An S1 nuclease-sensitive homopurine/homopyrimidine domain in the c-Ki-ras promoter interacts with a nuclear factor

(protooncogene/transcription/gene regulation)

ERIC K. HOFFMAN*†, STEPHEN P. TRUSKO*†, MAUREEN MURPHY*, AND DONNA L. GEORGE*†

*Department of Human Genetics and †Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104–6145

Communicated by Robert P. Perry, January 26, 1990 (received for review December 18, 1989)

ABSTRACT To gain insight into the normal controls mediating expression of the c-Ki-ras protooncogene, we have identified DNA sequence elements within its promoter that are essential for transcriptional activity. Transient expression assays using the bacterial chloramphenicol acetyltransferase gene were used initially to localize regions directing primary promoter function. Stepwise deletion of 5' promoter sequences resulted in a gradual decrease in the ability to drive transcription of the reporter gene, suggesting that this promoter is composed of multiple cis-acting elements. Gel mobility-shift and DNase protection studies involving a 166-base-pair DNA fragment allowed the identification of protein-binding sites corresponding to these multiple regulatory elements. One element demonstrating particular transcriptional influence exists as a homopurine/homopyrimidine-rich region that *in vitro* exhibits S1 nuclease sensitivity and binds at least one nuclear protein. Data from competition binding experiments suggest that this nuclear factor may be influential in the regulation of other essential growth-control genes as well.

The c-Ki-ras protooncogene is a member of the highly conserved *ras* gene family, whose protein products are believed to play a significant role in signal transduction and the regulation of cellular proliferation (1). This gene appears to be expressed constitutively at low levels in all cell types examined. However, modest but perhaps critical changes in mRNA levels have been reported to occur in association with certain normal processes of development, differentiation, and cell growth (2–5). Various mutagenic events, among them gene amplification (6) and retroviral integration (7, 8), can serve to effectively override this gene's normal transcriptional controls and have been implicated in the genesis of some tumors. Moreover, increased expression of an activated *ras* gene has been correlated with an augmentation of its transforming potential (9, 10). It might be expected that the expression of a gene with such a potentially pivotal role in growth-control pathways would be carefully regulated. Thus far, however, the regulatory elements that mediate transcription of *ras* genes in normal or neoplastic cells are poorly understood.

To begin to identify the DNA sequences that represent control elements for c-Ki-ras expression, we previously characterized the structure of the mouse gene and identified a 5' promoter region (11). The latter is G+C-rich, lacks an obvious TATA or CCAAT box, and contains multiple start sites for transcription. These features are common to an increasing number of characterized genes with "housekeeping" or growth-related functions in the cell. At present, the promoter requirements for transcriptional initiation and activation of housekeeping-type genes are less than well understood. Indeed, with the possible exception of Sp1, the

search for transcriptional mediators common to this set of genes has proven them thus far elusive. In the present study, we have utilized the mouse c-Ki-ras promoter as a prototypic promoter for the class of TATA-less housekeeping genes expressed ubiquitously at low levels. We have identified a homopurine/homopyrimidine-rich region whose deletion markedly reduces the transcriptional activity of remaining c-Ki-ras promoter sequences. This region exhibits nuclease sensitivity *in vitro* and acts as a binding site for at least one nuclear factor. Competition binding experiments have provided initial evidence that this nuclear factor may bind similar domains in the promoters of other housekeeping genes, such as those encoding the epidermal growth factor receptor (EGF-R) and the insulin receptor (I-R).

MATERIALS AND METHODS

Plasmid Constructions. Restriction fragments with various deletions of the c-Ki-ras promoter region were derived from the previously described construct pSX11 (11). Promoter deletion fragments were subcloned into the chloramphenicol acetyltransferase (CAT) vector pSVAOcat (11) by ligation at a blunt-ended *Hind*III site located 5' of the CAT gene. Plasmid DNAs were prepared by alkaline lysis and further purified by two rounds of CsCl density gradient centrifugation.

