N-ras oncogene causes AP-2 transcriptional self-interference, which leads to transformation

Perry Kannan, Reinhard Buettner, Paul J. Chiao, Sun O. Yim, Mona Sarkiss, and Michael A. Tainsky

Department of Tumor Biology, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas 77030 USA

Genetic alterations in elements of normal signal transduction mechanisms are known to be oncogenic events often resulting in aberrant activation of programs of gene transcription. We have investigated the effect of N-ras oncogene-induced tumorigenic transformation on the transcription factor AP-2. N-ras oncogene-induced transformation of human teratocarcinoma cells PA-1 results in sixfold elevated AP-2 mRNA levels. However, the level of AP-2-mediated trans-activation is dramatically inhibited in these cells. We show here that the high-level expression of AP-2 ultimately results in transcriptional "self-interference". The activation domain of AP-2, when fused to the DNA-binding domain of GAL4, is sufficient for self-interference. Non-N-ras PA-1 cells constitutively expressing AP-2 or GAL4-AP-2 fusion protein from an SV40 promoter exhibit reduced AP-2-mediated transcriptional activation, inhibition of differentiation, and promotion of anchorage-independent growth, properties that are similar to N-ras-transformed PA-1 cells. Thus, AP-2 is placed in the N-ras signal transduction pathway, and many of the biological effects of N-ras can be accomplished by overexpression of AP-2. This is the first evidence that inhibition of the activity of a transcription factor by self-interference contributes to a physiological process.

[Key Words: Transcription factor AP-2; N-ras oncogene; ras signal transduction; retinoic acid; transcriptional self-interference]

Received January 31, 1994; revised version accepted April 19, 1994.

DNA-binding transcription factors are often the nuclear targets for signals transduced in response to extracellular stimuli for growth and differentiation (Sen and Baltimore 1986; Treisman 1986; Chiu et al. 1987; Lee et al. 1987; Montminy and Bilezikjian 1987; Miyamoto et al. 1988; Hyman et al. 1989). Growth factors and hormones act in conjunction with specific receptors to enhance transcription of defined set of target genes (Yamamoto 1985; Carpenter 1987; Evans 1988). Such changes in the transcriptional activity of genes are frequently achieved by modulating the expression and activity of specific transcription factors. The transcription factor AP-2 appears to play a crucial role in a human teratocarcinoma cell line, N Tera 2, in response to cell differentiation signals induced by the developmental morphogen retinoic acid (RA; Williams et al. 1988; Lüscher et al. 1989). AP-2 mediates transcriptional activation in response to two other signal transduction pathways, the phorbol ester/ diacylglycerol-inducible protein kinase C [PKC] pathway and the cAMP-dependent protein kinase A pathway (Chiu et al. 1987; Imagawa et al. 1987; Hyman et al. 1989). Increased transcriptional activity in these two signal transduction pathways, unlike the effect of RA on AP-2, is not associated with the elevated levels of AP-2 expression in HeLa cells. The AP-2 mRNA levels are repressed by TPA, calcium ionophores, or any agent that increases intracellular cAMP concentration (Lüscher et al. 1989). The transcription factor AP-2 is a 52-kD protein that functions as a dimer, recognizing a palindromic sequence 5'-GCCNNNGGC-3' [Williams and Tjian 1991a,b]. The SV40 transcriptional control region and the human metallothionin II A (hMtIIA) gene contain AP-2-binding sites. Functional AP-2-binding sites are found in the regulatory regions of many other genes, including the murine major histocompatibility complex H-2 Kb, the collagenase, the human growth hormone, the human proenkephalin, and the human keratin K14 genes (Haslinger and Karin 1985; Lee et al. 1987; Mitchell et al. 1987; Williams et al. 1988; Hyman et al. 1989; Leask et al. 1991).

The three forms of ras, H-ras, K-ras, and N-ras, are thought to participate in signal transduction associated with growth control in mammalian cells. Oncogenic mutations of these ras genes induce uncontrolled cell proliferation or differentiation [e.g., PC12] depending on the cell background (Weinberg 1992). The ras genes code...
for GTP–GDP-binding proteins of 21 kD with a slow intrinsic GTPase activity. They serve as signal transducers by switching from an inactive GDP-bound form to an active GTP-bound form. The proteins Grb2-Sem-5, Shc, and Sos have been implicated in the signaling pathway from tyrosine kinase receptors to Ras [Egan et al. 1993]. The mechanism by which signaling passes downstream of Ras [for review, see Bos 1989; Gibbs and Marshall 1989; Santos and Nebreda 1989; Bollag and McCormick 1991] is not completely understood. However, evidence is emerging for the involvement of a cascade of protein kinases, including {\textit{raf}} and a series of MAP kinases, which act to transmit incoming signals to the nucleus [Medema and Bos 1993]. The nuclear events leading to {\textit{ras}}-mediated transformation are even less well understood.

