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Characterization of the promoter region of the human O^6-methylguanine-DNA methyltransferase gene

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ABSTRACT

O^6-methylguanine-DNA methyltransferase (MGMT)is a ubiquitous protein responsible for repair of O^6-alkylguanine, a mutagenic, carcinogenic and toxic lesion. To characterize the elements responsible for the regulation of the MGMT gene, a 2.6 kb SstI fragment isolated from a genomic clone, was shown to contain 5′ flanking sequences of the gene. The promoter activity of this fragment as well as various subfragments were tested in NIH 3T3 mouse fibroblasts by transient expression of the bacterial chloramphenicol acetyltransferase (CAT) gene linked to these fragments. Maximal promoter activity was observed in a 1.2 kb 3′ terminal fragment, which contains the first untranslated exon. The transcription initiation site was identified in this fragment by primer extension and S1 mapping. Sequence analysis of this fragment showed the absence of TATA and CAAT boxes but an abundance of extremely GC-rich sequences, including ten GC hexanucleotide motifs 5′CCGCCC. Reduced CAT expression with the minimal promoter sequence suggests the presence of multiple regulatory elements.

INTRODUCTION

The DNA adducts O^6-alkylguanine and O^4-alkylthymine, produced by simple monofunctional alkylating agents such as alkylnitrosoureas, are mutagenic and carcinogenic (1). Similarly, the cytotoxic, antitumor effects of chloroethylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea are believed to involve formation of O^6-guanine adducts (2). O^6-methylguanine-DNA-methyltransferase (MGMT, EC.2.1.1.63) specifically removes these DNA lesions by directly transferring the alkyl group to a cysteine acceptor residue within the protein itself in a stoichiometric autoinactivating reaction (3,4). All normal human cells and tissues express the MGMT protein, although levels vary appreciably according to cell and tissue type (5). A proportion of human tumor cells however (~20%) appear to be completely deficient in MGMT and these cells (termed Mer$^-$ or Mex$^-$) are hypersensitive to the carcinogens and drugs that induce O^6-guanine adducts (6,7,8). Accordingly, there is considerable interest in the molecular basis of regulation of the MGMT gene in normal tissues and its apparent absence in some malignant tumor cells.

Recent isolation of the MGMT cDNA (9,10,11) and development of human MGMT-specific antibodies (12) has facilitated molecular characterization of the gene and its expression in human cell lines. Evidence from our laboratories (13) and others (14,15) increasingly suggests that expression is regulated at the level of transcription because in all but one of the Mer$^-$ cell DNA samples studied to date (9), the MGMT gene was present in Southern analysis with no gross rearrangements or deletions whereas the mRNA and protein are undetectable by Northern and Western analyses, respectively.

Although events other than transcription, may result in mRNA deficiency in Mer$^-$ cells (e.g., RNA processing in the nucleus, mRNA transport from the nucleus and mRNA stability) (16), we have chosen to investigate transcriptional regulation by examining the 5′-flanking region of the human MGMT gene. Here we describe the characterization of promoter elements in the 5′-untranslated sequences and the identification of the transcription start site.

MATERIALS AND METHODS

Molecular biology

All techniques not described below, such as routine subcloning were performed according to Sambrook et al. (17).

DNA sequencing

DNA to be sequenced was ligated into M13mp18, 19, or pUC18 and subjected to sequencing by the dideoxy chain termination method (18) using Sequenase 2.0 (United States Biochemicals).

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Computer analyses were performed using the Intelligenetics suite of programs on a MicroVax 3600.

Primer extension

Oligonucleotide primers for extension reactions were synthesized using standard phosphoramidite chemistry and [^{32}P]-end labelled using T4 polynucleotide kinase. Hybridizations to CEM-CCRF human lymphocyte total RNA were performed in 50% formamide, 0.4 M NaCl, 40 mM PIPES pH 6.8, 1 mM EDTA at 35°C for 16 hours prior to extension with avian myeloblastosis virus (AMV; Boehringer), Moloney murine leukemia virus (M-MLV; Gibco-Bethesda Research Laboratories) or *Thermus thermophilus* (rTth; Perkin Elmer) reverse transcriptase (RTase). All enzymes were used under the conditions specified by the manufacturer except that AMV RTase reactions were also performed at 47°C. Reaction products were analyzed by denaturing polyacrylamide gel electrophoresis and detected by autoradiography.

