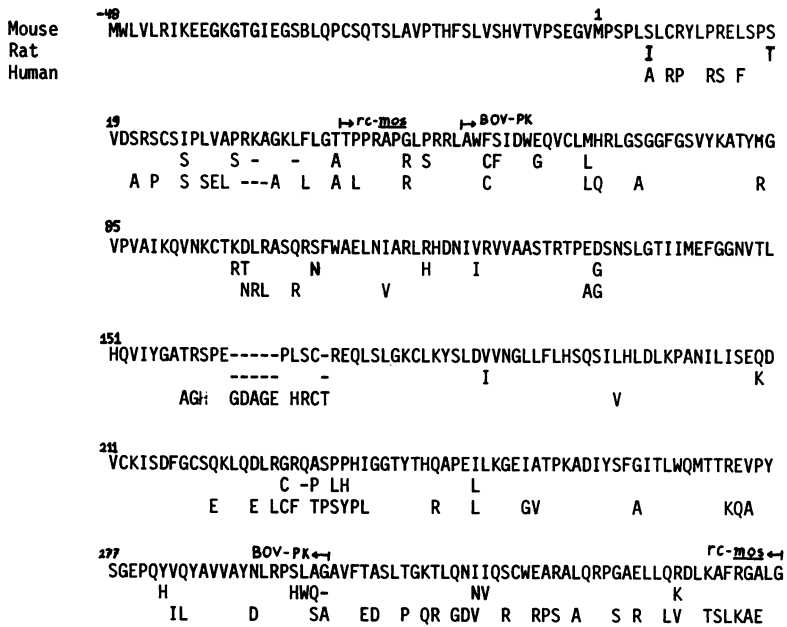


Figure 3. Comparison between mos proteins of mouse, rat and human origin

The amino acid sequences have been published (11, 12, 17) for mouse and human c-mos. The amino acid sequence of c-mos (rat) was derived from the nucleotide sequence shown in Fig. 2. Only differences in the amino acid sequences of c-mos (rat) and c-mos (human) compared to c-mos (mouse) are shown. The arrows denote the boundaries of the c-mos (mouse) regions shown to be present in rc-mos (22) or having homology to bovine protein kinase (16). The first internal methionine residue of c-mos (mouse) was defined as number 1. Bars denote deletions.

are sequences covering amino acid residues 50 to 155 and 240 to 280. Domains harboring the largest numbers of amino acid site substitutions are located as follows : amino acid residues 1 to 40, 220 to 235 and 290 to 343.

Conserved sequences between mos and src proteins

Recently the nucleotide sequence of c-src has been published (32). Van Beveren et al. (17) have pointed out the existence of homology between parts of proteins of c-mos (mouse) and v-src. The determination of the sequences of c-mos (human) (11) and c-mos (rat) allowed us to investigate whether or not the regions of homology between the proteins of c-mos (mouse) and v-src have also been conserved in the more distantly related

|     |                                                        |     |  |  |  | intron # c-src |               |
|-----|--------------------------------------------------------|-----|--|--|--|----------------|---------------|
| ↪10 | IASGMAYVERMNYVHRDLRAANILVGENLVCKVADFLGLARLIEDNEYTARQGA | ↪11 |  |  |  |                | c-src         |
|     | VVN LFLHSQSIL L KP IS QD IS CSQKLQ LRC-- AS            |     |  |  |  |                | c-mos (mouse) |
|     | VN LFLHSQSIL L KP IS KD IS CSQKLQVLRG-- -PS            |     |  |  |  |                | c-mos (rat)   |
|     | VVN LFLHSQSI L KP IS QD IS CSEKL LLC--F TP             |     |  |  |  |                | c-mos (human) |
|     |                                                        |     |  |  |  |                |               |
|     |                                                        |     |  |  |  | intron # c-src |               |
| ↪11 | GAKFPI----KWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPGMV    | ↪12 |  |  |  |                | c-src         |
|     | ASPHH GGTYTHQ ILKGEIA P A IY T WQM RE- S EP            |     |  |  |  |                | c-mos (mouse) |
|     | PSLPH GGTYTHQ LLKGEIA P A IY T WQM RE- S EP            |     |  |  |  |                | c-mos (rat)   |
|     | TPSY LGGTYTHR LLKGEV P A IY A T WQM Q-A S ER           |     |  |  |  |                | c-mos (human) |

