Nucleotide sequence of two *ras*^{H} related-genes isolated from the yeast *Saccharomyces cerevisiae*

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ABSTRACT

A complete nucleotide sequence of two ras-related yeast genes (c-ras^{sc-1} and c-ras^{sc-2}) isolated from the yeast strain Saccharomyces cerevisiae is reported. They encode predicted polypeptides of 40,000 and 41,000 daltons, respectively. The N-terminal 170 amino acids from both genes show extensive amino acid homology to other ras genes from vertebrates, whereas their C-termini have diverged. These genes should be useful in the elucidation of a normal biological function of ras-related genes in a simple system like yeast.

INTRODUCTION

Harvey and Kirsten murine sarcoma virus (Ha-MUSV and Ki-MUSV) are transforming retroviruses which were originally isolated during the passage of murine leukemia virus (MULV) in rats[1]. The transforming genes of these viruses have been named v-ras^{H} and v-ras^{K}, respectively, and represent 2 members of the ras oncogene family[2]. Cellular homologs (c-ras^{H} and c-ras^{K}) to both of these oncogenes have been detected in a large number of vertebrates, including humans[3,4]. A third member of the ras gene family, n-ras, has also been detected in a human neuroblastoma[5].

Recently, activated forms of the c-ras^{H}, c-ras^{K} and n-ras oncogenes have been isolated from different human tumors, and have been shown to contain single point mutations in their genes[5-11]. These mutations have been mapped to either one of two positions: 1) amino acid residue 12 or 2) amino acid residue 61[11]. More recently, it has been shown that a nitrosomethyl urea (NMU) induced mammary carcinoma of rats contains an activated c-ras^{H} gene[12].

Ras genes encode a polypeptide of approximately 21,000 daltons (p21^{RAS}) [12]. The protein is synthesized as a precursor and later processed at its c-terminus to form a mature p21 polypeptide[13]. This protein binds to the inner plasma membrane of the cell and has guanine nucleotide binding activity [14]. The p21 proteins encoded by v-ras^{H} and v-ras^{K} are phosphorylated at a

unique threonine residue, occupying amino acid position 59, whereas the p21 proteins encoded by the cellular homologs are not phosphorylated, having an alanine at residue 59[15]. The different ras genes are highly conserved evolutionarily, being found in both vertebrate and invertebrate cells[3], and also in lower eucaryotic cells such as yeast[16,17]. The finding of ras related genes in yeast provides a valuable system in which to perform genetic studies, both in haploid and diploid cells, such that the regulation and function(s) of these genes and their products may be better understood.

MATERIALS AND METHODS

The isolation of two different ras-related genes (c-ras^{sc-1} and c-ras^{sc-2}) from the yeast strain Saccharomyces cerevisiae has been described elsewhere[16]. Using labeled v-ras^{H} as a probe against c-ras^{sc-1} and c-ras^{sc-2}, the regions of homology to the v-ras^{H} gene were narrowed to approximately 1Kbp fragments. Both these genes were subjected to DNA sequence analysis in order to better understand the nature of the polypeptides they encode. The nucleotide sequence of each was obtained from both strands of the DNA using the chemical degradation method of Maxam and Gilbert[20]. The alpha ^{32}P deoxynucleotide triphosphates and gamma ^{32}P ATP used were purchased from New England Nuclear. Specific activities were 3,000-7,000 curies mmole^{-1}.

RESULTS AND DISCUSSION

Figures 1 and 2 present the DNA sequence of c-ras^{sc-1} and c-ras^{sc-2}, respectively. Temeles et. al.(personal communication) have analyzed the mRNA's encoded by these genes and find them to be approximately 1150 nucleotides long. Since a consensus sequence for transcriptional promotion in yeast has not been identified, it is impossible to position a putative 5' end for the mRNA's from the sequence data. This is not the case for transcription termination in yeast. The sequence pTTTTTATA has been proposed to represent a consensus sequence usually located approximately 50 nucleotides upstream from yeast transcriptional termination sites[18]. In both c-ras^{sc-1} (Fig. 1, position 1133-1140) and c-ras^{sc-2} (Fig. 2, position 1216-1223) a stretch of 8 nucleotides is present which contains 7 out of 8 nucleotides (c-ras^{sc-1}) or 8 out of 8 nucleotides (c-ras^{sc-2}) common to the proposed yeast transcriptional termination consensus sequence[18]. Based on the size of the transcripts, the presence of a putative transcriptional termination sequence, and the absence of any yeast splice site junctions[19],

