Identification of a novel murine IAP-promoted placenta-expressed gene

Audrey Chang-Yeh, David E.Mold and Ru Chih C.Huang*

Department of Biology, The Johns Hopkins University, Baltimore, MD 21218, USA

Received March 4, 1991; Revised and Accepted April 29, 1991

EMBL accession nos X58523 and X58524

ABSTRACT

We have cloned and characterized a novel cellular gene that is promoted by an intracisternal A-particle (IAP) LTR and expressed in the mouse placenta (mouse IAP promoted placental gene, MIPP). A 1067bp cDNA clone containing an IAP LTR U5 region duplicated in its 5' terminus and an ORF coding for a potential 202 amino acids protein was isolated from an 8.5 day old mouse embryo cDNA library. Sequence analysis of the 5' region of a genomic clone revealed the presence of a solo IAP LTR with the same U5 duplication, and primer extension analysis confirmed that transcription of the MIPP gene is under the control of the IAP LTR. Expression of the MIPP gene parallels that of IAP genes in normal mouse tissues with abundant transcripts present in the placenta and also in the myeloma MOPC-315. The MIPP-encoded protein is composed of four 48-amino acid repeat units and shares homology with a vaccinia virus gene product. MIPP-related sequences were also detected in higher eukaryotic genomes including human.

INTRODUCTION

Mouse intracisternal A-particles (IAPs) are endogenous retrovirus-like structures containing a 7.2Kb genomic RNA, a 73KDa gag-equivalent protein, several other structural related polypeptides and a tightly bound reverse transcriptase activity. Genes coding for IAPs are members of a dispersed multigene family with approximately 1000 copies per haploid genome in *Mus musculus* (1). They have the same overall structural organization as infectious retroviruses genes (2,3) with 5' and 3' long terminal repeats and coding sequences for the gag and pol domains (4,5,6). These elements lack an env-like region and in this respect, IAP elements are similar to retrotransposons, such as Ty elements of yeast and copia of *Drosophila* (for a review, see ref. 7). The IAP LTR contains all of the presumptive regulatory signals for promotion, initiation, and polyadenylation of transcription (8). IAP genes are expressed during development in normal preimplantation embryos (9,10,11) and in many transformed cells including embryonal carcinomas (12), hybridomas (13) and plasmacytomas (14).

Our previous studies have further demonstrated that IAP LTRs vary in their promoter activity and some are capable of bidirectional transcription (15). In addition to directing their own transcription, IAP LTRs have the capability of promoting transcription of adjacent cellular sequences as found for LTRs of many retroviruses (16) and thus can act as endogenous mutagens and contribute to genetic variability. Two documented cases are the insertion of an IAP sequence into the intron of the kappa-light chain gene, thereby inactivating expression (17), and the activation of the *c-mos* gene in the plasmacytoma XRPC24 by the insertion of an IAP gene in the 5' end of the *c-mos* coding region (18,19,20). Recently it has been reported that the gene encoding the oncodevelopmental protein, oncomodulin, in the rat is promoted by a solo IAP LTR (21,22).

In this communication, we describe another example of cellular gene expression directed by an IAP LTR. A mouse IAP promoted placenta-expressed gene (MIPP) and cDNA were isolated. The entire MIPP cDNA sequence, along with the MIPP promoter sequence from a genomic clone were determined. A 'solo' IAP LTR was observed positioned directly ahead of the first exon of the MIPP gene. Northern blot hybridization analyses have further shown that MIPP mRNA and IAP specific RNAs are present in placenta but not in most other tissues studied. The initiation site of the transcript has been mapped to the U3/R boundary by primer extension analysis. The level of IAP expression in placenta in fact was found to be many fold higher than that in thymus, the only other tissue in which substantial levels of IAP transcripts were detected (23,24). In the myeloma MOPC-315 where the IAP promoter is active (25), we detected MIPP expression, although the RNA species detected is larger than the one observed in placenta. The MIPP gene is evolutionally conserved in mammals. Its function is currently under active investigation.