Transfections and CAT Assays. Plasmid DNAs were transfected into mouse NIH 3T3 cells by using the calcium phosphate coprecipitation method (12). For each construct assayed, at least three separate transfections were performed and each of these was done in triplicate. To control for possible variations in plasmid purity, each transfection experiment used independently prepared DNA preparations. CAT assays were performed essentially as described by Gorman *et al.* (13), with the following modifications. Cell extract (150 μ g of protein) was incubated with 4 mM acetyl CoA and 0.1 μ Ci (3.7 kBq) of [γ - 32 P]ATP. Probe DNA (0.5–1.0 ng) was incubated with 10 μ g of HeLa whole cell extract (BRL) and 1 μ g of poly(dI-dC)·poly(dI-dC) (Pharmacia) for 15 min at 23°C in 20 mM Tris, pH 8.0/100 mM KCl/1.5 mM MgCl₂/1 mM dithiothreitol/8% (vol/vol) glycerol. Samples were fractionated in 4% polyacrylamide gels at 150 V for 1.5

Gel Mobility-Shift Assays. Probes to be used in the mobility-shift assays (14) were prepared by end-labeling with phage T4 polynucleotide kinase and [γ - 32 P]ATP. Probe DNA (0.5–1.0 ng) was incubated with 10 μ g of HeLa whole cell extract (BRL) and 1 μ g of poly(dI-dC)·poly(dI-dC) (Pharmacia) for 15 min at 23°C in 20 mM Tris, pH 8.0/100 mM KCl/1.5 mM MgCl₂/1 mM dithiothreitol/8% (vol/vol) glycerol. Samples were fractionated in 4% polyacrylamide gels at 150 V for 1.5

hr in a buffer consisting of 90 mM Tris base (pH 8.5), 90 mM boric acid, and 2 mM EDTA. Following electrophoresis, gels were transferred to Whatman 3MM paper, dried, and exposed for autoradiography. Oligonucleotide competitor DNAs used in certain gel shift assays were pGEM-3 (nucleotides 7–37) and a 22-mer containing an Sp1 consensus binding site (5'-GATCGATCGGGGCGGGGCGATC-3') (15).

DNase I Protection Assays. These were performed by a modification of the procedure of Dynan and Tjian (16). Probes were prepared by 5' end-labeling with [γ - 32 P]ATP and T4 polynucleotide kinase followed by digestion with a second restriction enzyme and purification of the appropriate end-labeled fragment. Binding reactions were performed as described above. The reaction buffer was adjusted to 5 mM CaCl₂ and 10 mM MgCl₂, and 1 μ l of DNase I (100–200 μ g/ml) (Pharmacia) was added to each reaction mixture. Digestion of DNA–protein complexes was allowed to proceed for 60 sec at 23°C. Samples were loaded onto preparative polyacrylamide gels and electrophoresed as described for mobility-shift assays. DNA–protein complexes were localized by autoradiography, excised from the gel, and purified by electroelution, whereupon both complexes were pooled and analyzed in a denaturing 10% polyacrylamide gel.

RESULTS

We previously reported that the mouse *c-Ki-ras* promoter region is contained within a 1.1-kilobase (kb) *Xba*I–*Sma*I restriction fragment (11). This DNA fragment encompasses a 5' untranslated exon (exon 0), sequences upstream of this exon, and 345 base pairs (bp) of the first intron. To further delineate the location of cis-acting regulatory elements, we used the bacterial CAT gene as a reporter in transient transfection assays. For the studies reported here, linearly deleted segments of a 380-bp *Mst*II–*Aha*II restriction fragment (construct pKRS-413) were fused to the promoterless CAT gene in the vector pSVAOcat, and the resulting constructs were assayed for relative promoter strength.

Construct pKRS-413 (Fig. 1A) was derived from the 1.1-kb *Xba*I–*Sma*I fragment. It contains 380 bp of 5' flanking DNA (positions –413 to –33, where –1 represents the 3' boundary of exon 0), including the *c-Ki-ras* transcriptional start sites and most of exon 0, but lacks the intervening sequences as well as 400 bp of further 5' flanking material that is present in the larger fragment. This construct was able to drive CAT transcription at a level comparable to that displayed by the 1.1-kb fragment (data not shown), indicating that it contains the principal regulatory promoter elements detected in our previous work. Primer extension studies were performed to confirm that the CAT activities measured in these assays were derived from transcripts that initiated at sites solely within the *c-Ki-ras* promoter region. Primer extension of poly(A)⁺ RNA isolated from cells transfected with pKRS-413 produced multiple products whose 5' termini mapped solely within the region shown (11) to contain the multiple transcription initiation sites for the *c-Ki-ras* gene (data not shown).