S1 nuclease analysis

DNA probes were generated by annealing [^{32}P]-labeled oligonucleotides to single stranded (ss) M13 DNA containing the 772 bp PstI genomic fragment and extended with 5 units Taq DNA polymerase (Promega)and 200 μM each dNTP. Following digestion with SphI and isolation from a 4% denaturing Nusieve agarose gel (FMC Bioproducts), the probes were hybridized to 50 μg CEM-CCRF total RNA in 80% formamide, 400 mM NaCl, 1 mM EDTA, 40 mM PIPES (pH 6.8) at 35°C overnight. Following precipitation with ethanol, DNA-RNA hybrids were digested with either 2 or 8 units S1 nuclease (Gibco-BRL), in a final volume of 50 μl of 50 mM NaCl, 30 mM CH_3COONa, 1 mM $ZnSO_4$, 5% glycerol (pH 4.6) at 30°C for 1 hour. Reactions were stopped by extraction with phenol/chloroform. After ethanol precipitation, protected fragments were analyzed on a 6% denaturing polyacrylamide gel and detected by autoradiography.

Reporter gene analysis

Plasmids used for insertion of the 5′ sequences were pOCAT1 (a gift from Dr. D.D. Moore) (19) and pCATbasic (Promega), both of which contained the bacterial chloramphenicol acetyltransferase (CAT) gene and polyadenylation signals derived from herpes simplex virus (HSV) and simian virus 40 (SV40) genes, respectively. Mouse NIH 3T3 fibroblasts were transfected with the CAT vector (20 μg) using a modified calcium phosphate coprecipitation method under conditions described by the manufacturer (Stratagene) (20). The CAT enzyme assay, performed 48 hours post-transfection, was based on the method of Neumann et al. (21) using [^{3}H]-acetyl CoA (New England Nuclear). CAT containing plasmids were cotransfected with 10 μg of a β-galactosidase expression plasmid, pCH110 (Pharmacia-LKB), and the assay for β-galactosidase (22) was used to correct the CAT activity values for transfection frequency. Activities for both enzymes were calculated as activity per hour per mg total cell protein and the promoter activity was expressed as the ratio of CAT:β-galactosidase. The DNA construct that exhibited the highest normalized CAT activity was arbitrarily designated as having 100% promoter activity. A cytomegalovirus (CMV) CAT construct was used as a positive control.

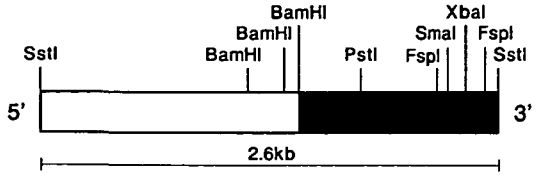

Figure 1. Diagram of the 2.6 kb fragment that hybridizes to human MGMT cDNA. The positions of various restriction endonuclease recognition sites are shown. The region for which nucleotide sequence was obtained is solid.

RESULTS

The screening of a human placental genomic DNA library (Clontech) with an 835 bp human MGMT cDNA containing 14 bp EcoRI adapters at both ends (9) resulted in the isolation of 12 independent clones. Two clones containing 2.6 kb SstI fragments that specifically hybridized with oligonucleotides corresponding to the 5′-untranslated region (K. Tano and S. Mitra, unpublished experiment) were subcloned into pUC18 and named pKT200. More extensive analysis with a variety of enzymes demonstrated that the exon homologous region was located within a 270 bp SmaI fragment (Fig. 1).

Identification of the promoter region

To identify the DNA sequences required for MGMT gene expression, various restriction fragments (Fig. 1) of the 5′-untranslated region were ligated upstream from the CAT gene in the plasmids pOCAT1 and pCATbasic.