MATERIALS AND METHODS

Material

Restriction endonucleases and DNA modification enzymes were obtained from New England Biolabs, Inc. and U.S. Biochemical Corp. Endonuclease digestions and DNA modifications were carried out as specified by the manufacturer. Isotopes were purchased from Amersham. The oligodeoxyribonucleotides used

* To whom correspondence should be addressed

for primer extension and sequencing were synthesized with an Applied Biosystems 380A DNA synthesizer. Nylon membranes were purchased from Amersham (Hybond-N). Chemicals were purchased from Research Organics and Sigma. The CF-1 mouse embryo cDNA library was a gift from Dr. Y.C. Hsu and a mouse genomic library constructed from a Swiss 3T3 cell line was a gift from Dr. M. Daniel Lane. MOPC-315 cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 100 U of penicillin per ml, and 100mg of streptomycin per ml.

Cloning and sequencing

DNA fragments were radiolabeled by the procedure of Feinberg and Vogelstein (26). Plating and screening of the cDNA and genomic libraries were carried out as previously described (27). Southernblots were performed as follows. Prehybridization was carried out at 65°C for 2 hours in 5×SSPE (1×=0.18M NaCl, 10mM sodium phosphate (pH 7.7), 1mM EDTA), 5×Denhardt's solution (1×=0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), 0.5% SDS, and 20ng/ml sheared denatured salmon sperm DNA. Hybridization was carried out at 65°C for 12 hours in the same solution with the addition of the denatured labeled probe at 1×10⁶ cpm/ml. Positive plaques were purified by three subsequent rounds of screening and phage DNA was isolated using a standard liquid lysate protocol (28). Lambda inserts were cloned into the plasmid pGEM3Z (Promega). Nested sets of deletions were constructed using exonuclease III and S1 nuclease as directed by the manufacturer (Promega Erase-A-Base). All DNA sequencing was performed using the Sequenase(tm) (United States Biochemical Corp.) dideoxy chain termination procedure according to manufacturer's instructions using synthetic oligonucleotides complementary to the Sp6 and T7 promoter regions as sequencing primers. The sequences were aligned using the NUCALN sequence homology computer program as previously described (8).

Animals and tissue preparation

Outbred CF-1 mice were obtained from Charles River Labs. Tissues were obtained from one month old mice of both sexes. To obtain placentas and embryos, CF-1 mice were mated, and the day a vaginal plug was found was designated as day 0.5. On day 12.5 the conceptuses were isolated from the gravid uteri, and the placentas and embryos were dissected free of decidua and membranes.

Isolation of RNAs, Northern blots, and primer extension

Total cellular RNA was prepared from tissues and cells according to the single step acid guanidium thiocyanate phenol chloroform extraction procedure of Chomczynski and Sacchi (29). Poly (A)⁺ RNA was isolated using Amersham's Hybond mAP according to the manufacturer's instructions. Total or poly (A)⁺ RNA was denatured in 50% dimethyl sulfoxide, 2.2M formaldehyde, 20mM sodium phosphate, pH 6.8, by heating at 65°C for 30 minutes. The denatured samples were separated on 1.5% agarose gels containing 2.2M formaldehyde, 20mM sodium phosphate (pH 6.8), stained in ethidium bromide in 0.05M sodium phosphate (pH 6.8), destained in the same buffer, photographed and transferred directly to a nylon membrane (Hybond-N). Prehybridization was carried out at 42°C in 5×SSPE, 50% formamide, 5×Denhardt's solution, 0.1% SDS, and 20ng/ml sheared denatured salmon sperm DNA. After 12

hours, the prehybridization solution was removed and replaced with fresh solution of $5\times$ SSPE, 50% formamide, $2\times$ Denhardt's solution, 0.1% (w/v) SDS, 20 ng/ml denatured salmon sperm DNA, and the radiolabeled probe at $1-3\times 10^6$ cpm/ml. Hybridization was carried out at 42°C for 12 hours. The filters were washed two times in $2\times$ SSPE, 0.1% SDS for 15 minutes at room temperature, two times in $0.1\times$ SSPE, 0.1% SDS, 15 minutes each, once at room temperature, and once at 50°C . Filters were stripped of the probe by boiling the filter in a solution of 0.1% SDS. As a control for loading and transfer, filters were stained in 0.04% methylene blue to visualize the RNA.