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                                                                  50
      TGT CTC TAT TAT CAT CTG TCT TGT TCT TTC TTG CAA TGC TTA ATT AAC TGC TGC CAC AAT TGA CTT CGG TTT
                                                                                                 150
                                    100                                   met gln gly asn lys ser thr ile arg
GGC TAT TTC ACG ATT GAA CAG GTA AAC AAA ATT TTC CCT TTT TAG AAC GAC ATG CAG GGA AAT AAA TCA ACT ATA AGA
                                                                 200
glu tyr lys ile val val val gly gly gly gly val gly lys ser ala leu thr ile gln phe ile gln ser tyr phe
GAG TAT AAG ATA GTA GTT GTC GGT GGA GGT GGC GTT GGT AAA TCT GCT TTA ACA ATT CAA TTC ATT CAA TCA TAC TTT
                          250                                                                   300
val asp glu tyr asp pro thr ile glu asp ser tyr arg lys gln val val ile asp asp lys val ser ile leu asp
GTG GAC GAA TAT GAC CCT ACT ATC GAA GAT TCT TAC AGA AAA CAA GTT GTC ATC GAT GAC AAA GTA TCC ATT TTG GAC
                                                350
ile leu asp thr ala gly gln glu glu tyr ser ala met arg glu gln tyr met arg thr gly glu gly phe leu leu
ATT CTA GAT ACT GCT GGA CAA GAA GAG TAT TCT GCG ATG AGA GAA CAG TAC ATG AGG ACT GGG GAA GGT TTC CTA CTG
                    400                                                                 450
val tyr ser val thr ser arg asn ser phe asp glu leu leu ser tyr tyr gln gln ile gln arg val lys asp ser
GTC TAT TCC GTC ACC TCT AGA AAT TCC TTT GAT GAG TTA CTG TCT TAT TAT CAG CAA ATT CAA AGA GTA AAA GAT TCT
                                        500
asp tyr ile pro val val val val gly asn lys leu asp leu glu asn glu arg gln val ser tyr glu asp gly leu
GAC TAC ATT CCT GTA GTC GTG GTA GGT AAC AAA TTG GAC CTT GAA AAT GAA AGA CAA GTC TCT TAT GAA GAC GGG TTA
          550                                                                 600
arg leu ala lys gln leu asn ala pro phe leu glu thr ser ala lys gln ala ile asn val asp glu ala phe tyr
CGC TTG GCC AAG CAG TTG AAT GCA CCC TTT CTA GAA ACG TCT GCG AAA CAA GCC ATC AAC GTA GAC GAG GCC TTT TAT
                                  650
ser leu ile arg leu val arg asp asp gly gly lys tyr asn ser met asn arg gln leu asp asn thr asn glu ile
AGC CTT ATT CGT TTG GTA AGG GAC GAC GGT GGG AAA TAC AAT AGC ATG AAT CGT CAA CTG GAT AAT ACG AAT GAA ATA
   700                                                                 750
arg asp ser glu leu thr ser ser ala thr ala asp arg glu lys lys asn asn gly ser tyr val leu asp asn ser
AGA GAT TCG GAG CTA ACC TCA TCT GCA ACA GCG GAT AGA GAA AAA AAG AAC AAC GGG TCT TAT GTA CTC GAT AAT TCT
                                800                                                             850
leu thr asn ala gly thr gly ser ser lys ser ala val asn his asn gly glu thr thr lys arg thr asp glu
TTG ACC AAT GCT GGC ACT GGC TCC AGT TCA AAG TCA GCC GTT AAC CAT AAC GGT GAA ACT ACT AAA CGA ACT GAT GAA
                                                900
lys asn tyr val asn gln asn asn asn asn glu gly asn thr lys tyr ser ser asn gly asn gly asn arg ser asp
AAG AAT TAC GTT AAT CAA AAC AAT AAC AAT GAA GGA AAT ACC AAG TAC TCC AGT AAC GGC AAC GGA AAT CGA AGT GAT
                          950                                                                   1000
ile ser arg gly asn gln asn asn ala leu asn ser arg ser lys gln ser ala glu pro gln lys asn ser ser ala
ATT AGT CGT GGT AAT CAA AAT AAT GCC TTA AAT TCG AGA AGT AAA CAG TCT GCT GAG CCA CAA AAA AAT TCA AGC GCC
                                      1050
asn ala arg lys glu ser ser gly gly cys cys ile ile cys
AAC GCT AGA AAA GAA TCT AGT GGT GGT TGT TGT ATA ATT TGT TGA AAA TGA TCC TGC TCT CTT GAT ATG ACA TGG TTT
                1100                                                                  1150
TGA TTG CAA GTC TAT TCT TAC TTG TTC AAT TGG TTT CAC CAG TAT CTT TTT GTA TCC ATT TTA CTA TGC TGT GTA TCT
                                        1200
AGT AAA AGA GTT GCT TGC CAC CAA ACA TTA ATA CTA ATG GTT AAT CTG CCA CCT TTT GTA AGC TT
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Figure 1. Nucleotide sequence of c-ras^{sc-1}. The amino acid sequence has been put above the nucleotide sequence beginning at position 124. The above sequence is 1229 nucleotides long and has been numbered every 50 nucleotides. The arrows show the position of RNA splice sites when this sequence is compared to the human c-ras^{H} gene.