Fig. 1A shows the structure of the deletion fragments derived from the construct pKRS-413, as well as the percent relative CAT activity exhibited by these fragments. The endpoints of the deletion fragments are defined by negative numbers, with –1 representing the 3' boundary of the untranslated exon 0. Progressive removal of 5' sequences from the 380-bp promoter fragment resulted in a stepwise decrease in CAT activities, indicative of the involvement of several cis-acting factors. Deletion of the 5'-most 166 nucleotides (–413 to –247) significantly reduced CAT activity, suggesting that this region is necessary for optimal promoter function.

FIG. 1. Deletion analysis of the mouse *c-Ki-ras* promoter region. (A) Organization of the mouse *c-Ki-ras* promoter and the 5' deletion fragments analyzed in CAT assay reactions. Top line represents the *c-Ki-ras* promoter region. The exon 0 donor splice site is marked by an arrow and the transcription initiation sites are represented by dots. The 3'-most nucleotide of exon 0 is designated as –1, and upstream sequences are assigned consecutive negative numbers. Restriction sites: Ah, *Aha* II; S, *Sma* I; P, *Pst* I; Sa, *Sau*3A; A, *Apa* I; B, *Bgl* I; M, *Mst* II. Illustrated below the map are the various deletion fragments assayed for promoter activity. The CAT activity exhibited by the –413 to –33 (construct pKRS-413) fragment has been arbitrarily assigned a value of 100%; activities of the deletion constructs are expressed relative to this activity, with standard deviations shown in parentheses. (B) Representative thin-layer chromatograph of [14 C]chloramphenicol (CM) and its acetylated products (3AC and 1AC) produced in CAT assay reactions. Lane pSVAN shows the CAT activity present in NIH 3T3 cell extracts following transfection with a promoterless CAT vector, pSVAOcat. Remaining lanes illustrate the CAT activities generated by constructs with various 5' deletions in the *c-Ki-ras* promoter region. Numbers below each lane indicate 5' deletion endpoints for each construct as defined in A.

The 166-bp regulatory domain (–413 to –247) contains a 29-bp segment (nucleotides –318 through –290) with an unusual sequence composition (see Fig. 6). Referred to as a homopurine/homopyrimidine (pur/pyr) motif, this region is composed of one strand containing primarily pyrimidine residues and a complementary purine-rich strand. Additionally, situated within the *c-Ki-ras* motif is a pair of 12-bp mirror repeat sequences (5'-TCCCTCCCTCCC-3'). Because we and others (17–20) have noted similar pur/pyr motifs in several other promoters, we chose to specifically focus on this element in a deletion construct. Removal of the pur/pyr motif from pKRS-413 to generate construct pKRSΔ –322/–288 resulted in a marked decrease in promoter activity (Fig. 1A). (This deletion would encompass three complete turns of a B-form DNA helix, thereby conserving periodicity.) Although the absolute promoter strength of this deletion construct cannot be determined from these studies, data obtained in the CAT assays are consistent with the idea that the pur/pyr motif constitutes a functionally significant element within the promoter.

Binding of Protein Factors to the *c-Ki-ras* Promoter Region. To determine whether the 166-bp promoter region (–413 to

–247) contains recognition sites for DNA-binding proteins, this segment of DNA was used as a probe in gel mobility-shift assays. When radiolabeled 166-bp double-stranded DNA was incubated with HeLa whole-cell extract, two discrete DNA–protein complexes (B1 and B2) were resolved (Fig. 2). The formation of both complexes was inhibited when increasing amounts of unlabeled 166-bp DNA were included in the binding reactions. The addition of nonspecific competitor DNA (pGEM) had no effect on complex formation. These results indicate that the complexes observed are due to the binding of one or more proteins to specific sequences present within the 166-bp promoter fragment.

