Table 2. Molecular constants (cm^{-1}) and effective bond distances (Å) for C_5. For the first column the uncertainties in the last digit or digits are 2 SD (SD of the fit was 0.0036 cm^{-1}).

Parameter	This work	Ab initio
ν_3	2169.4404 (18)	2344
B''	0.085305 (31)	0.0866
B'	0.084900 (32)	
r_0*	1.2833 (5)	1.271, 1.275† 1.277, 1.280‡

*Ground state–effective C–C bond length, compared with ab initio equilibrium values. †From (*12*). ‡From (7).

and surmise that the average structure does not become dramatically more bent in the $v = 1$ upper state.

During the course of this project we learned of the detection of C_5 in the carbon star IRC+10216 by Bernath and co-workers (*24*). This discovery highlights the importance of carbon cluster chemistry in astrophysical contexts. The C_5 molecule is a well-known product of carbon condensation and is even observed in low-pressure benzene-O_2 and C_2H_2-O_2 flames under sooting conditions (*3*). In addition, the well-known interstellar molecules, the polycyanoacetylenes (HC_nN; n = 3, 5, 7, 9, and 11), are easily produced in a nitrogen- and hydrogen-rich condensing carbon environment (*2, 22*). It is possible that the bare carbon clusters, as well as the cyanoacetylenes, all originate from a similar chemical environment. A short-term goal of such work is to study other carbon clusters (C_4, C_6, C_7, and so forth) that are likely to exist in space and play an important role in interstellar chemistry. Our experiment is quite general and should certainly be applicable to these other carbon clusters. For both future laboratory and astronomical studies, reliable estimates are needed of the absolute absorption strength for the ν_3 band of C_5 as well as for allowed transitions in other carbon clusters. Such results would allow molecular densities to be extracted from the observations. Such work should also stimulate quantum chemists to begin high-level calculations for the small carbon clusters.

Note added in proof: We have received a preprint by A. R. W. McKellar *et al.* (*25*) reporting the observation of these same spectra in a discharge.

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AP1/*jun* Function Is Differentially Induced in Promotion-Sensitive and Resistant JB6 Cells

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Tumor promoters may bring about events that lead to neoplastic transformation by inducing specific promotion-relevant effector genes. Functional activation of the transacting transcription factor AP-1 by the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) may play an essential role in this process. Clonal genetic variants of mouse epidermal JB6 cells that are genetically susceptible (P^+) or resistant (P^-) to promotion of transformation by TPA were transfected with 3XTRE-CAT, a construct that has AP-1 cis-enhancer sequences attached to a reporter gene encoding chloramphenicol acetyltransferase (CAT). Transfected JB6 P^+, but not P^- variants, showed TPA-inducible CAT synthesis. Epidermal growth factor, another transformation promoter in JB6 cells, also caused P^+ specific induction of CAT gene expression. These results demonstrate an association between induced AP-1 function and sensitivity to promotion of neoplastic transformation.

BY MEANS OF GENETICALLY BRED carcinogenesis-sensitive or resistant strains of mice, it was demonstrated that susceptibility to promotion of neoplastic transformation in vivo is inheritable and genetically controlled (*1*). Colburn *et al.* subsequently developed the JB6 mouse epidermal cell system of clonal genetic variants of promotion-sensitive (P^+) and promotion-resistant (P^-) cells that permitted the study of genetic susceptibility to transformation promotion at the molecular level (*2*). In P^+ JB6 cells TPA and EGF induce the formation of large, tumorigenic, anchorage-independent colonies in soft agar at a high frequency (10 to 40% of the cell population). In contrast, the P^- cells exhibit a response to the tumor-promoting agents that is 0.1 to 1% that of P^+ cells, and the colonies are much smaller (*3*). From the promotion-sensitive variants, two cloned sequences, designated *pro*-1 and *pro*-2, were isolated, either of which confers promotion-sensitivity when transfected into the P^- cells (*4*).