one would expect the 5' end of the mRNAs to be present close to the N-terminus of the polypeptides. A more accurate determination of message size is in progress using S1 mapping.

Putative protein products

The DNA sequence of c-ras^{sc-1} shows an open reading frame sufficient to encode a 309 amino acid polypeptide, with its N-terminus at position 124 (Fig. 1). The sequence of c-ras^{sc-2} shows an open reading frame of 322 amino acids, beginning at position 142 (Fig. 2). This predicts that these genes encode proteins with molecular weights of approximately 40,000 and 41,000 daltons, respectively. It has recently been reported that Saccharomyces

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                                                                    50
                  TCA TCC ACT CTT TAT CTG ACT CTT CTG CAC TAT ATT AAT CAA CTA GGA GAA AAT TAC TTG AGC
                                                 100
AGA AAG ATA CGA GAG AAT TAC GGA TAA AAA AAC CAA GTT AAC CGT TTT CGA ATT GAA AGG AGA TAT ACA GAA AAA AAA
      150                                                                   200
met pro leu asn lys ser asn ilu arg glu tyr lys leu val val val gly gly gly gly val gly lys ser ala leu
ATG CCT TTG AAC AAG TCG AAC ATA AGA GAG TAC AAG CTA GTC GTC GTT GGT GGT GGT GGT GTT GGT AAA TCT GCT TTG
                                    250
thr ilu gln leu thr gln ser his phe val asp glu tyr asp pro thr ilu glu asp ser tyr arg lys gln val val
ACC ATA CAA TTG ACC CAA TCG CAC TTT GTA GAT GAA TAC GAT CCC ACA ATT GAG GAT TCA TAC AGG AAG CAA GTG GTG
 300                                                                   350
ilu asp asp glu val ser ilu leu asp ilu leu asp thr ala gly gln glu glu tyr ser ala met arg glu gln tyr
ATT GAT GAT GAA GTG TCT ATA TTG GAC ATT TTG GAT ACT GCA GGG CAG GAA GAA TAC TCT GCT ATG AGG GAA CAA TAC
                                  400                                                                   450
met arg asn gly glu gly phe leu leu val tyr ser ilu thr ser lys ser ser leu asp glu ser met thr tyr tyr
ATG CGC AAC GGC GAA GGA TTC CTA TTG GTT TAC TCT ATA ACG TCC AAG TCG TCT CTT GAT GAG CTG ATG ACT TAC TAT
                                                 500
gln gln ilu pro arg val lys asp thr asp tyr val pro ilu val val val gly asn lys ser asp leu glu asn glu
CAA CAG ATA CCG AGA GTC AAA GAT ACC GAC TAT GTT CCA ATT GTG GTT GTT GGT AAC AAA TCT GAT TTA GAA AAC GAA
                            550                                                                   600
lys gln val ser tyr gln asp gly leu asn met ala lys gln met asn ala pro phe leu glu thr ser ala lys gln
AAA CAG GTC TCT TAC CAG GAC GGG TTG AAC ATG GCA AAG CAA ATG AAC GCT CCT TTC TTG GAG ACA TCT GCT AAG CAA
                                                650