The CAT plasmid DNA was cotransfected with pCH110 DNA into NIH 3T3 mouse fibroblasts. We chose NIH 3T3 cells as the recipient for these constructs because they represent a stable diploid line that expresses stable levels of MGMT activity and is known to be transfectable with good efficiency. Figure 2 shows relative CAT activity values that were normalized for transfection frequency by comparison with β-galactosidase activity. These results demonstrate that the maximal promoter activity is

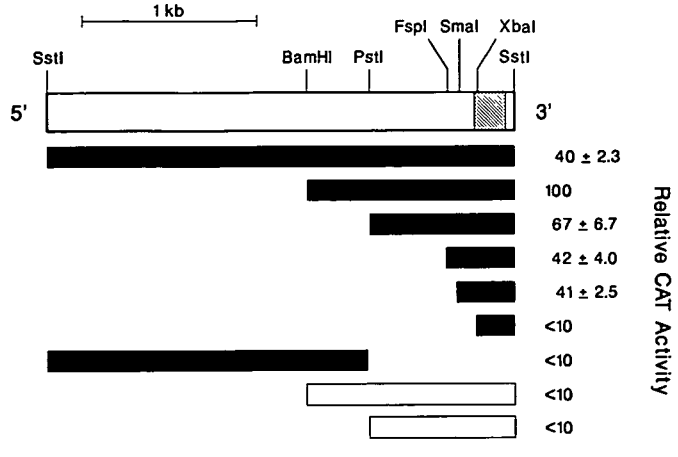

Figure 2. Relative CAT activity produced by various fragments of the MGMT gene 5′-untranslated region. Values normalized for transfection frequency are the mean from 3 or more independent transfection experiments, with standard errors. Solid boxes represent fragments orientated correctly with respect to the CAT gene in the plasmid. Open boxes indicate fragments in the inverted orientation. The shaded area represents the position of the first exon.

produced by the 1.2 kb BamHI fragment. All other CAT activities are expressed as a percentage of this value. The BamH1 fragment's promoter activity was generally 2–4% of a control CMV-CAT construct and at least 10-fold greater than a promoterless control. CAT expression was lost when the BamH1 fragment was inserted into the plasmid in the opposite orientation. The full-length 2.6 kb SstI fragment had appreciable promoter activity, although considerably less than that of the 1.2 kb BamH1 fragment. Promoter activity was not detected when the region 3′ to the PstI site was deleted; however, the reciprocal construct, PstI to SstI had 67% the activity of the BamH1-SstI fragment. Further deletion of this 1.2 kb fragment from the 5′ end to the FspI site or to the SmaI site reduced the activity to 41%. The 183 bp XbaI fragment, which contained the majority of the first exon and some intron sequence, yielded < 10% of the CAT activity produced by the 1.2 kb BamHI fragment.

DNA sequence analysis and the start of transcription

To identify the exon and any potential promoter elements within this DNA, we sequenced a region extending 1157 bp 5′ from the SstI site to a BamHI site. The very high GC content of the cloned DNA presented problems during initial sequencing using the M13 −40 universal primer. Frequent abberant chain termination and anomalous fragment mobility during electrophoresis were seen, presumably caused by secondary structure within the sequence. Three approaches were adopted to overcome these problems: oligonucleotide primers (17- or 20-mers) homologous to sequences within the insert were constructed; dITP or 7-deaza-dGTP were used in conjunction with dGTP during sequencing reactions; and numerous restriction fragments were subcloned into M13mp18 and 19 for resequencing. The results of these analyses are shown in Figure 3. The sequence is numbered 1–1157, starting at the recognition site for the restriction enzyme BamHI.

The first exon is located at positions 955–1116 (Fig. 3). This area includes the region between 1044 and 1116 which is homologous to the cDNA sequence published by Tano et al. (excluding the 14 nucleotide adapter sequence) (9). Two extensively GC-rich regions are present at base numbers 823–936 (89%) and 979–1031 (90%) and are separated by a run of pyrimidines (936–951). No obvious TATA box is present in the sequence.