The expression of genes transcriptionally induced by TPA is among the events thought to be required to implement the process of tumor promotion. The list of such inducible genes has grown to include at least 30 (*5, 6*), among them, proto-oncogenes [such as c-*myc* and c-*fos* (*5*)] and genes encoding proteases, including collagenase and plasminogen activator (*5*). All of these genes are observed at elevated levels of expression in the genesis or maintenance of neoplasia (*6*). The AP-1 protein is a transacting transcription factor that controls expression of some of these genes in cells treated with TPA (*7, 8*). The AP-1 transcript is itself induced by TPA (*9*), epidermal growth factor (*10*), and serum (*9, 11*) and is encoded by a recently discovered proto-

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oncogene designated as c-*jun* (*12*). If AP-1 controls a set of effector genes required for tumor promoter–induced transformation and if events required for promotion are ultimately controlled by activated *pro* genes, then P^+ and P^- cells would be expected to display differential induction of AP-1–dependent genes in response to tumor promoters such as TPA.

To test this prediction, mouse JB6 P^+ and P^- variants were treated with tumor promoters after transient transfection with 3XTRE-CAT, a construct that contains three tandem TPA-responsive cis-enhancer elements attached to the herpes simplex virus–thymidine kinase (HSV-TK) promoter and a CAT reporter gene (*7*). Induced CAT gene expression in this system depends on tumor promoter–mediated activation of cellular AP-1 activity (*13*). Initial assays for CAT activity of transient transfectants indicated substantial differences in basal CAT activity (fivefold) between the transfected P^+ and P^- variants. We therefore determined whether this was caused by differences in transfection efficiency. For each cell line, DNA uptake was quantitated as a function of plasmid dose. Dosages could then be adjusted so that DNA uptake was equalized, thus permitting direct comparison of the P^+ and P^- variants. To equalize plasmid uptake, each cell line was transfected (*4*) with several dosages of pBLCAT2, a construct identical to 3XTRE-CAT, but without the AP-1 enhancer sequences. Plasmid uptake was then quantitated by CAT hybridization as described in Table 1. The ratio of transfection efficiencies for P^+ Cl 41 and P^- Cl 30 cells transfected with 1.5 μg of DNA is 5:1. At this dosage, P^+ Cl 41 incorporates a mean of 810 copies per cell, and P^- Cl 30 incorporates a mean of 160 copies per cell, at 20 hours after transfection. These values are within the range of those measured for plasmid copy number per cell in previous transient transfection experiments (*14*).

Independent corroboration of this hybridization analysis was achieved by showing that when DNA uptake was equalized, expression of the constitutively expressed plasmid pRSVCAT was also equalized. pRSVCAT (*15*), which is driven by the Rous sarcoma virus long terminal repeat (RSV-LTR), displays a linear dose-response curve for CAT activity in P^+ Cl 41 cells (Fig. 1A). In P^- Cl 30 cells, 9 μg of transfected pRSVCAT is required to generate the same level of CAT activity as 1.5 μg of transfected pRSVCAT in Cl 41 cells (Fig. 1B). These DNA dosages are within the linear range for plasmid uptake as a function of added DNA dosage (*16*). This 6:1 ratio in transfection efficiencies for Cl 41 and Cl 30 shows agreement with the 5:1 ratio measured for dot blot hybridizations. This determination of relative transfection efficiencies thus provided information sufficient to adjust dosages in order to equalize DNA uptake between the P^+ and P^- cell lines.

To verify in the JB6 system that specificity for TPA-inducible expression resided within the AP-1 cis element, we compared expression in cells transfected with plasmids containing or lacking the AP-1 cis element. pRSVCAT has no AP-1 binding sites but displays a high constitutive level of activity over an extended time course (250 to 300 units of CAT enzyme activity). Its activity is uninducible by TPA (Fig. 2), and is nearly equal for Cl 30 and Cl 41 cells taking up equalized quantities of plasmid. The pBLCAT2 plasmid (*7*) is identical to 3XTRE-CAT, but lacks an AP-1 binding cis element. Its expression was uninducible in Cl 41 cells by TPA; both for P^- Cl 30 and P^+ Cl 41 over a 24-hour time course it was uninducible and virtually constant (at a level only 7.5 to 10% that of 3XTRE-CAT basal expression). This finding verifies that, in the JB6 system, specificity for TPA-inducible CAT expression resides within the AP-1 cis-enhancer element (*17*).