PA-1 human teratocarcinoma-derived cells are represented by a series of subclones with differing propensities to differentiate and potency to induce tumors in nude mice [Zeuthen et al. 1980; Tainsky et al. 1988]. Clonal sublines representing the early stages of tumorigenesis have been isolated and maintained in culture [Tainsky et al. 1988]. Early passage nontumorigenic sublines of PA-1 cells are capable of exhibiting RA-induced differentiation, and like normal cells, they are resistant to N-\textit{ras} oncogene-induced transformation. They lose the resistance to N-\textit{ras} after undergoing ∼25 additional passages in culture yet remain nontumorigenic and RA responsive. Transfection of \textit{v-\textit{myc}} oncogene also makes the early passage PA-1 cells sensitive to\textit{ras}-induced transformation. The early passage nontumorigenic \textit{ras}-resistant PA-1 cells progress through a coordinate increase in responsiveness to growth factors and susceptibility to N-\textit{ras} oncogene-induced transformation [Chiao et al. 1991]; these changes were attributed to the loss of a tumor suppressor gene function [Krizman et al. 1990]. Late passage cells, on the other hand, are tumorigenic in nude mice and contain an N-\textit{ras} oncogene spontaneously acquired during 100 passages in culture [Tainsky et al. 1984]. This oncogene has a G → A point mutation that changes the codon 12 amino acid from glycine to aspartate. The N-\textit{ras}-transformed PA-1 tumor cells cannot differentiate in response to RA. In this study we have explored changes in the expression and activity of the differentiation-sensitive transcription factor AP-2 after N-\textit{ras} oncogene-induced transformation and have found that the expression of AP-2 is induced to a higher level. This was intriguing because RA, which induces differentiation and growth arrest of human teratocarcinoma cells N Tera 2 and PA-1 [Williams et al. 1988; Lüscher et al. 1989; Tainsky et al. 1991], also induces the expression of AP-2. The increased levels of AP-2 mRNA in N-\textit{ras}-transformed PA-1 cells, however, do not result in induction of AP-2 trans-activation activity but, rather, inhibition of AP-2 activity. Therefore, this increase in AP-2 mRNA levels has an inherently different result from that attributable to RA treatment. In non-N-\textit{ras} PA-1 cell lines, constitutive overexpression of AP-2 also led to inhibition of AP-2 trans-activation activity and conferred phenotypic effects similar to N-\textit{ras} oncogene-transformed cells. These results strongly suggest a role for AP-2 in the N-\textit{ras} pathway of signal transduction. Here, we describe that inhibition of AP-2 activity at high expression levels is attributable to transcriptional self-interference. Analysis of this phenomenon and possible explanations are discussed in this report. This is the first evidence for the physiological significance of transcriptional self-interference in cell growth and tumor progression.