An oligonucleotide, PSEQ2, homologous to the noncoding strand (position 1044–1028), was used for both primer extensions and for the production of a ssDNA probe for S1 nuclease analysis. Because of the high GC content of the target sequence, reverse transcription was attempted with several enzymes (AMV, M-MLV and rTth) under various reaction conditions. The use of AMV RTase at a higher temperature (47°C) has been shown to be useful in overcoming problems caused by secondary structure within the transcribed sequence (23). Since the rTth RTase is active at 70°C (24) it also would be expected to function efficiently on GC-rich sequences. However, very little extended product was seen with either the AMV RTase or rTth RTase (data not shown), whereas M-MLV RTase at 37°C generated easily detectable signals (Fig. 4A).

An extension product of 89 bp, produced from PSEQ2, was consistent with transcription initiated at position 955. To confirm this result, S1 nuclease analysis using an M13-derived ssDNA probe was performed. A 195 bp [^{32}P]-end labeled fragment (PSEQ2-SphI, positions 1044–849) was annealed to CEM-CCRF total RNA and subjected to analysis as described in Materials and Methods. Although multiple bands are apparent on the autoradiograph (Fig. 4B), probably the combined result of incomplete S1 nuclease digestion and secondary structure generated by the GC-rich areas, one band clearly corresponds to that generated by the primer extension, consistent with transcription initiation at position 955.

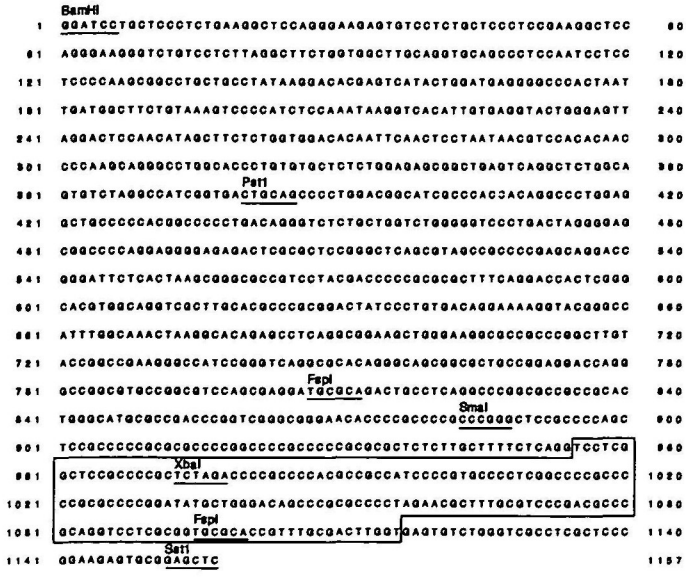

Figure 3. Nucleotide sequence of the human MGMT gene 5′-untranslated region. The first exon is boxed.

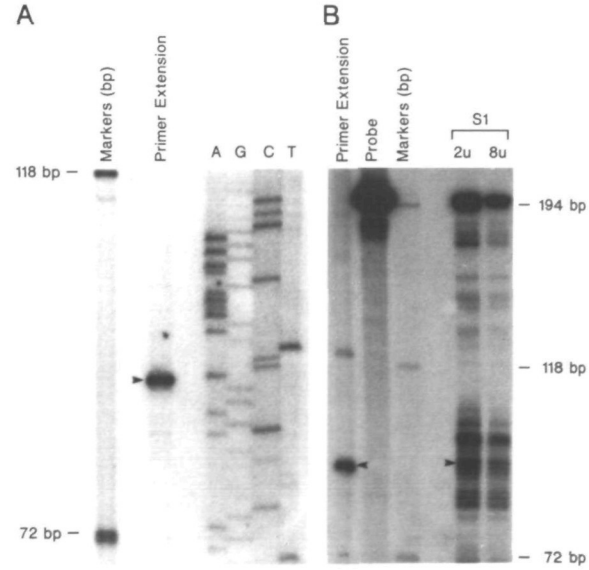

Figure 4. Autoradiographs of primer extension and S1 nuclease protection reactions with the oligonucleotide PSEQ2. The position of markers correspond to HaeIII fragments of ϕX174. A)Alignment of the primer extension product with the nucleotide sequence ladder generated from PSEQ2. The 89 bp extended fragment (see arrows) corresponds to adenine at position 955 on the complementary strand. B)Alignment of the primer extension and S1 nuclease protection products. Reactions contained 2 or 8 units of S1 nuclease.