ala ilu asn val glu glu ala phe tyr thr leu ala arg leu val arg asp glu gly gly lys tyr asn lys thr leu
GCA ATC AAC GTG GAA GAG GCG TTT TAC ACT CTA GCA CGT TTA GTT AGA GAC GAA GGC GGC AAG TAC AAC AAG ACT TTG
                  700                                                                   750
thr glu asn asp asn ser lys gln thr ser gln asp thr lys gly ser gly ala asn ser val pro arg asn ser gly
ACG GAA AAT GAC AAC TCC AAG CAA ACT TCT CAA GAT ACA AAA GGG AGC GGT GCC AAC TCT GTG CCT AGA AAT AGC GGT
                                      800
gly leu arg lys met ser asn ala ala asn gly lys asn val asn ser ser thr thr val val asn ala arg asn ala
GGC CTC AGG AAG ATG AGC AAT GCT GCC AAC GGT AAA AAT GTG AAC AGT AGC ACA ACT GTC GTG AAT GCC AGG AAT GCA
       850                                                                   900
ser ilu glu ser lys thr gly leu ala gly asn gln ala thr asn gly lys thr gln thr asp arg thr asn ilu asp
AGC ATA GAG AGT AAG ACA GGG TTG GCA GGC AAC CAG GCG ACA AAT GGT AAG ACA CAA ACT GAT CGC ACC AAT ATA GAC
                                    950
asn ser thr gly gln ala gly gln ala asn ala gln ser ala asn thr val asn asn arg val asn asn asn ser lys
AAT TCC ACG GGC CAA GCT GGT CAG GCC AAC GCT CAA AGC GCT AAT ACG GTT AAT AAT CGT GTA AAT AAT AAT AGT AAG
1000                                                                  1050
ala gly gln val ser asn ala lys gln ala arg ser lys gln ala ala pro gly gly asn thr ser glu ala ser lys
GCC GGT CAA GTT TCA AAT GCT AAA CAG GCT AGG AGC AAG CAA GCT GCA CCC GGC GGT AAC ACC AGT GAA GCC TCC AAG
                              1100
ser gly ser gly gly cys cys ilu ilu ser                                                         1150
AGC GGA TCG GGT GGC TGT TGT ATT ATA AGT TAA TAA AAA GGA AAT AGT TGT AGA AAC GCT AAG ACG AAA AGA ACT CTA
                                                  1200
TAA AGT TGA AAC GAG TAC ACA CAT TTA TAA ATA TAT ACA AAA GTA AAT AAA AAA GTG ACT GTT TTT ATA TTG CTT ATT
GCC ATT TGC
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Figure 2. The nucleotide sequence of c-ras^{sc-2}. The above sequence is 1241 nucleotides long and is numbered every 50 nucleotides. The amino acid sequence has been put above the DNA sequence beginning at position 142. The arrows show the position of RNA splice sites when this sequence is compared with the human c-ras^{H} gene.

cerevisiae synthesizes proteins related to the p21 gene product of mammalian ras genes[21]. The cross reactive proteins found migrate in the 30,000 dalton region on 12% urea-SDS polyacrylomide gels. Since our sequence data predict primary protein products of 40,000 and 41,000 daltons, it is possible that the reported protein species represent processed forms of our predicted protein products. The possibility of such a relationship is being actively investigated.