**Results**

\textbf{Induction of AP-2 gene expression by N-\textit{ras} transformation}

Tumor progression of PA-1 cells from a nontumorigenic stage is associated with certain phenotypic alterations. The cells develop responsiveness to the mitogenic effect of growth factors, and they exhibit sensitivity to N-\textit{ras} oncogene-induced transformation [Tainsky et al. 1988; Chiao et al. 1991]. As with other teratocarcinoma cell lines, differentiation can be induced in PA-1 cells by treatment with the morphogen RA [Tainsky et al. 1991]. When the PA-1 cells are transformed by an activated N-\textit{ras}, their ability to exhibit RA-induced differentiation and differentiation-associated changes in gene expression are greatly reduced [Buettnner et al. 1991; Tainsky et al. 1991]. Stimulation of AP-2 mRNA and protein levels has been observed in RA-treated N Tera 2 teratocarcinoma cells [Williams et al. 1988; Lüscher et al. 1989]. We investigated the pattern of expression of the AP-2 gene in PA-1 sublines that display various levels of tumor progression and different levels of sensitivity to RA. Northern blot analysis revealed the existence of several distinct AP-2-specific messages in PA-1 cells (Fig. 1A). The levels of these AP-2 transcripts were very low in early passage PA-1 sublines clone 6 and clone 6 N-\textit{ras} [lanes 1 and 2, respectively]. Clone 6 cells contain an N-\textit{ras} suppressor function [Krizman et al. 1990]. Increased expression of two AP-2-specific messages (∼3.5 and 2.5 kb) was observed in clone 1 and clone 6 myc PA-1 cells [lanes 3 and 4, respectively], which have lost the presumptive N-\textit{ras} suppressor function [Krizman et al. 1990; Chiao et al. 1991]. Tumorigenic transformation of PA-1 cells by a spontaneously activated N-\textit{ras} gene resulted in more than a sixfold increase in the level of these transcripts [lane 6, 9113]. To confirm whether the induction of AP-2 expression is attributable to the N-\textit{ras} oncogene, we tested the subline 6928 that was constructed by transfection of the spontaneously activated N-\textit{ras} gene of 9113 into clone 1 cells [Tainsky et al. 1984]. The level of AP-2 expression in subline 6928 was more than fivefold higher than the parent cell clone 1 [cf. lanes 3 and 5]. Line 9117 is a tumorigenic but differentiation incompetent subline of PA-1 cells but does not contain an activated N-\textit{ras}; these 9117 cells did not have an elevated level of AP-2 gene expression (lane 7), indicating that other mechanisms of tumorigenicity can occur independent of any increase in AP-2 gene expression. Figure 1A also reveals the existence of an additional AP-2-specific mRNA.
The correlation between N-ras transformation and overexpression of the AP-2 gene was confirmed using somatic cell genetics. Hybrid cells were derived from fusion of tumor suppressor-containing cells [clone 6] and tumorigenic PA-1 cells, 9113, that carry an activated N-ras (Tainsky et al. 1991). The hybrid cells were initially nontumorigenic because of a dominant tumor suppressor function derived from the clone 6 cells. Tumor suppressor function, however, does not affect N-ras gene expression (Tainsky et al. 1991). After 15 passages in culture, tumorigenic hybrid variants were obtained as a result of loss of chromosome 19 (Tainsky et al. 1991), the presumptive site of the N-ras suppressor function. The level of AP-2 gene expression in the nontumorigenic hybrids is as low as that in clone 6 cells, with the 5.4-kb mRNA predominating [Fig. 1B, cf. lanes 1, 3, and 5]. Tumorigenic hybrids resulting from chromosome loss that display full N-ras oncogene-induced tumorigenicity contain elevated levels of expression of 3.5- and 2.5-kb AP-2 mRNAs [cf. lanes 2, 4, and 6]. These results substantiate that N-ras transformation, not just expression, leads to increased expression of the transcription factor AP-2. In addition, the 5.4-kb AP-2 mRNA that is seen in nontumorigenic hybrids was lost when the hybrids became tumorigenic. These results suggest that the tumor suppressor function may control, either directly or indirectly, the expression and processing of AP-2 mRNAs.

**Inhibition of AP-2 trans-activation activity by N-ras transformation**

RA treatment leads to growth arrest and induction of differentiation in sensitive PA-1 cells but not in N-ras-transformed PA-1 tumor cells. RA has been shown to induce transient AP-2 gene expression, with a concomitant increase in AP-2-driven transcriptional activity in human teratocarcinoma cell line N Tera 2 [Lüscher et al. 1989] as well as in RA-sensitive PA-1 cells [see below]. Because these two pathways promote opposite effects on the fate of cells, proliferation versus differentiation, both increase AP-2 mRNA expression, we became interested in understanding the functional significance of induced AP-2 expression in N-ras PA-1 cells. We therefore measured the trans-activation activity of AP-2 in PA-1 sublines using an AP-2 chloramphenicol acetyl transferase (CAT) reporter construct [Fig. 2A]. The N-ras suppressor-containing PA-1 clone 6 cells that showed very low levels of AP-2 expression had a significant level of AP-2 trans-activation activity. The CAT activity was 12-fold higher than the parental vector containing only the thymidine kinase [tk] promoter. Clone 1 PA-1 cells that had lost the N-ras suppressor gene had a secondfold higher CAT activity than the tkCAT. Little AP-2-induced CAT activity was detected in tumorigenic sublines of PA-1 cells 9113 and 6928. These cells are tumorigenic and have either spontaneously activated [9113] or gene-transferred [6928] N-ras oncogene and express high levels of AP-2 mRNAs. These results are not attributable to transfection efficiency or defective CAT gene expression because Rous sarcoma virus long terminal repeat (LTR)-driven CAT activity is equivalent in N-ras PA-1 cells and non-N-ras PA-1 cells.