DNase I protection assays were performed to further characterize the sequences involved in protein binding. The 166-bp promoter fragment was 5′-end-labeled on either the coding or the noncoding strand, combined with HeLa whole-cell or nuclear extract, and subjected to partial DNase I digestion. The initial experiments carried out on the 166-bp fragment demonstrated that the two DNA–protein complexes (B1 and B2) generated an identical DNase I protection pattern, whether assayed individually or as a pooled sample. The results presented in Fig. 3 were obtained using a sample of the pooled material. Two protected regions (sites 1 and 2) were detected on both strands (Fig. 3 A and B). Site 1 encompasses nucleotides –288 to –311 on the coding strand and nucleotides –305 to –339 on the noncoding strand. Site 2 extends from –348 to –365 on the coding strand and from –346 to –378 on the noncoding strand. Each protected region falls within one or more of the deleted domains found to contribute to promoter activity in transient expression assays.

Site 2 contains a stretch of nucleotides (AGGGCGGGAA) that shows a high degree of similarity to the Sp1 consensus sequence (15, 21), raising the possibility that this protein is involved in protecting at least part of site 2 from nuclease digestion. Protection of these sequences (–346 to –358) was, in fact, detected when DNase I protection assays were carried out in the presence of partially purified Sp1 (data not shown). Other protected sequences within this region do not exhibit significant homology to previously characterized protein-binding elements. Interestingly, the protected sequences within site 1 on the coding strand encompass 21 of 29 bp of the pur/pyr motif described above.

S1 Nuclease Sensitivity in the c-Ki-ras Promoter Region. Sensitivity to S1 nuclease is associated with functionally important regions of several eukaryotic promoters (18, 20, 22–24). Additionally, S1 sensitivity has been shown to be a

FIG. 2. Gel mobility-shift analysis of nuclear proteins binding to the 166-bp c-Ki-ras promoter fragment. The 166-bp promoter fragment (–413 to –247) was end-labeled with ^{32}P and incubated with 10 μg of HeLa whole-cell extract in the presence of 1 μg of poly(dI-dC) as nonspecific competitor DNA. Included in the reaction mixtures were various amounts (molar excess) of specific (166 bp) or nonspecific (pGEM-3 nucleotides 1653–1772) competitor DNAs. DNA–protein complexes are labeled B1 or B2, and unbound probe is labeled F.

FIG. 3. DNase I protection studies of the c-Ki-ras promoter region. The 166-bp promoter fragment (–413 to –247) was end-labeled on either strand and used as a probe in DNase I protection studies. DNA labeled on the coding strand (A) or the noncoding strand (B) was incubated with HeLa whole-cell extract and partially digested with DNase I. Boxes represent sequences that are protected from DNase I digestion. Arrowheads mark DNase I-hypersensitive sites induced by protein binding. Lanes: M, products of Maxam and Gilbert G+A sequencing reaction of the 166-bp fragment; F, free probe DNA; B, bound DNA.

property of pur/pyr elements assayed *in vitro* (for a recent review see ref. 19). Such nuclease sensitivity may be attributed to the ability of involved sequence elements to adopt unorthodox DNA conformations, such as triple-helical H-DNA or slipped helices. We performed S1 nuclease digestion studies on supercoiled plasmid DNAs containing c-Ki-ras promoter sequences. The 166-bp restriction fragment (–413 to –247) utilized in the gel mobility-shift and DNase I protection assays was subcloned into pGEM-3 (construct pK166) for use as a substrate. The restriction endonuclease *Xmn* I linearizes pK166 by cleavage once within vector sequences. When supercoiled plasmid DNA was digested first with S1 nuclease and subsequently by the restriction enzyme *Xmn* I, two DNA fragments, 1790 and 1210 bp in size, were detected (Fig. 4A, lane 4). These fragments were not seen when the DNA supercoiling was removed by linearizing the plasmid with *Xmn* I prior to exposure to S1 nuclease (lane 2) or when the DNA was digested with *Xmn* I in the absence of S1 nuclease treatment (Fig. 4A, lane 3). Moreover, these DNA fragments were not detected when control plasmid DNA, lacking the 166-bp promoter region, was digested in the same manner (data not shown). These results indicate that the 166-bp promoter fragment does indeed exhibit S1 nuclease sensitivity. Additional experiments were conducted to determine specifically which sequences within this region are susceptible to S1 nuclease cleavage.