Once the P^+ and P^- cells were equalized for plasmid uptake, we directly compared the basal and the induced levels of CAT expression. Within 3.5 hours after starting TPA treatment (but not by 1.5 hours), P^+

Table 1. Determination of CAT plasmid uptake in P^+ and P^- JB6 cell lines by hybridization analysis of transient transfectants. Cells were plated at 1×10^5 per 60-mm dish and incubated in 5% fetal calf serum overnight. Cells were transfected (*4*) with plasmid pBLCAT2, incubated for 4 hours, switched to 2% serum, and incubated for an additional 16 hours. After harvesting and cell counting (six times), Hirt supernatants were generated (*29*), and isolated DNA (*7*) was blotted onto nitrocellulose (*30*). Under conditions of high stringency, blots were hybridized with a ^{32}P-labeled CAT probe, a 553-bp Hind III–Nco I fragment from plasmid pSV2CAT (*15*). Blot hybridization was quantitated by liquid scintillation counting of excised spots. Copies of pBLCAT2 taken up by P^+ Cl 41 and P^- Cl 30 cells transfected with 1.5 μg of plasmid are shown in the table below. Radioactivity hybridized to blotted Hirt supernatants ranged from 200 to 1000 cpm for the indicated number of cells. Within each experiment, we included a set of standards containing known quantities of plasmid ranging from 10 pg to 10 ng, and another at the same dosages premixed with 4×10^4 cells and subjected to Hirt extraction, both of which gave linear standard curves. The ratio of these standards enabled us to internally control for fractional yield of plasmid, which averaged 50% of theoretical yield. The calculated values for total picograms of transfected plasmid took this fractional yield into account. The copies per cell at 20 hours were computed as the calculated value for picograms of pBLCAT2 per cell × (1 copy/5.5×10^{-6} pg).

Cell line	Cell no. ($\times 10^{-4}$)	Total picograms of pBLCAT2	Picograms of pBLCAT2 per cell	CAT copies per cell at 20 hours
P^+ Cl 41	7.7	347	4.5×10^{-3}	810
P^- Cl 30	5.0	46	9.1×10^{-4}	160