Primer extension analysis was used to map the 5' terminus of the RNA transcript. Thirty micrograms of total placental RNA was hybridized in 40mM PIPES (pH 6.4), 1mM EDTA (pH 8.0), 0.4M NaCl, and 80% formamide overnight with the 5' end labeled specific oligonucleotide 5'AGTACAGTCAAAGAT3' at 47°C. The primer extension was performed essentially as described by Sambrook *et al.* (28). The product of the extension reaction was analyzed on a 7M urea/6% polyacrylamide denaturing gel.

Zoo blot

Genomic DNAs were a gift from Steven Quirk. 10 μ g of genomic DNA was digested with EcoRI and run out on a 1% agarose gel. Southern analysis was performed as described above in the screening and cloning section. The low stringency wash was a 1 $\times$ SSPE/0.1% SDS 50°C for 30 minutes and the high stringency wash was in 0.1 $\times$ SSPE/0.1% SDS at 50°C for 30 minutes.

Figure 1. Complete nucleotide and predicted amino acid sequence of the MIPP cDNA. An asterisk indicates the stop codon. The 15mer (nt146 to nt160) used in the primer extension, as well as the polyadenylation signal, are underlined. The arrowhead at position 166 denotes the beginning of the deletion clone used to probe Northern and the genomic library (MIPPd4).

RESULTS

Sequence analysis of MIPP cDNA reveals the presence of two U5 regions of IAP LTR at its 5' terminus

To search for cellular genes that may be transcriptionally regulated through the IAP promoters during early mouse development, a restriction fragment containing only the U5 region from the IAP LTR was used to screen an 8.5 day mouse embryo cDNA library. Twelve lambda clones were chosen and plaque purified. Southern hybridization with probes specific to the internal regions of the IAP genome and the U3 region of the IAP

LTR revealed that 11 of the 12 clones contained IAP genes. One cDNA clone, however, failed to hybridize to these probes and therefore represented a possible candidate for a U5-linked cellular gene. This cDNA, designated MIPP, was subcloned into the sequencing vector pGEM 3Z and both strands of a subclone were sequenced in its entirety (Fig. 1). The sequence of the MIPP cDNA features characteristics of eukaryotic mRNAs such as a perfect AATAAA polyadenylation signal located 18 bases upstream from the 'CA' polyadenylation site and a partial poly (A) tail in its 3' end. In the upstream region of MIPP are two copies of IAP LTR U5 sequences along with 12 bases of the 3' end of the corresponding R region. The first U5 is a complete U5 whereas the second copy has the first 5' 13 bases of U5 missing and is referred to as the deleted U5 (dU5). The five bases, ATTGG, separate the two U5 copies. One of the three forward reading frames of MIPP is free of multi-termination codons and has the potential of coding for a polypeptide. The longest potential open reading frame within the MIPP cDNA begins with an AUG at nucleotide position 143 and terminates with a stop codon at position 749, representing a putative protein of 202 amino acids.

Figure 2(a). Restriction map of the SalI 14kb genomic clone and the location of the first two exons. (▽) Sma I (□) Sac I (●) Xba I (■) Eco RI. Exon 1 and 2 are noted in the sequence as solid blocks. (b). Nucleotide sequence of the 5' region of the MIPP gene. The U3-R-U5-dU5 region is indicated. The exons are depicted in upper case. The amino acid sequence appear below the coding sequence. The six base pair direct repeats that surround the MIPP LTR are denoted with arrows. Various motifs in the U3 region are underlined. The splicing sites, (SA) splice acceptor (SD) splice donor, are labeled and underlined. The BI homologous element is noted with an arrow in the direction of its transcription.

Figure 3. Northern blot analysis of various tissues. Twenty-five micrograms of total cellular RNAs were loaded and probed with (A) MIPPd4. The same filter was stripped and probed with (B) IAP 1.4 BamHI fragment. (C) represents a methylene blue staining of the nylon filter. The lanes are as follows: L: liver H: heart S: spleen Ts: testes Ty: thymus P: placenta E: embryo 315: MOPC-315. Triangles mark the migration of the 28S and 18S ribosomal RNAs and the arrows mark the migration of the transcripts with the corresponding size (Kb).

One complete, solo IAP LTR is found to be linked to the mouse MIPP gene

A MIPP specific probe (MIPPd4, Fig. 1) containing only the internal region of MIPP and lacking any of the IAP LTR sequence was used to screen one million plaques of a mouse library. One positive clone, containing a single 14Kb insert was isolated and mapped (Fig. 2a). Sequences homologous to various regions of the cDNA were found in the genomic clone and no hybridization with IAP internal sequences was detected in the 14Kb insert.