Similar results were obtained when the somatic cell hybrids were tested for their ability to trans-activate the AP-2 reporter construct. The activity observed in nontumorigenic hybrids decreased when the cells became tumorigenic [Fig. 2B]. The N-ras oncogene is expressed equally in all of the hybrid cell lines but has its full
N-ras oncogene modulates transcription factor AP-2

PA-1 cells overexpressing AP-2 exhibit many properties of N-ras-transformed cells

N-ras PA-1 cells have high-level expression of AP-2 mRNA, but AP-2 trans-activation is inhibited. We cloned and analyzed AP-2 cDNA from one of the N-ras PA-1 cells, 6928. The AP-2 clone did not have any mutations in the 1347-bp coding region and matched perfectly with the human cDNA sequence reported previously [Williams et al. 1988] and the exon sequences found in the human AP-2 gene sequence [Bauer et al. 1994]. This AP-2 cDNA was cloned adjacent to a Droso-

PA-1 cells overexpressing AP-2 exhibit many properties of N-ras-transformed cells

N-ras oncogene expression directly affects AP-2 gene expression in PA-1 cells

AP-2 trans-activation activity is inhibited in PA-1 cells carrying an activated N-ras gene. We therefore analyzed the consequences of transient N-ras oncogene expression on AP-2 trans-activation activity in non-N-ras PA-1 cells. Increasing amounts of an activated N-ras expression plasmid progressively reduced AP-2 trans-activation activity to more than sixfold [Fig. 3A]. N-ras PA-1 cells have inherent low AP-2 trans-activation activity. The role of the activated N-ras on AP-2 activity was tested in these cells using an anti-sense N-ras expression construct. In transient assays, a sixfold restoration of AP-2 trans-activation activity was observed in N-ras PA-1 cell 9113 [Fig. 3B]. These results confirm that N-ras-induced transformation affects AP-2-mediated transcriptional activity in PA-1 cells and establish a direct link between N-ras and AP-2.

Figure 3. Transient expression of N-ras oncogene directly affects AP-2 trans-activation activity. N-ras oncogene cloned adjacent to a β-actin promoter was cotransfected with 4 µg of AP-2 reporter plasmid into PA-1 cells, and the CAT activity was determined as described in Materials and methods. (A) Effect of varying amount of pNras transfection on AP-2 trans-activation activity. The endogenous level of AP-2 activity is taken as 1 to calculate the effect of N-ras gene expression, and the values are shown at the top of each assay. Plasmid pβactin 16 is the parent vector of pNras. (B) Expression of antisense N-ras transcripts restores AP-2 trans-activation activity in N-ras PA-1 cells 9113. pNrasAS [4 µg] and AP-2 reporter plasmid [4 µg] were transfected into 9113 PA-1 cells. The fold induction of activity over the control reporter plasmid tkCAT [pBLCAT2] is shown (top).

Figure 2. Trans-activation activity of AP-2 in PA-1 cells and hybrids. AP-2 reporter plasmid [4 µg] or AP-2RE-tk CAT (AP-2–tkCAT) or 4 µg of parent plasmid pBLCAT2 (tkCAT) was transfected into PA-1 cells. After 48 hr CAT activity in 8 µg of freeze-thaw lysed cell extract was measured as described in Materials and methods. (A) AP-2 trans-activation activity of various PA-1 sublines; (B) AP-2 trans-activation activity of PA-1 cell hybrids. (NT) Nontumorigenic hybrid cells; (T) tumorigenic hybrid cells.

The cell line 9117 provides a useful tumor cell control. It does not contain an activated N-ras and has a high level of AP-2 activity. As shown earlier, AP-2 gene expression in 9117 was also not as high as in N-ras PA-1 cells. Consistent with other cells that have low levels of AP-2 mRNA, 11-fold AP-2 trans-activation activity was detected in these cells (Fig. 2A). These results suggest that only N-ras-induced transformation and not just tumorigenicity leads to increased AP-2 expression and reduced AP-2 trans-activation activity.

Oncogenic effect in the tumorigenic hybrid cells after the loss of a suppressor function [Tainsky et al. 1991].