DNA and amino acid sequence comparisons

Comparison of the DNA sequence of the two genes reveals approximately 45 percent homology at the nucleotide level (Fig. 1, position 124 to 660 and

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                          1                 10                  20                  30             ↓    40
c-ras^sc-1  M Q G N K S T I R E Y K I V V V G G G G V G K S A L T I Q F I Q S Y F V D E Y D P T I E D S Y R K Q
c-ras^sc-2    P L       N I R       L         G                       L T   S H
c-ras^H     - - - - - - - M T       L         A                       L I   N H
N-ras       - - - - - - - M T       L         A                       L I   N H

                        50                  60                  70                  80                  90
c-ras^sc-1  V V I D D K V S I L D I L D T A G Q E E Y S A M R E Q Y M R T G E G F L L V Y S V T S R N S F D E L
c-ras^sc-2          D E V S I                                 E         N           L   Y S I T S K S   L D E S
c-ras^H             G E T C L                                 D         T           C   F A I N N T K   F E D I
N-ras               G E T C L                                 D         T           C   F A I N N S K   F A D I

                   ↓  100                 110                 120                   130                 140
c-ras^sc-1  L S Y Y Q Q I Q R V K D S D Y I P V V V V G N K L D L E N E R Q V S Y E D G L R L A K Q L N A P F L
c-ras^sc-2  M T   Y Q     P         T   Y V   I   V         S     E N E K K   S Y Q D G L N M   K Q M N A   F L
c-ras^H     H Q   R E     K         S   D V   M   L         C     A A - R T   E S R Q A Q D L   R S Y G I   Y I
N-ras       N L   R E     K         S   D V   M   L         C     P T - R T   D T K Q A H E L   K S Y G I   F I

                         150↓               160    164
c-ras^sc-1  E T S A K Q A I N V D E A F Y S L I R L V R|D D G G K Y N S M N R Q L D N T N E I R D S E L T S S A
c-ras^sc-2            Q A I N   E E       T   A   L V  |  E           K T L T E N D N S K Q T S Q D T K G S G A
c-ras^H               T R Q G   E D       T   V   E I  |- - - - - - - - - - - - - - - - - - - - - - - - - - - -
N-ras                 T R Q G   E D       T   V   E I  |- - - - - - - - - - - - - - - - - - - - - - - - - - - -

c-ras^sc-1  T A D R E K K N N G S Y V L D N S L T N A G T G S S S K S A V N H N G E T T K R T D E K N Y V N Q N
c-ras^sc-2  N S V P R N S G G L R K M S N A A N G K N V N S S T T V V N A R N A S I E S K T G L A G N Q A T N G
c-ras^H     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
N-ras       - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                                                                                                            165
c-ras^sc-1  N N N E G N T K Y S S N G N G N R S D I S R G N Q N N A L N S - - - - - - - - - - - - - - - R S K|Q
c-ras^sc-2  K T Q T D R T N I D N S T G Q A G Q A N A Q S A N T V N N R V N N N S K A G Q V S N A K Q A
c-ras^H     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -|
N-ras       - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -|

                   170                 180               189
c-ras^sc-1  S A E P Q K N S S A N A R K E S S G G C C I I C
c-ras^sc-2  - A A P G G   T S - E A S K S G S G G C C I I S
c-ras^H     H K L R K L   P P D E S G P G C M S C K   V L S
N-ras       Y R M K K L   S S D D G T Q G C M G L P   V V M
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Figure 3. Comparison of amino acid sequence of c-*ras*$^{sc-1}$ and c-*ras*$^{sc-2}$ with c-*ras*^{H} and n-*ras*. The amino acids have been numbered according to the c-*ras*^{H} numbering system. The dashed lines are positions where no amino acids are present. Empty spaces represent regions where amino acids are identical between all four genes. Between position 164 and 165 amino acids are present only in c-*ras*$^{sc-1}$ and c-*ras*$^{sc-2}$. Arrows indicate positions where introns occur in the nucleotide sequence of c-*ras*^{H}.