Figure 4. Primer extension analysis. An end-labeled 15mer oligonucleotide was hybridized for 12 hours at 47°C to 30 micrograms of RNA from (1) mouse placenta or (2) yeast RNA. The product of the extension reaction was analyzed on denaturing polyacrylamide gel with HpaII digest of pBR322 as size markers (M).

However, homology to an IAP LTR was localized to a 2.7Kb EcoRI fragment. This 2.7Kb R1 fragment was then subcloned and sequenced for further characterization (Fig. 2b). Comparison of the sequence with the Genbank data bank revealed that the MIPP LTR has 97.3% homology to a previously sequenced IAP LTR (30). Like the majority of other IAP LTRs, the MIPP LTR is terminated by a perfect complementary inverted repeat of four base pairs (TGTT and AACA). It is also flanked by a six pair direct repeat (ATTATT). Unlike other IAP LTRs, it contains the same duplicated U5 sequence (U5,dU5) organization as found in the MIPP cDNA and is directly linked to eleven nucleotides of what is the MIPP gene first exon. The first intron is 298 base pairs long and is flanked by a near perfect splice donor site sequence (ATGTAAGT) at position 824 and a splice acceptor sequence (TTTTCCCTTTTAAATCAGG) at position 1107. The MIPP protein coding sequence begins with the ATG at position 1138 within the 138 base pair second exon. Sequencing upstream of the MIPP LTR revealed no other promoter-like elements or IAP internal sequences. There is, however, an inverted B1 repetitive element present at position 147.

MIPP gene and IAP gene are both specifically expressed in mouse placenta and myeloma

Northern blot analysis were used to evaluate transcription products of the MIPP gene. Because the cDNA clone was isolated from an embryo library that was made from the embryos and their placenta (personal communication YC Hsu), we decided to search for MIPP expression in these two tissues, along with other normal tissues and also in MOPC-315 RNA, a known source of IAP transcripts (25). In total RNA, we were able to detect the 1.2 Kb RNA species only in placenta and not in any of the other normal tissues tested (Fig. 3a) in a 14 hour exposure. This RNA species is likely polyadenylated since it is present in oligo d(T) retained RNA (data not shown). In the myeloma RNA, a major 2.2Kb RNA species was observed along with a minor 4.4Kb RNA species. Upon overexposure (36 hours), the same 2.2Kb transcript can be seen in the placenta RNA although at much lower levels. The minor bands that co-migrate with the 28S and 18S ribosomal RNA are most likely due to trapping of the MIPP message by the large amount of ribosomal RNA present in the unfractionated total RNA.

The presence of an IAP LTR linked to the MIPP gene raised the possibility that expression of the MIPP gene might parallel that of IAP genes. Using a 1.4Kb BamHI fragment (25) from the IAP internal region, we probed the same tissue blot and were able to detect the 5.4Kb IAP transcript in the placenta with a

MIPP	7	ECYDPVTKQWTTVASMNHPR	CGLGVVCVCYGAIIYALGGWVGAEIGNTI	53
C2L	235	THYSPRTEYWTTVGNTRQFYNNAN--VLHNC	LYIIGGMINNRHVYSV	279
MIPP	54	ERFDPDENKWEVVGSMVAVSRY	YFGCEMQGLIYAVGGISNEGLELRSF	101
C2L	280	SRVDLETKKWKTVTNTMSSLKSEVSTCVNDG	KLYVIGGL-EFSISTGVA	326
MIPP	102	EYVDPLSKRWSPLPPMGTRRAYLGVAA-LND	CIYAIGGWNETQDALHTV	149
C2L	327	EYLKHGTSKWKIRLPNLITPR-YSGASVFVND	DIYVMGGVYTTYEKYVVL	374
MIPP	150	EKY-SFEEEEKWEVASMKVPRAGMCAVTVNG	LIVSVGGRS-SSHDF-LAPG	197
C2L	375	NDVECFTKNRWIKKSPMPRHHSIVYAVEYD	GDIYVITGITHETRN-YL	421

Figure 5. Deduced Protein Sequence from MIPP and a vaccinia virus protein, C2L. The homologous amino acids between the two proteins are denoted by ':' and the repeated domain is boxed.