DISCUSSION

The transcription start site and the minimal promoter sequences of the human MGMT gene were located in a 1157 bp region of a genomic clone. The promoter region has features similar to the promoters of many so-called 'housekeeping' genes, such as hypoxanthine phosphoribosyltransferase (25) and adenosine deaminase (26), whose activity is required in all cells and is not subject to environmental control. Promoters of these genes usually contain very GC-rich sequences and, unlike most other genes (27), lack a TATA box. The human MGMT 5'-flanking sequence also lacks a TATA box and is GC-rich, particularly in the two regions between 823–936 (89%) and 979–1031 (90%). The sequence believed to be the minimal promoter is between residue numbers 886–955 and thus contains the majority of the first GC-rich region. The second occurs 3' of the transcription start site and is within the first untranslated exon. In the absence of a TATA box, which usually determines the start of transcription (27), there must be other sequences in the MGMT gene that perform the same function and aid the binding of RNA polymerase II (28).

A promoter element or putative CCAAT box (ACACCC) is present at positions 870–876, identified only by its homology to the rabbit beta globin gene promoter (29). A direct repeat is located at positions 6–32 and 41–67, although the relevance of these regions is at present unclear. The sequence CCGCCC, which is present as a tandem array in both SV40 and HSV promoters (30,31), occurs five times within a 53 bp region, at 875–928, which spans the SmaI site. Such regions have been shown to act as promoter/enhancer-type elements in these viruses (32) but are also present in other eukaryotic gene promoters, e.g., 3-hydroxy-3-methylglutaryl coenzyme A reductase (33) and the epidermal growth factor receptor (34). A stem-loop structure could form from the sequences 862–868 and 874–880, which may be important in transcriptional regulation.

The DNA sequence was searched for known recognition sequences for transcription activating binding proteins to determine whether any potential transcription regulatory elements were present. An AP1 site (TGAGTCAG) (35) is located at position 344–351, and the region 151–156 demonstrates 6/8 homologous bases with this search sequence. An AP2 site (G(CG)(CG)(TA)G(GC)CC) (36) is present at 420–427 and at position 602–609 a second possible site which has 6/8 bases homologous to the search sequence is present. AP1 and AP2 are responsible for upregulation of expression mediated by TPA and cAMP (35,36), although no evidence that these modulators affect the activity of the MGMT gene currently exists. An SP1 site (GGGCGG) (37) is present at 862–867; however, if this regulatory element is recognized on the complementary strand, the CCGCCC sequences described above would also be SP1 binding sites. There is partial homology to the glucocorticoid responsive element (GGTACANNNTGTTCT) (38) at positions 28–42 and 63–77; and to the consensus sequence for the eukaryotic heat shock promoter element (CNNGAANNTT-CNNGA) (39) at position 577–591. However, little is known about the cellular response of the MGMT gene to hormones or stress. The relative locations of all the above elements are summarized in Figure 5.

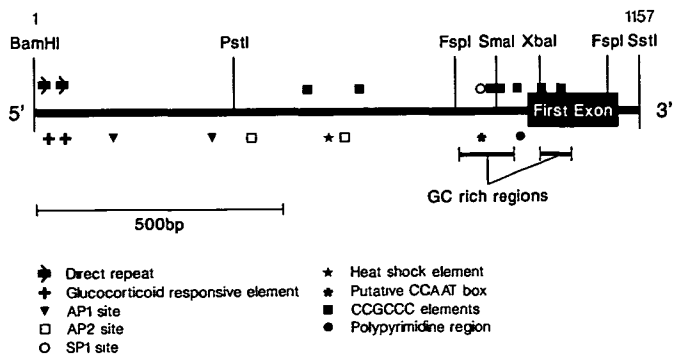

Figure 5. Diagram of the 1157 bp sequenced fragment of human MGMT gene 5'-untranslated region showing the location of potential regulatory elements on the sense strand.