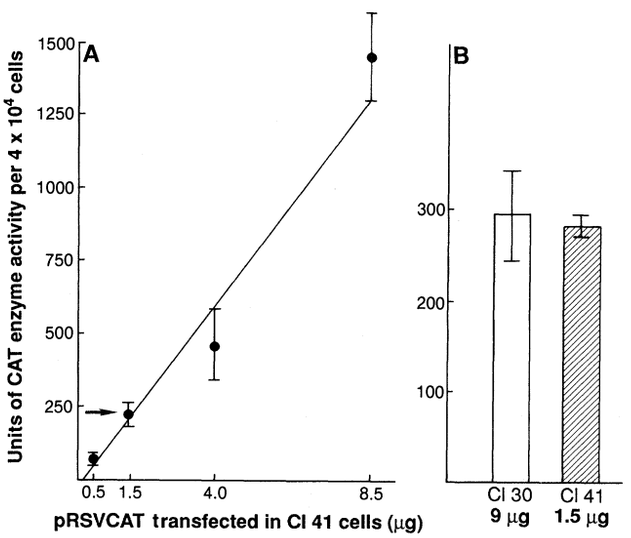

Fig. 1. Equalization of plasmid uptake in P^+ and P^- cells equalizes pRSVCAT activity. P^+ Cl 41 cells were transfected with 0.5, 1.5, or 8.5 μg of plasmid pRSVCAT; P^- Cl 30 cells were transfected with 9 μg. Cells were incubated and harvested and then assayed for CAT activity. The line and bar graphs indicate an equivalence point at which the units of CAT activity for 9 μg of plasmid in Cl 30 are equal to the activity from 1.5 μg transfected into Cl 41. Graphs represent the mean of two independent transfections, whose individual values are indicated by the range bars. CAT assay methods: Cell extracts were prepared (*15*) and activity was measured by the new rapid CAT assay developed by Du Pont, Biotechnology Systems (*31*), with some modifications. Sonicated cell extracts (4×10^4 cell equivalents) incubated with substrates gave significant levels of activity within the linear range of the reaction after 4 hours at 37°C. Added substrates were 0.1 m*M* [^{14}C]acetyl coenzyme A [0.1 μCi per reaction mix; 50 mCi/mmol (*31*)], and 1 m*M* chloramphenicol. Reaction mixtures were extracted and partitioned with ethyl acetate. The upper phase, containing only incorporated label, was counted directly by liquid scintillation counting without further manipulations.

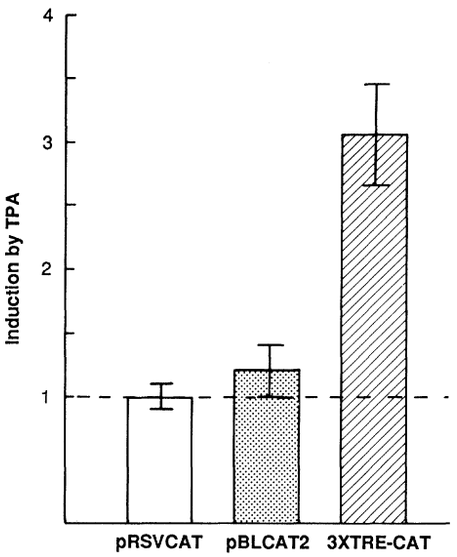

Fig. 2. Inducible expression of CAT by TPA specifically requires a consensus AP-1 cis-enhancer element. P^+ Cl 41 cells were transfected with 1.5 μg of pRSVCAT, pBLCAT2, or the AP-1–dependent 3XTRE-CAT. Cells were transfected and switched to 2% serum 4 hours later. After 16 hours, they were treated with 10 ng of TPA per milliliter (16 n*M*) (*3*) in 0.1% DMSO solvent or with DMSO alone in fresh 2% serum for 24 hours and assayed for CAT activity. We chose 2% serum since maximum induction was observable at that serum concentration. Induction represents multiples of the basal value (fold-induction). Results are expressed as the mean of two independent transfection experiments plus or minus half the range.

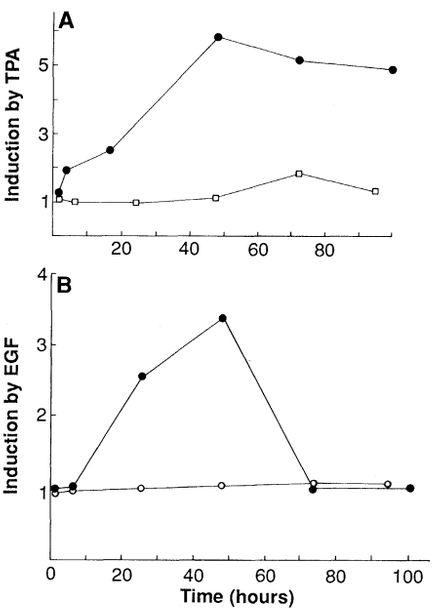

Fig. 3. (**A**) TPA induces AP-1–dependent CAT expression in P^+ cells but not in P^- cells. P^- Cl 30 cells were transfected with 8 μg of 3XTRE-CAT; P^+ Cl 41 cells with 1.5 μg of 3XTRE-CAT. After transfection and switching to 2% serum for 16 to 18 hours of incubation, we changed cells to fresh 2% serum with 10 ng of TPA per milliliter (16 n*M*) or DMSO (solvent control). At the end of each time point cells were harvested and assayed for CAT activity. Basal CAT activity for P^+ Cl 41 cells ranged from 79 to 152 units per 4×10^4 cells, and for P^- Cl 30 cells ranged from 61 to 88 units per 4×10^4 cells. In some experiments basal activity in P^- cells averaged slightly higher than in P^+ cells. Results showing differential inducibility were obtained in all of three independent experiments. Indicated results were for a representative experiment, and were expressed as the means of multiples of the basal values for induction for two independent transfections, which for Cl 41 averaged within 22% of the values shown, and differed by not more than 41%. For Cl 30, induction values averaged within 7% and differed by not more than 15%. (**B**) EGF induces AP-1–dependent CAT expression in P^+ cells but not in P^- cells. P^- Cl 30 and P^+ Cl 41 cells were treated as described in the legend for (A), except that cells were treated with 20 ng/ml EGF [3.2 n*M* (*3*)] instead of TPA. Differential inducibility by EGF was reproducibly observed in each of two independent transfection experiments, one of which showed differential inducibility by EGF with maximum CAT induction in Cl 41 cells of 4.8-fold and in Cl 30 cells of 1.0-fold.