3 hour exposure. With a longer exposure (14 hours), similar transcripts were present in thymus and testis RNA but in greatly reduced amounts (Fig. 3b). As a control for the amount of RNA loaded and the efficiency of transfer, the filter was stained to visualize the RNA (Fig. 3c).

Transcription of the MIPP gene is initiated at the U3/R boundary of the LTR

Primer extension experiments were used to determine the initiation site for the MIPP transcript in placenta (Fig. 4). A 15 base pair oligonucleotide complementary to the 5' end of the coding sequence (Fig. 1), generated a major extended product of approximately 213 base pairs. The result is consistent with initiation occurring at the U3/R boundary of the LTR. This is the characteristic start site for transcription promoted by IAP LTRs (15).

MIPP protein has a forty-eight amino acid repeat unit

The amino acid sequence of this protein is interesting in that it consists of four 48 amino acid repeat units. A vaccinia virus protein sequence, C2L, (31,32) that contains a similar repeat structure was discovered by a search of Genbank. The two proteins share 24.3% identity in a 189 amino acid overlap within this region. An alignment of the repeated structures is shown in Figure 5. The function of both MIPP and C2L remains unknown.

MIPP sequence is conserved evolutionally in mammals

High molecular weight DNA was prepared from tissues from a variety of species and digested with Eco R1 restriction endonuclease. Complete digestion of DNA was monitored by evaluation of ethidium bromide stained gel patterns. Hybridization after Southern transfer was carried out at 65°C for 12 hours, followed by a low stringency wash and a high stringency wash (see Materials and Methods). After the high stringency wash, hybridization bands were seen in human, monkey, hamster, mouse, but not in the DNAs of chicken, nematode, fruitfly, yeast,

or T5 phage, even upon overexposure of the autoradiogram (Fig. 6). Some hybridization was seen to chicken after the low stringency wash (data not shown), suggesting that less homology exists between MIPP and the chicken sequence, which is reflective of the greater evolutionary distance between the two species.

DISCUSSION

In this study, we present an example of a mammalian gene transcribed in the placenta by a retrovirus-like promoter with the cloning and characterization of the MIPP gene. This set of circumstances presumably arose as the result of a germline retrotransposition of an IAP element. A likely sequence of events that might have occurred is that a complete IAP element transposed via reverse transcriptase to the MIPP locus and the two IAP LTRs subsequently underwent homologous recombination removing the body of the IAP element, leaving behind the solo U3-R-U5-dU5 arrangement. The partial U5 duplication may have arisen in this manner if during crossing over, the alignment of the LTRs was shifted by forty-four base pairs. In this regard it is significant that the two U5s are identical and separated by the first five bases of the IAP internal sequence (ATTGG). This type of event could also explain the presence of the six base pair direct repeats that flank the MIPP LTRs. Similar repeats are found at the 5' and 3' LTR boundaries flanking complete IAP elements. These short direct repeats are believed to be generated when the element is inserted into a staggered break in the DNA duplex and are hallmarks of a transposition event (16). Other solo murine IAP LTRs have been reported and are assumed to have been generated from an originally complete IAP element by unequal crossing over at the LTRs (33). Recently, it has been reported that the tumor-associated, calcium-binding protein oncomodulin expressed in rat placenta is under the control of a solo LTR that is related to the Syrian Hamster IAP element (21,22).

It is known that certain regions of the DNA more prone to transpositional events are known as 'hot spots' in the chromosomes (16). The fact that we have a B1 element, another known repetitive element that is suspected of transposing throughout the genome (34) leads us to believe that the MIPP locus may indeed represent a 'hot spot' for genetic rearrangement.

In the mouse, IAP genes are poorly expressed in normal tissues with the exception of thymus, where expression is strain-specific (24). We report here the finding of IAP transcripts in mouse placenta along with MIPP transcripts. Of the normal CF-1 mouse tissues we studied, abundant IAP transcripts were found only in the placenta. This result is in agreement with the recent report of Djaffar *et al.* (35), who detected high levels of IAP related transcripts in rat placental tissues. A high degree of placental expression has also been reported for the IAP promoted oncomodulin gene in the rat (21,22). IAP genes are also known to be highly transcribed in many mouse tumor cells. We have shown that in MOPC-315 where IAP transcription is abundant (25), a 2.2Kb RNA species hybridizes to the MIPP probe at about the same intensity as the 1.2Kb placental transcript. Upon longer exposures, the 2.2Kb transcript can be seen in the placenta RNA, although at much lower intensity. This larger transcript could represent an unprocessed or alternately processed form of the MIPP RNA. Another possibility is the larger transcript represents expression of a different allele of MIPP. Current work is ongoing to better characterize this larger transcript.