The cell line 9117 provides a useful tumor cell control. It does not contain an activated N-ras and has a high level of AP-2 activity. As shown earlier, AP-2 gene expression in 9117 was also not as high as in N-ras PA-1 cells. Consistent with other cells that have low levels of AP-2 mRNA, 11-fold AP-2 trans-activation activity was detected in these cells (Fig. 2A). These results suggest that only N-ras-induced transformation and not just tumorigenicity leads to increased AP-2 expression and reduced AP-2 trans-activation activity.

N-ras oncogene expression directly affects AP-2 gene expression in PA-1 cells

AP-2 trans-activation activity is inhibited in PA-1 cells carrying an activated N-ras gene. We therefore analyzed the consequences of transient N-ras oncogene expression on AP-2 trans-activation activity in non-N-ras PA-1 cells. Increasing amounts of an activated N-ras expression construct were cotransfected with an AP-2 reporter plasmid into 9117 cells. In these transient assays the N-ras expression plasmid progressively reduced AP-2 trans-activation activity to more than sixfold [Fig. 3A]. N-ras PA-1 cells have inherent low AP-2 trans-activation activity. The role of the activated N-ras on AP-2 activity was tested in these cells using an anti-sense N-ras expression construct. In transient assays, a sixfold restoration of AP-2 trans-activation activity was observed in N-ras PA-1 cell 9113 [Fig. 3B]. These results confirm that N-ras-induced transformation affects AP-2-mediated transcriptional activity in PA-1 cells and establish a direct link between N-ras and AP-2.
We transfected this expressor plasmid into Drosophila PA-1 clone 1 and 9117 cells that had been shown to produce a functional AP-2 protein. This cDNA clone was able to produce a functional AP-2 protein (Fig. 4A). The AP-2 cDNA was cloned adjacent to a SV40 promoter, and this expressor construct pSAP2 produced the correctly sized 52-kD protein (Mitchell et al. 1987) in in vitro translation experiments (not shown). We transfected this expressor plasmid into non-N-ras PA-1 clone 1 and 9117 cells that are devoid of AP-2 protein. This indicates strongly that high expression of AP-2 leads to inhibition of its own activity.

We addressed the effect of constitutive expression of AP-2 on cells by establishing stable transfectants of plasmid pNAP2, a pSV2neo derivative of pSAP2, in clone 1 cells (a non-N-ras and nontumorigenic PA-1 subline). In our initial screening two AP-2 overexpressor cell lines were identified by reverse transcriptase–polymerase chain reaction analysis (data not shown). The levels of expression and integrity of AP-2 protein in these two AP-2 expressor clone 1/AP-2a and 1/AP-2k cell lines were confirmed by Western blot analysis. Nuclear extracts of both AP-2 expressors showed elevated levels of a 52-kD protein that comigrated with the in vitro-translated AP-2 protein [Fig. 5A]. The trans-activation activity of AP-2, like N-ras-transformed cells, was inhibited in the two AP-2 stable transfectants, clone cells 1/AP-2a and 1/AP-2k relative to the parent clone 1 and to pSV2neo-transfected clone 1 cells [Fig. 5B]. In addition, these stable transfectants exhibited many properties of N-ras-transformed PA-1 cells. RA treatment is not effective in arresting the growth of N-ras PA-1 cells, but the growth of clone 1 PA-1 cells is reduced by >80% (Tainsky et al. 1991, Fig. 5C). The two AP-2 overexpressor cell lines were refractory to the growth-inhibitory effect of RA comparable to N-ras PA-1 cells. Unlike their parent clone 1 cells, both of the AP-2 overexpressor cell lines exhibited anchorage-independent growth equivalent to tumorigenic PA-1 cells [Table 1]. These results indicate that AP-2 causes self-interference at high expression levels, which results in transformation and resistance to RA-induced growth arrest. The inverse relationship of AP-2 expression and activity is similar in N-ras PA-1 cells and cell lines constitutively expressing AP-2. This correlation implies strongly that AP-2 plays a crucial role in downstream signal transduction of N-ras-induced cellular transformation.