Cl 41 cells showed significant inducibility and reached a maximum at 48 hours (Fig. 3A). Others have also observed induction of 3XTRE-CAT (in HeLa cells) within 12 hours of TPA treatment (*7*), but an extensive time course has not yet been reported. High levels of TPA-induced activity (fivefold) persisted for at least 100 hours. JB6 cells display an irreversible commitment to promotion by TPA after 96 hours (*18*). This raises the possibility that TPA-induced functional activation of AP-1 over an extended time course might be required to bring about transformation events.

In contrast to the P^+ cells, CAT activity in the P^- cells showed little inducibility by TPA at any time point tested. This phenotype-specific induction was also observed in two additional independent P^+ and P^- clonal JB6 cell variants (*19*). These data are consistent with the hypothesis that a defect in AP-1 function renders the P^- cell unresponsive to promotion stimuli. The basal levels of expression of 3XTRE-CAT for Cl 30 and Cl 41 cells were similar once the uptake of DNA was equalized (see legend to Fig. 3); this similarity suggests that the AP-1 protein is present in P^- cells and is capable of behaving as a functional transcriptional factor. The defect in inducible gene regulation in P^- cells therefore appears to be on the protein kinase C (PKC) signal transduction pathway elicited by TPA, not in the AP-1 protein itself. The observed unresponsiveness of P^- cells cannot, however, be attributed to lack of PKC activity or a PKC substrate (*20*). Finally, the difference in inducibility cannot be a function of differences in growth rates, since we observe that TPA induces AP-1–dependent CAT expression under conditions in which the growth rates of P^+ and P^- cells are equal. Since it has been shown that TPA induces expression of AP-1/*jun* mRNA (*10*) and protein, we are currently investigating the possibility that in P^- cells its induction is defective. Recent data suggest that P^- cells exhibit such a defect at the level of TPA inducible AP-1/*jun* protein synthesis (*19*). P^+ and P^- cells were [^{35}S]methionine-labeled under steady-state conditions. Cell lysates were immunoprecipitated with specific antisera to AP-1/*jun* (*21, 22*). P^+ Cl 41 cells exhibited 2.5- and 3.1-fold increases in AP-1/*jun* protein after 7 and 44 hours of TPA treatment. In contrast, P^- Cl 30 cells showed no increases (0.5- and 1.2-fold, respectively) in AP-1/*jun* protein after TPA treatment.

Epidermal growth factor (EGF), like TPA, reproducibly stimulates expression of 3XTRE-CAT in P^+ cells (Fig. 3B). P^+ cells were induced by EGF after 6.5 hours. Maximum induction was observed at 48 hours, as for TPA, and was 3.4-fold the basal level. Expression returned to basal levels by 72 hours. In P^- Cl 30 cells, expression of transfected 3XTRE-CAT was not induced at any time points tested. The P^- defect in AP-1–dependent transactivation of gene expression by EGF parallels insensitivity of the P^- cell to its transformation-promoting activity.

Vila and Weber have recently reported evidence suggesting a role for PKC in mediating certain cellular responses to EGF (*23*). It is possible that EGF may induce AP-1 function along a PKC-mediated pathway. EGF is also known to increase intracellular cyclic AMP levels, but cyclic AMP does not induce AP-1 binding to its enhancer (*24*). The pathway by which EGF induces transactivation of gene expression by AP-1 remains to be elucidated.