Figure 6. Zoo Blot: Ten micrograms of genomic DNA were digested with EcoR1, probed with MIPPD4, and washed at a high stringency as described in Materials and Methods. The lanes are as follows; H:human Mk:monkey Ha:hamster M:mouse C:chicken F:fruitfly N:nematode Y:yeast T:T5 phage. Dashes at right mark the position of a λ HindIII DNA size marker.

In the case of the MIPP gene, we are confident that transcription is driven by the IAP LTR in the placenta for the following reasons: i) U5 sequences are present at the 5' end of the cDNA and these are identical to those of the LTR in the genomic clone. ii) the MIPP is transcribed in the same mouse tissues as IAP genes. iii) The MIPP transcript size (1.2Kb) is consistent with the size expected (given a poly (A) tail of 250 residues) if initiation is within the IAP LTR. iv) Primer extension with a specific primer maps the initiation site to the U3/R boundary of the LTR.

The MIPP protein consists of four segments which appear to have diverged from a common ancestral domain. A strikingly similar homologous repeat was found in the vaccinia virus C2L protein. Repeating motifs are characteristic of many ligand-binding protein families. The lipocortin protein family has a characteristic 70 amino acid unit that is repeated multiple times within the protein. This highly conserved consensus region is thought to be important for calcium/phospholipid binding (36). The macrophage mannose receptor contains eight repeats related in sequence to the C-type carbohydrate binding domain of animal lectins (37,38). Two other endocytic receptors, the low density lipoprotein receptor (39) and the cation-independent mannose-6-phosphate receptor also have repeated binding motifs. In an analogous manner, MIPP amino acid repeat could be a region of some yet unknown biological functional importance.

We have shown that MIPP-related DNA sequences are present in all of the higher eukaryotes we tested, including human. It will be interesting to know what pattern of expression of the MIPP gene is found in other mammals. Our preliminary Northern analysis of human placenta RNA shows that a human counterpart of the MIPP transcripts does exist. If the same placental specific pattern of expression is seen in humans, an examination of the human MIPP promoter could lead to important insights into the role of retroviruses in evolution. Further experiments to assess MIPP's role in mouse placenta and other species are necessary.

ACKNOWLEDGEMENTS

We thank Dr. Karen Beemon, Dr. Robert Christy, Jerry Stitzel, and Michael Zierler for their helpful discussion during the course of this study. This investigation was supported by Public Health Service grant 5ROICA48263 from the National Institutes of Health. A.C.Y. was supported by National Research Service Predoctoral Training grant 5T32GMO7231 from the National Institutes of Health.