Promoter elements necessary for basal level transcription are generally found in the region −25 to −110 from the transcription start site, and at least three protein-binding sites are necessary to promote transcription (31). Because the SmaI-SstI fragment has significant promoter activity and the transcription start is positioned at 955, the MGMT promoter elements are expected to reside between positions 886 and 930. This sequence contains three of the CCGCCC elements.

The promoter activity was not increased by a fragment extending 76 bp 5' from the SmaI site to the FspI site. Since this fragment includes an SP1 site, the suggested CCAAT box and possible stem-loop structure, the role of these elements in regulating the expression of this gene is questionable.

Further 5' extension by 426 bp to the PstI site increased promoter activity by 60%, suggesting that this region contains elements important for modulation of expression (40). This region includes several potential transcription factor binding sites; two AP2 recognition sequences at positions 420–427 and at 602–609, a CCGCCC element and the region 577–591 which shows homology to the heat shock protein regulatory element.

Addition of a further 385 bp up to the BamH1 site produced a 50% increase in promoter activity, which may be attributed to a direct repeat present at positions 4–32 and 37–67. Such repeated sequences having been shown to be important for the control of gene expression (27). In addition, a potential AP1 site at 344–351 and two possible glucocorticoid responsive elements at 28–42 and 63–77 in this region may be important for producing the enhancer-like activity. Extension to the 5' SstI site had an inhibitory effect on the transcription efficiency suggesting that repressor-type elements may be present in these additional sequences. The region from the 5' SstI site to the PstI site was deficient in promoter activity confirming that the minimal promoter region is located 3' to this area.

The human MGMT promoter does not function when the BamHI or PstI fragments are inverted with respect to the CAT gene. This is consistent with the usual unidirectional function of promoter sequences. The progressive increase in promoter activity with 5' extension from the minimal (SmaI-SstI) to the maximal (BamH1-SstI) promoter indicates that the larger fragment contains multiple elements (promoter or enhancer) that are required for an efficient transcriptional unit (41).

All the data for promoter activity in this report were obtained by transfections into murine NIH 3T3 cells, however, preliminary experiments with the same CAT constructs by us and by Dr. Bernd Kaina at Kernforschungszentrum, Karlsruhe (personal communication) have confirmed their promoter activity in human cells. Moreover, there appears to be no obvious difference

between Mer^+ and Mer^- cells in their capacity to support the promoter activity.

In conclusion, the human MGMT promoter belongs to the class of GC rich, TATA-free, promoters and further investigation of its transcription factors and the sequences to which they bind should contribute to understanding the mechanism of regulation in this unusual family of genes.