Fig. 2, position 142 to 677). The DNA sequence also shows a high degree of third base changes between the two genes. Sequences both upstream and downstream of the two genes have no detectable homology. The regions which correspond to the N-terminal half of the putative proteins have 140 out of 170 amino acids identical (Fig. 3). This represents approximately 83% amino acid homology. However, it should be noted that the majority of amino acid differences between the two genes in this region are neutral, and would not effect secondary structure. In contrast to the high conservation in the N-terminal portion of these proteins, the C-terminal halves have diverged considerably, with approximately 20% of the C-terminal amino acids being identical and less than one third of the remaining 80% being conservative changes. It is interesting to note that the major point of divergence between

ras^{H}p21 and *ras*^{K}p21 is in the C-terminal portion of the proteins. Another very apparent characteristic of the C-termini of the two putative yeast proteins is that they are very asparagine rich. The significance of this is unknown.

When the two yeast *ras* genes are compared to v-*ras*^{H}, one finds approximately 30% homology at the nucleotide level. However, approximately 60% of the amino acids are identical between *ras*^{H}p21 and the putative c-*ras*$^{sc-1}$ and c-*ras*$^{sc-2}$ encoded proteins. Of the remaining 40%, half constitute conservative amino acid changes. In comparing the putative yeast proteins and the n-*ras* and *ras*^{H}p21's (Fig. 3) several features are apparent:

1) The c-*ras*$^{sc-1}$ and c-*ras*$^{sc-2}$ encoded proteins have 7 additional N-terminal amino acids and 117 and 129 additional C-terminal amino acids, respectively. These additional C-terminal amino acids are present downstream from the mature mammalian p21. It is not known whether these extra amino acids act in some specific processing events necessary for the yeast proteins, whether they serve some additional unrelated function in yeast, or if they represent a gene region which has been spliced onto a different gene in higher eucaryotic cells.

2) In some places, where amino acids differ between the yeast proteins, one or the other will match either one or both of the amino acid residues at the analogous position in n-*ras* or *ras*^{H}p21 (Fig. 3, residues 24, 25, 28, 49, 74, 84, 90, 106, 109, 133, 135, 141, 142, 153, 158). In other positions, the amino acid residues of all four proteins are different. (Fig. 3, residues 94, 95, 128, 132).

3) The mammalian *ras* proteins have 3 cysteines between amino acid residues 1 and 164 (mature p21). While the nature of disulfide linkages has yet to be determined for these proteins, it is interesting to note that there are no cysteines in the analogous positions in the yeast proteins.

4) Amino acid 12 is a glycine and amino acid 61 is a glutamine in both of the yeast proteins, which is analogous to the amino acids found at these positions in the normal cellular p21. The 12 and 61 positions are known mutation sites for the activation of *ras* oncogenes in different tumors. The viral *ras*^{H}p21 has an arginine at position 12, and other activated *ras* oncogenes have amino acids different than glycine at this site. Glycine is a known breaker of alpha helices and any change from this amino acid at position 12 would result in a change in the structure of the protein. Interestingly, the yeast proteins have four glycines in a row around the 12 position, so that mutation of two glycine residues in this region may be

required to alter the biological activity of these gene products.

5) There are only two amino acid differences between v-*ras*^{H} and c-*ras*^{H}; one is at position 12 and the second is at 59. The second site is a threonine in v-*ras*^{H} and it is phosphorylated whereas, in c-*ras*^{H} it is an alanine and as a result, is not phosphorylated. Both the yeast *ras* genes have an alanine at position 59.