REFERENCES

- Ono, M., Cole, M.D., White, A.T. and Huang, R.C.C. (1980) *Cell* **21**, 465-473.
- Cole, M.D., Ono, N. and Huang, R.C.C. (1981) *J. Virol.* **38**, 680-687.
- Kuff, E.L., Smith, L.A. and Lueders, K.K. (1981) *Mol. Cell. Biol.* **1**, 216-217.
- Chiu, I.M., Huang, R.C.C. and Aaronson, S.A. (1985) *Virus Res.* **3**, 1-11.
- Paterson, B.M., Segal, S., Lueders, K.K. and Kuff, E.L. (1978) *J. Virol.* **27**, 118-126.
- Mietz, J.A., Grossman, A., Lueders, K.K. and Kuff, E.L. (1987) M17551, *J. Virol.* **61**, 3020-3029.
- Kuff, E.L. and Lueders, K.K. (1988) *Adv. Cancer Res.* **51**, 183-276.
- Christy, R.J., Brown, A.R., Gourlie, B.B. and Huang, R.C.C. (1985) X01705, *Nucleic Acids Res.*, **13**, 289-302.
- Calarco, P.G. and Szollosi, D. (1973) *Nature (London) New Biol.* **243**, 91-93.
- Chase, D.G. and Piko, L. (1973) *J. Natl. Cancer Inst.* **51**, 1971-1973.
- Kelly, F. and Condamine, H. (1982) *Biochim. Biophys. Acta.* **651**, 105-141.
- Morgan, R.A., Christy, R.J. and Huang, R.C.C. (1988) *Development* **102**, 23-30.
- Cohen, J.B., Unger, T., Rechavi, G., Canaani, E. and Givol, D. (1983) *Nature (London)* **306**, 797-799.
- Morgan, R.A. and Huang, R.C.C. (1984) *Cancer Res.* **44**, 5234-5241.
- Christy, R.J. and Huang, R.C.C. (1988) *Mol. Cell Biol.* **8**, 1093-1102.
- Weiss, R., Teich, N., Teich, H.E., Varmus, H.E. and Coffin, J. (eds.). (1982) *Molecular Biology of Tumor Viruses* 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Hawley, R.G., Shulman, M.J., Muraldo, H., Gibson, D.M. and Hozumi, N. (1982) *Proc. Natl. Acad. Sci.* **79**, 7425-7429.
- Canaani, E., Dreazen, O., Klar, A., Rechavi, G., Ram, D., Cohen, S.B. and Givol, D. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7118-7122.
- Kuff, E.L., Feenstra, A., Lueders, K., Smith, L., Hawley, R., Hozumi, N. and Shulman, M. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1992-1996.
- Rechavi, G., Givol, E. and Canaani, E. (1982) *Nature (London)* **300**, 607-611.
- Banville, D. and Boie, Y. (1989) X15839, *J. Mol. Biol.* **207**, 481-490.
- Furter, C.S., Heizmann, C. and Berchtold, M.W. (1989) *J. Biol. Chem.* **264**, 18276-18279.
- Grossman, Z., Mietz, J.A. and Kuff, E.L. (1987) *Nucleic Acids Res.* **15**, 3823-3843.
- Kuff, E.L. and Fewell, J.W. (1985) *Mol. Cell. Biol.* **5**, 474-483.
- Wujcik, K.M., Morgan, R.A. and Huang, R.C.C. (1984) *J. Virol.* **52**, 29-36.
- Feinberg, A.P. and Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6-13.
- Huynh, T.V., Young, R.A. and Davis, R.W. (1985) In Glover, D.M. (ed.), *DNA Cloning-A Practical Approach*. IRL Press, Washington, D.C., Vol. I, pp. 49-78.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edition. Cold Spring Harbor Press, Cold Spring Harbor.
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156-159.
- Augenlicht, L.H., Kobrin, D., Pavlovic, A. and Royston, M.E. (1984) M13376, *J. Biol. Chem.* **259**, 1842-1847.
- Goebel, S.J., Johnson, G.P., Perkins, M.E., Davis, S.W., Winslow, J.P. and Paoletti, E. (1990) M35027, *Virology* **179**, 247-266.
- Kotwal, G.J. and Moss, B. (1988) *Virology* **167**, 524-537.
- Man, Y.M., Delius, H. and Leader, D.P. (1987) *Nucleic Acids Res.* **15**, 3291-3304.
- Moshier, J.A., Deutch, A.H. and Huang, R.C.C. (1987) M18260, *Gene* **58**, 19-27.
- Djaffar, I., Dianoux, L., Leibovich, S., Emanoil-Ravier, R. and Peries, J. (1990) *Biochim. Biophys. Res. Commun.* **169**, 222-231.
- Pepinsky, R.B., Tizard, R., Mattaliano, R.J., Sinclair, L.K., Miller, G.T., Browning, J.L., Chow, E.P., Burne, C., Huang, K.S., Pratt, D., Wachter, L., Hession, C., Frey, A.Z. and Wallner, B.P. (1988) *J. Biol. Chem.* **263**, 10799-10817.
- Taylor, M.E., Conary, J.T., Lennartz, M.R., Stahl, P.D. and Drickamer, K. (1990) J0550, *J. Biol. Chem.* **265**, 12156-12162.
- Tong, P.Y., Gregory, W. and Kornfeld, S. (1989) *J. Biol. Chem.* **264**, 7962-7969.
- Russell, D.W., Brown, M.S. and Goldstein, J.L. (1989) *J. Biol. Chem.* **264**, 21682-21688.