The 1790-bp and 1210-bp fragments, generated by sequential digestion of pK166 with S1 nuclease and *Xmn* I, were excised from a low-melting-point agarose gel, purified, and end-labeled with ^{32}P . The labeled fragments were then digested with a restriction enzyme (*Eco*RI or *Bam*HI) that cleaves once within polylinker sequences adjacent to the subcloning site, so that the distance to the S1-sensitive site relative to these respective polylinker sites could be measured (Fig. 4B). The digests were fractionated in a nondenaturing 12% acrylamide gel and exposed for autoradiography (Fig. 4A, lanes 5–7). *Eco*RI digestion of the 1210-bp *Xmn* I/S1 fragment generated a population of small DNA fragments ranging in size from about 116 bp to 132 bp. The 3′ termini of

FIG. 4. Mapping of an S1 nuclease-sensitive site in the c-Ki-ras promoter region. (A) Lanes 1–4 show an ethidium bromide-stained 1% agarose gel. Lane 1, *Hind*III-digested λ , *Hae* III-digested ϕ X marker DNA; lane 2, pK166 DNA sequentially digested, first with *Xmn* I and then with S1; lane 3, *Xmn* I-digested pK166 DNA; lane 4, pK166 DNA sequentially digested, first with S1 nuclease and then *Xmn* I. Visible in lane 4 are two fragments, 1790 bp and 1210 bp in size, as well as residual linear plasmid DNA. The 1790-bp and 1210-bp fragments were excised, purified, and 32 P-end-labeled for further analysis. Lanes 5–7 show an autoradiographic exposure of end-labeled DNA fragments fractionated in a 12% acrylamide gel. Lane 5, *Eco*RI-digested 1210-bp fragment; lane 6, *Bam*HI-digested 1790-bp fragment; lane 7, 32 P-end-labeled *Msp* I-digested pBR322 marker DNA. (B) Physical maps of pK166 and digestion products. The top map illustrates the structure of intact pK166. The thin line represents vector sequences and the open rectangle represents the cloned 166-bp c-Ki-ras promoter fragment (nucleotides –413 to –247). The middle map defines the structure of the 1790-bp and 1210-bp fragments generated by sequential digestion of pK166 DNA with S1 nuclease and *Xmn* I. The stippled box approximates the location of the S1 nuclease-sensitive region (positions –311 to –288) and corresponds with the location of the pur/pyr motif. The lower map further defines the endpoints of the population of small DNA fragments generated by *Eco*RI digestion of the 1210-bp fragment or *Bam*HI digestion of the 1790-bp fragment. X, *Xmn* I; E, *Eco*RI; B, *Bam*HI.

these fragments, generated by S1 cleavage, are located between nucleotides –311 and –295. Digestion of the 1790-bp *Xmn* I/S1 fragment with *Bam*HI generated subfragments ranging in size from about 45 bp to 57 bp. The 5' ends of these molecules map between nucleotides –301 and –288. Taken together, these data indicate that an S1-sensitive region of $\approx$ 23 bp is situated between nucleotides –311 and –288, directly coinciding with the position of the pur/pyr motif (Fig. 4B and Fig. 6). This result, coupled with the CAT assays and DNase I protection data outlined above, highlights the functionality of this element in transcriptional regulation.

Binding of Protein Factors to Double-Stranded Oligonucleotides Representing the Pur/Py Motif. As noted previously, the pur/pyr motif represents a sequence element shared to varying degrees by the promoter regions of a number of genes, in particular certain growth-control genes that possess G+C-rich promoters. These include the EGF-R (18) and I-R (25, 26) genes. To determine whether these sequence similarities reflect functional similarities, mobility-shift assays were performed using oligonucleotides representing pur/pyr motifs in these genes. Oligonucleotide D30 is composed of sequences that encompass the entire c-Ki-ras pur/pyr domain (–318 to –290). In gel shift assays with oligonucleotide D30 as a probe, three discrete complexes were detected; two of these (B1 and B3 in Fig. 5A) involve sequence-specific binding. Competitor oligonucleotides

FIG. 5. Gel mobility-shift analysis of DNA-protein interactions associated with the c-Ki-ras pur/pyr motif. (A) Competition binding studies with the D30 oligonucleotide probe. The 32 P-end-labeled D30 oligonucleotide probe was incubated with 10 μ g of nuclear extract in the absence of competitor DNA (lane 1) or in the presence of a 50-fold molar excess of unlabeled D30 (lane 2), EGF-R pur/pyr motif 31-mer (lane 3), I-R pur/pyr motif 35-mer (lane 4), or nonspecific pGEM 30-mer (lane 5). (B) Nucleotide sequences of the double-stranded oligonucleotides used in the mobility-shift assays. Vertical lines between the sequences designate conserved base pairs common to all three oligonucleotides.