In summary, *Saccharomyces cerevisiae* contains two active *ras*-like genes, both coding for a similar protein which is highly homologous to the mammalian p21. In addition, it has been found that these two genes constitute an essential gene family in family (K. Tatchell *et. al.*, in press). If either one of the two genes is inactivated expression of the other gene suffices for continued viability of the yeast cells. Considering the evolutionary distance that exists between yeast and mammalian cells, the degree of amino acid conservation between the yeast *ras*-related genes and mammalian *ras* is striking. This suggests that these genes may have a common biological function. While it is not yet clear what function these genes and their product(s) perform in yeast, one can now carry out genetic and biological experiments in yeast cells such that a function may soon be found.

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REFERENCES

1) Ellis, R.W., Lowy, D.R., Scolnick, E.M. Advances in Viral Oncology Vol. 1, ed. George Klein, 107-126 (1982).
2) Coffin, J.M., Varmus, H.F., Bishop, J.M., Essex, M., Hardy, W.D., Martin, G.D., Rosenberg, N.E., Scolnick, E.M., Weinberg, R.A., Vogt, P.K., J. Virol. 40, 953-957, (1981).
3) Weiss, R.A., Teich, N.M., Varmus, H.E., Coffin, J.M., (eds) "RNA Tumor Viruses", Cold Spring Harbor Laboratories, New York (1982).
4) Cooper G.M., Science 218, 801-804 (1982).
5) Taparowsky, E, Shinizu, K., Goldfarb, M., Wigler, M., Cell 39, 581-586 (1983).
6) Tabin, C., Bradley, S., Bargmann, C., Weinberg, R., Papageorge, A., Scolnick, E., Dhar, R., Lowy, D and Chang, E. Nature 300 143-148 (1982).
7) Reddy, E.P., Reynolds, R.K., Santos, E., Barbacid, M., Nature 300, 149-152 (1982).
8) Taparowsky, E, Suard, Y., Fasano, O., Shimizu, K., Goldfarb, M and Wigler, M., Nature 300, 762-765 (1982).
9) Shimizu, K., Birnbaum, D., Ruley, M.A., Fasano, O., Suard, Y., Edlund, L., Taparowsky, E., Goldfarb, M., and Wigler, M., Nature 304, 497-500 (1983).

10) McGrath, J.P., Capon, D.J., Smith, D.H., Chen, E.Y., Seeburg, P.H., Golddel, D., and Levinson, A.D., Nature 304, 501 (1983).
11) Yuasa, Y., Srivastara, S.K., Dunn, C.Y., Rhim, J.S., Reddy, E.P. and Aaronson, S.A., Nature 303, 775-779 (1983).
12) Sukumar, S, Notario, V, Martin-Zanca, D., Barbacid, M., Nature 306, 658-661 (1983).
13) Shih, T.Y., Weeks, M.O., Gruss, P., Dhar, R., Oroszlan, S., Scolnick, E.M., J. Virol. 42, 253-261, (1982).
14) Scolnick, E.M., Papageorge, A.G., Shih, J.Y., Proc. Natl. Acad. Sci. USA 76, 5355-5359 (1979).
15) Shih,T.Y., Stokes, P.E., Smythers, G.W., Dhar, R., Oroszlan, S., J. Biol. Chem. 257, 11767-11773 (1982).
16) Defeo-Jones, D., Scolnick, E.M., Koller, R., Dhar, R., Nature 306, 707-709, (1983).
17) Gallwitz, D., Donath, C., Sandy, C., Nature 306, 704-707 (1983).
18) Herrikoff, S., Kelley, J.D. and Cohen, E.H., Cell 33, 607-614 (1983).
19) Langford, C, Gallwitz, D., Cell 33, 519-527 (1983).
20) Maxam, A., and Gilbert, W. Proc. Natl. Acad. Sci. USA, 74, 560 (1977).
21) Papageorge, A.G., DeFeo-Jones, D., Robinson, P., Temeles, G., Scolnick, E.M., Molecular & Cellular Biology, 4, 23-29, (1984).