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REFERENCES

1. Margison, G.P. and O'Connor, P.J. (1990) In Grover, C.S. and Grover, P.L. (eds.), *Handbook of Experimental Pharmacology* **94**, 547–571.
2. Brent, T.P. (1985) *Pharmac. Ther.* **31**, 121–140.
3. Foote, R.S., Mitra, S. and Pal, B.C. (1980) *Biochem. Biophys. Res. Comm.* **97**, 654–659.
4. Olsson, M. and Lindahl, T. (1980) *J. Biol. Chem.* **255**, 10569–10571.
5. Myrnes, B., Nordstrand, K., Giercksky, K., Sjunneskog, C. and Krokan, H. (1984) *Carcinogenesis* **5**, 1061–1064.
6. Day, R.S. III, Ziolkowski, C.H., Scudiero, D.A., Meyer, S.A., Lubiniecki, A.S., Girardi, A.J., Galloway, S.M. and Bynum, G.D. (1980) *Nature* **288**, 724–727.
7. Sklar, R. and Strauss B. (1981) *Nature* **289**, 471–420.
8. Day, R.S., Babich, M.A., Yarosh, D.B. and Scudiero, D.A. (1987) *J. Cell. Sci.* Suppl. **6**, 333–353.
9. Tano, K., Shiota, S., Collier, J., Foote, R.S. and Mitra S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 686–690.
10. Rydberg, B., Spurr, N. and Karran, P. (1990) *J. Biol. Chem.* **265**, 9563–9570.
11. Hayakawa, H., Koike, G. and Sekiguchi, M. (1990) *J. Mol. Biol.* **213**, 739–747.
12. von Wronski, M.D., Shiota, S., Tano, K., Mitra, S., Bigner, D.D. and Brent, T.P. (1991) *J. Biol. Chem.* **266**, 1064–1070.
13. Ostrowski, L.E., von Wronski, M.A., Bigner, S.H., Rasheed, A., Schold Jr., S.C., Brent, T.P., Mitra, S. and Bigner, D.D. (1991) *Carcinogenesis* **12**, 1739–1744.
14. Pieper, R.O., Futsher, B.W., Dong, Q., Ellis, T.M. and Erickson, L.C. (1990) *Cancer Commun.* **2**, 13–20.
15. Fornace Jr., A.J., Papathanasiou, M.A., Hollander, M.C. and Yarosh, D.B. (1990) *Cancer Res.* **50**, 7908–7911.
16. Darnell, J.E. Jr. (1982) *Nature* **297**, 365–371.
17. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. Second Edition. Cold Spring Harbor University Press, Cold Spring Harbor.
18. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
19. Prost, E. and Moore, D.D. (1986) *Gene* **15**, 107–111.
20. Chen, C. and Okayama, H. (1987) *Mol. Cell. Biol.* **7**, 2745–2752.
21. Neumann, J.R., Morency, C.A. and Russian, K.O. (1987) *Biotechniques* **5**, 444–447.
22. Herbomel, P., Bourachot, B. and Yaniv, M. (1984) *Cell* **39**, 653–662.
23. Shimomaye, E. and Salvato, M. (1989) *Gene Anal. Techn.* **6**, 25–28.
24. Myers, T.W. and Gelfand, D.H. (1991) *Biochemistry* **30**, 7661–7666.
25. Patel, P.I., Framson, P.E., Caskey, C.T. and Chinault, A.C. (1986) *Mol. Cell. Biol.* **6**, 393–403.
26. Valerio, D., Dnynesteyn, M.G.C., Dekker, B.M.M., Weeda, G., Berkvens, T. M., van der Voorm, L., van Ormondt, H. and van der Eb, A.J. (1985) *EMBO J.* **4**, 437–443.
27. Breathnoch, R. and Chambon, P. (1981) *Ann. Rev. Biochem.* **50**, 349–383.
28. Mitchell, P.J. and Tjian, R. (1989) *Science* **245**, 371–378.
29. Dierks, P., van Ooyen, A., Cochran, M.D., Dobkin, C., Reiser, J. and Weissmann, C. (1983) *Cell* **32**, 695–706.
30. Byrne, B.J., Davis, M.S., Yamaguchi, J., Bergsma, D.J. and Subramanian, K.N. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 721–725.
31. McKnight, S. and Kingsbury, R. (1982) *Science* **217**, 316–324.
32. Barneva-Saldana, H., Takahashi, K., Vigneron, M., Wildeman, A., Davidson, L. and Chambon, P. (1985) *EMBO J.* **4**, 3839–3849.
33. Reynolds, G.A., Basu, S.K., Osborne, T.H., Chin, D.J., Gil, G., Brown, M.S., Goldstein, J.L. and Luskey, K.L. (1984) *Cell* **38**, 275–285.
34. Ishii, S., Xu, Y-H., Stratton, R.H., Roe, B.A., Merlino, G.T. and Pastan, I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4920–4924.
35. Lee, W., Mitchell, P. and Tjian, R. (1987) *Cell* **49**, 741–752.
36. Imangawa, M., Chin, R. and Kavin, M. (1987) *Cell* **51**, 251–260.
37. Gidoni, D., Dynan, W.S. and Tjian, R. (1984) *Nature* **312**, 409–413.
38. Jantzen, H-M., Strahle, U., Gloss, B., Stewart, F., Schmid, W., Boshast, M., Miksicek, R. and Schutz, G. (1987) *Cell* **49**, 29–38.
39. Lindquist, S. (1986) *Ann. Rev. Biochem.* **55**, 1151–1191.
40. Schirm, S., Jiricny, J. and Schaffner, W. (1987) *Genes and Development* **1**, 65–74.
41. Dynan, W.S. (1989) *Cell* **58**, 1–4.