comprising unrelated DNA sequences, including a pGEM-3 30-mer (Fig. 5A, lane 5) and an Sp1 consensus binding site (data not shown), failed to compete with D30 for binding. Competition studies were then extended to include oligonucleotides representative of similar pur/pyr motifs located in the 5' regions of the EGF-R and I-R genes; both of these regions were previously shown to be influential in the promoter activity of these genes (18, 25, 26). The sequences of these oligonucleotides are compared in Fig. 5B. As evident in Fig. 5A, each of these two competitor oligonucleotides effectively inhibited the formation of complex B1 and reduced to varying degrees the formation of complex B3. These results indicate that one or more protein factors can bind to the c-Ki-ras pur/pyr domain and at least one of these can also interact with similar domains found in the 5' flanking regions of the EGF-R and I-R genes.

DISCUSSION

In the present report we have extended our previous studies (11) to analyze in greater detail the sequence elements involved in the expression of the c-Ki-ras gene and the DNA-protein interactions associated with these elements (Fig. 6). We report that a 380-bp DNA fragment of the 5' region of the mouse c-Ki-ras gene that includes the major transcription start sites encompasses the principal promoter elements of this gene. Mobility-shift and DNase protection assays demonstrated that a 166-bp subfragment binds several nuclear proteins *in vitro*, one of which is likely to be Sp1. This region also binds one or more as yet uncharacterized nuclear factors. Finally, we report the existence of a region $\approx$ 100 bp upstream of the primary *ras* mRNA start sites that contains a pur/pyr domain and exhibits both S1 nuclease sensitivity and DNase I hypersensitivity *in vitro*. Deletion of this pur/

FIG. 6. Summary of DNA-protein interactions associated with the 166-bp c-Ki-ras promoter region. The nucleotide sequence of both strands of the 166-bp promoter fragment is shown, summarizing the DNA-protein interactions observed in this study. Sequences protected from DNase I digestion are indicated with brackets. Protected regions on the coding (upper) strand are marked by brackets above the sequence, while protected regions on the noncoding (lower) strand are designated by brackets below the sequence. Bold underline marks the region found to be sensitive to S1 nuclease.

pyr motif results in a markedly reduced ability of remaining promoter sequences to drive CAT transcription. Additionally, this motif specifically binds one or more nuclear factors. Taken together, these results argue strongly that this pur/pyr motif represents a critical element regulating the transcription of the c-Ki-ras protooncogene.

This study is not the first to ascribe a biologically relevant role to pur/pyr repeats. Studies on the human EGF-R promoter demonstrated the existence of an S1 nuclease-sensitive site associated with a pur/pyr motif (18). DNase protection studies suggested that at least two proteins, Sp1 and another referred to as TCF, are able to bind this region, specifically to the loose consensus TCCTCCTCC. Our data, however, indicate that Sp1 does not seem to be involved in the DNase I protection observed over the pur/pyr motif of the c-Ki-ras promoter. Additionally, Postel *et al.* (20) showed that a pur/pyr motif plays a functional role in the transcriptional activity of P_2 , the principal c-myc promoter. This group partially purified the DNA-binding protein PuF, which binds the sequence GGGAGGG contained within the pur/pyr region and enhances the activity of P_2 when assayed in an *in vitro* reconstituted transcription reaction. Hay *et al.* (17) similarly found that a separate pur/pyr motif in the c-myc protooncogene, ≈ 100 bp upstream from the P_1 promoter, acts as a positive effector on the transcriptional activity of P_1 . Pur/pyr motifs thus constitute functionally significant regulatory elements in the promoters of housekeeping genes such as c-Ki-ras and the EGF-R gene, as well as in the more classical TATA-containing c-myc promoter.