Serum plays a role in the P^+ cellular response to TPA. When TPA treatment was conducted in 1% serum instead of 2% serum the induction response was abolished altogether, as was the transformation response (*25*). Thus a serum cofactor is required to induce AP-1 function. At concentrations of 10% or higher, serum induces CAT expression two- to threefold without added TPA or EGF. Thus, serum also appears to have a stimulating factor of its own. P^- cells did not exhibit serum-responsive CAT induction. Like TPA and EGF, serum (at high doses) is a promoter of transformation in JB6 cells (*2*).

Our observation of P^+-specific, inducible AP-1 function by three different JB6 transformation promoters is consistent with the hypothesis that AP-1 function is required in the signal transduction pathway for promotion of neoplastic transformation. While AP-1 may be required, it is probably not sufficient for promotion; there is reason to

expect that other proteins, including Fos (*21, 26*), may participate in effecting this and other AP-1–dependent responses. Our results do not rule out the possibility that AP-1 can also play a role in mitogenic responses, as has been suggested by Ryseck *et al.* (*11*) and by Lamph *et al.* (*9*). In JB6 cells, however, promotion and mitogenesis have been dissociated (*27*). Under the (log phase) conditions of our experiments, TPA and, EGF are not mitogens but they are transformation promoters (*27, 28*). These experiments thus measure parameters of AP-1 function related to promotion and do not address mitogenesis-related events.

This report provides evidence for an association between AP-1–induced function and promotion of neoplastic transformation, and suggests that a defect in AP-1 activity may render the cell unresponsive to promotion stimuli. Recent experiments indicate that transfer of an activated *pro* gene to a P^- cell can reconstitute, not only the P^+ phenotype (*4*), but also AP-1–dependent transactivation of CAT gene expression induced by TPA (*19*). This suggests that *pro* genes can execute control over the activity of AP-1. Such investigations promise to shed light upon mechanisms of the signal transduction pathway for promotion of neoplastic transformation by TPA, EGF, and other tumor promoters.

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Selective Amplification and Cloning of Four New Members of the G Protein–Coupled Receptor Family

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An approach based on the polymerase chain reaction has been devised to clone new members of the family of genes encoding guanosine triphosphate–binding protein (G protein)–coupled receptors. Degenerate primers corresponding to consensus sequences of the third and sixth transmembrane segments of available receptors were used to selectively amplify and clone members of this gene family from thyroid complementary DNA. Clones encoding three known receptors and four new putative receptors were obtained. Sequence comparisons established that the new genes belong to the G protein–coupled receptor family. Close structural similarity was observed between one of the putative receptors and the 5HT1a receptor. Two other molecules displayed common sequence characteristics, suggesting that they are members of a new subfamily of receptors with a very short nonglycosylated (extracellular) amino-terminal extension.

THE INITIAL DISCOVERY THAT THE β-adrenergic receptor is structurally and evolutionarily related to the visual pigment opsin (*1*) has led to the identification of a growing number of members of this gene family. These have in common the presence of seven transmembrane segments and the ability to interact with G proteins. To clone the thyrotropin receptor [which is coupled to adenylyl cyclase via Gs (*2*)], we have devised a method based on the polymerase chain reaction (PCR) (*3*).

Polyadenylated [poly(A)$^+$] RNA prepared from human thyroid tissue was reverse transcribed and the resulting cDNAs were subjected to amplification by PCR with the use of a set of highly "degenerate" primers (Fig. 1). These were devised from the compilation of sequences corresponding to the third and sixth transmembrane segments of the following receptors: β_1-, β_2-, and α_2-adrenergic receptors (*4–6*); M1 muscarinic receptor (*7*); substance K receptor (*8*); and the serotonin receptor subtype G-21 (*9*) [now known as the 5HT1a receptor (*10*)]. The sequence similarity between any two of the receptors in this region ranged from 52 to 80% (Fig. 1A). Therefore, each primer consisted of a mixture of oligonucleotides with a number of degeneracies allowing a 78% match, or better, with any of the receptors. Nevertheless, the choice of the

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