Figure 4. Conserved domains in c-mos and c-src proteins

A comparison is made between the amino acid sequences of c-src introns 10 and 11 (32) and parts of the c-mos protein sequences shown in Figure 3, based on the observations of Van Beveren et al. (17). The boundaries of the introns are indicated as well as the protein kinase active site of c-src (35). Only differences between c-mos proteins and c-src protein are indicated for the c-mos proteins. Bars denote deletions.

rat and human c-mos proteins. Large homology would suggest that these domains play an essential role in some unknown function(s) of the mos protein. For this purpose part of the amino acid sequences of the mos proteins and the amino acid sequence coded for by exons 10 and 11 of c-src (32) were aligned as shown in Figure 4. As expected the homology regions between the c-mos (mouse) and c-src proteins are located in those domains of the mos gene that are evolutionary best conserved, namely domains covering amino acid residues 170 to 220 and 240 to 280.

## DISCUSSION

In the characterization of c-mos (rat) we made use of the presence of a Hind III site in c-mos (rat) to distinguish it from c-mos (mouse) (3), a highly related oncogene. The nucleotide sequence of c-mos (rat) presented here confirmed the presence of this diagnostic restriction site. A single point mutation destroyed one Pst I site (in c-mos (mouse)) and created the partially overlapping Hind III site (in c-mos (rat)). The sequence also revealed the near to perfect identity in restriction enzyme maps between c-mos (mouse), a readily transforming oncogene when activated by enhancer-promoter sequences (32), and c-mos (rat), which does not give rise to stable transformation of tissue culture cells (3). This allowed us to construct several hybrid mos genes containing sequences of mouse and rat

origin in order to study regions required for stable transformation.

The c-mos (rat) nucleotide sequence revealed the point mutation converting the first ATG of c-mos (mouse) (position -48, Fig. 3) to TTG in c-mos (rat). As is the case for c-mos (human) (11) it appeared that the N-terminus of c-mos (rat) is defined by the first internal methionin of c-mos (mouse) (position 1, Fig. 3). Recently Papkoff and Hunter (34) observed the presence of a 33 k mos specific protein in cells expressing c-mos (mouse). This is approximately 5 k less than the expected size for the c-mos (mouse) protein. If, however, for c-mos (mouse) the translation would initiate on the first internal ATG the discrepancy in size disappears. Therefore we suggest that for the three species examined translation of mos sequences starts at the first internal ATG of c-mos (mouse). We define this methionine residue as number 1 (see Figure 3).

The analysis of the relationship between mos proteins and the catalytic subunit of BOV-PK as reported by Barker & Dayhoff (16) can now be extended : a) we have observed the conservation in all mos proteins of the lysine residue (position 90 in the c-mos (mouse) protein and position 71 in BOV-PK) that binds ATP and b) we detected the conservation of the amino acid sequence APE (position 244, Fig. 3) that forms part of the sequence KWTAPEA thought to confer tyrosine protein kinase activity to pp60<sup>src</sup>. All mos proteins and BOV-PK have replaced the last alanine in the sequence KWTAPEA by isoleucine (BOV-PK and c-mos (mouse)) or leucine (c-mos of rat and human origin). The lack of tyrosine protein kinase activity associated with mos proteins (15) could be explained by data of Bryant and Parsons (35), who showed by site-directed mutagenesis of v-src that replacement of the last alanine residue by threonine completely abolished the tyrosine kinase activity. However Kloetzer et al. (36) described the temperature sensitive autophosphorylation of serine and threonine residues of the 85 k gag-mos polyprotein encoded by ts-110 Moloney murine sarcoma virus, suggesting that mos encoded proteins do have an associated protein kinase activity.

The detection of a biologically active recombined c-mos (mouse) gene in a plasmacytoma (22) defined more closely the sequences necessary for transformation: Amino acids found to be dispensible are located in the region starting at position -48 and ending at position 41 (Fig. 3). In agreement with these data we observed a high mutational rate in the domains covering amino acid residues -48 to 40. However, additional mutations found in rat and human mos genes apparently abolish the ability of mos protein to



stably transform cells. Mutations in two domains might be responsible for this : the domain extending from amino acid residue 220 to 240 and the domain covering the C-terminus of the mos protein. In the former domain the transforming gene v-mos has no amino acids substitutions compared to c-mos- (mouse) and in the latter domain v-mos has only 4 amino acid substitutions (17). A similar situation was reported by Van Straaten et al. (37) for the inactive c-fos (human) gene and the transforming v-fos gene which differ at their C-terminus. These and our data suggest that replacement site substitutions, destroying the transforming activity of a protein, are perfectly allowed to occur during evolution with the prerequisite that they leave intact the open reading frame and thereby unknown functions of these oncogenes.

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