A particularly striking result is our finding that certain pur/pyr motifs present in the EGF-R and I-R gene promoters effectively compete with the c-Ki-ras motif for specific binding of a nuclear factor. This fact thus functionally establishes the relatedness of these transcriptionally influential motifs and should serve as a step toward unifying ideas regarding the regulation of expression of this class of genes as a whole. Interestingly, each of the pur/pyr motifs in these three genes is located 80–100 bp upstream of the major transcription start sites, implying the existence of a spatial component to their function. It is further intriguing to speculate that the potential of pur/pyr regions to deviate from a normal double-helical conformation has implications for the expression of the genes in which they are contained; such conformations may allow for the maintenance of these promoters in a structure poised for transcription, thereby potentiating basal levels of constitutive expression.

Though compelling, our initial result requires further investigation before the identity of the nuclear factors binding these pur/pyr motifs as either identical or crossbinding members of a family of such proteins can be established. Toward that goal, we have screened a cDNA expression library and have isolated

several promising candidates for genes encoding proteins that bind specifically to the c-Ki-ras pur/pyr motif.

We thank Mark Pykett for experimental assistance, Alan Kiniburgh for helpful discussions, and Susan Kelchner for help in preparation of the manuscript. We are grateful to Steve Jackson and Robert Tjian for providing partially purified Sp1. M.M. is supported by a Public Health Service predoctoral training grant (5-732-GMO7229) from the National Institutes of Health.

1. Barbacid, M. (1987) *Annu. Rev. Biochem.* **56**, 779–827.
2. Müller, R., Slamon, D. J., Adamson, E. D., Tremblay, J. M., Müller, D., Cline, M. J. & Verma, I. M. (1983) *Mol. Cell. Biol.* **3**, 1062–1069.
3. Campisi, J., Gray, H. E., Pardee, A. B., Dean, M. & Sonenshein, G. E. (1984) *Cell* **36**, 241–247.
4. Goyette, M., Petropoulos, C. J., Shank, P. R. & Fausto, N. (1984) *Mol. Cell. Biol.* **4**, 1493–1498.
5. Leon, J., Guerrero, I. & Pellicer, A. (1987) *Mol. Cell. Biol.* **7**, 1535–1540.
6. Schwab, M., Alitalo, K., Varmus, H. E., Bishop, J. M. & George, D. (1983) *Nature (London)* **303**, 497–501.
7. George, D. L., Glick, B., Trusko, S. & Freeman, N. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1651–1655.
8. Trusko, S. P., Hoffman, E. K. & George, D. L. (1989) *Nucleic Acids Res.* **17**, 9259–9265.
9. Pulciani, S., Santos, E., Long, L. K., Sorrentino, V. & Barbacid, M. (1985) *Mol. Cell. Biol.* **5**, 2836–2841.
10. Winter, E. & Perucho, M. (1986) *Mol. Cell. Biol.* **6**, 2562–2570.
11. Hoffman, E. K., Trusko, S. P., Freeman, N. & George, D. L. (1987) *Mol. Cell. Biol.* **7**, 2592–2596.
12. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52**, 456–467.
13. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
14. Fried, M. & Crothers, D. M. (1981) *Nucleic Acids Res.* **9**, 6505–6523.
15. Kadonaga, J. T. & Tjian, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5889–5893.
16. Dynan, W. S. & Tjian, R. (1983) *Cell* **35**, 79–87.
17. Hay, N., Bishop, J. M. & Levans, D. (1987) *Genes Dev.* **1**, 659–671.
18. Johnson, A. C., Jinno, Y. & Merlino, G. T. (1988) *Mol. Cell. Biol.* **8**, 4174–4184.
19. Wells, R. D., Collier, D. A., Hanvey, J. C., Shimizu, M. & Wohlrab, F. (1988) *FASEB J.* **2**, 2939–2949.
20. Postel, E. H., Mango, S. E. & Flint, S. J. (1989) *Mol. Cell. Biol.* **9**, 5123–5133.
21. Briggs, M. R., Kadonaga, J. T., Bell, S. P. & Tjian, R. (1986) *Science* **234**, 47–52.
22. Larsen, A. & Weintraub, H. (1982) *Cell* **29**, 609–622.
23. Nickol, J. M. & Felsenfeld, G. (1983) *Cell* **35**, 467–477.
24. Schon, E., Evans, T., Welsch, J. & Efstratiadis, A. (1983) *Cell* **35**, 837–848.
25. Seino, S., Seino, M., Nishi, S. & Bell, G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 114–118.
26. Tewari, D. S., Cook, D. M. & Taub, R. (1989) *J. Biol. Chem.* **264**, 16238–16245.