RA induces AP-2 gene expression and trans-activation activity only in non-N-ras PA-1 cells

As part of normal signal transduction pathway in differentiation-competent PA-1 cells (clone 1 and 9117), RA treatment increased the expression of AP-2 only in sensitive cells. In RA-treated 9117 PA-1 cells the AP-2 mRNA is transiently induced and peaks after 24 hr of treatment [Fig. 6A]. A concomitant increase in AP-2 trans-activation activity, about fourfold higher than untreated cells, was observed in the RA-treated cells [Fig. 6B], similar to that reported for N Tera 2 cells (Lüsch er et al. 1989). RA treatment of N-ras PA-1 cell 6928, how-

Figure 4. Effect of AP-2 overexpression on non-N-ras PA-1 cells in transient assays. Transfection of non-N-ras PA-1 cells and measurement of CAT activity were performed as described in Materials and methods. [A] Drosophila actin promoter-driven AP-2 expressor plasmid produces functional AP-2 protein in AP-2 null Schneider cells. [B] Dose-dependent effect of overexpression of AP-2 in clone 1 and 9117 PA-1 cells and the control plasmid pSAP2/FS21 in 9117 PA-1 cells. 3x AP-2RE-tkCAT reporter plasmid [4 µg] was transfected in all assays. Transfected pSAP2 or pSAP2/FS21 DNA is shown [in µg] [top]. All of the DNA amounts were equalized with the addition of pBluescript plasmid DNA. The percentage conversion to acetylated forms of chloramphenicol by the endogenous level of AP-2 is taken as one to compare with the cotransfections.
N-ras oncogene modulates transcription factor AP-2

Figure 5. Effect of constitutive overexpression of AP-2 in PA-1 cells. (A) Western blot analysis of clone 1 cells expressing AP-2. Western blot analysis was performed with 6 μg of nuclear extract of PA-1 cells as described in Materials and methods. The blot was incubated with 1:1000 dilution of AP-2 (C-18) antibody and visualized using 1 μCi/ml of [125I]-labeled goat-anti-rabbit antibody. HeLa cell nuclear extract is included for comparison. In vitro-translated AP-2 protein from an AP-2 expressor plasmid, pSAP2 (i.v.t. AP-2), is included to identify the position of AP-2 protein migration (arrowhead). (B) Trans-activation activity of AP-2 in AP-2 stable cell lines. Experiments were carried out as described in the legend to Fig. 2. tkCAT, pBLCAT2; AP-2-tkCAT, 3X AP-2RE-tkCAT. [C] Effect of RA on cell growth of AP-2 or GAL4–AP-2 overexpressor cell lines. Experiments were carried out as described in Materials and methods, and the surviving cells were counted. The growth of 10–5 M RA-treated cells for 10 days is shown as a percentage of respective untreated cell control.

Table 1. The effect of constitutive expression of AP-2 or GAL4–AP-2 on anchorage-independent growth of PA-1 cells

| PA-1 subline | Colony formation/10⁴ cells |
|--------------|---------------------------|
| Clone 1     | 0                         |
| Clone 1–neo | 2                         |
| 6928 (N-ras/GT) | 444                      |
| 9113 (N-ras/SP) | 750                      |
| Clone 1 AP-2a  | 424                      |
| Clone 1 AP-2k  | 364                      |
| Clone 1 GAL4–AP-2d | 1424                    |
| Clone 1 GAL4–AP-2k | 544                    |

Colony formation in soft agar was measured as described in Materials and methods, and the results are averages of at least two experiments. [N-ras/GT] Gene-transfected N-ras oncogene; [N-ras/SP] spontaneously activated N-ras.

Low trans-activation activity of AP-2 in N-ras PA-1 cells is not attributable to the absence of AP-2 protein or loss of sequence-specific DNA-binding ability of AP-2

High levels of AP-2 mRNA lead to inhibition of AP-2 trans-activation activity in N-ras PA-1 cells. To address this paradox we measured the level of AP-2 protein in the nuclear extracts of these cells. Western blot analysis showed the two N-ras PA-1 cells 6928 and 9113 had higher level of AP-2 protein when compared with non-N-ras PA-1 cells and HeLa cells (Fig. 7A). These results
The activation domain of AP-2 can mediate transcriptional self-interference

Preliminary experiments suggested that the amino-terminal region of AP-2 that includes the activation domain is involved in self-interference (not shown). To obtain conclusive evidence we fused the amino-terminal region of AP-2 from amino acids 11–226 to the DNA-binding domain of GAL4 (pGLAP2/11-226) and tested the role of the fusion protein in self-interference. Amino acids 11–226 contain the activation domain of AP-2 and the basic region that is necessary but not sufficient for DNA binding. As expected, 9117 PA-1 cells did not exhibit endogenous GAL4-specific CAT activity when the GAL4 reporter construct alone was transfected into these cells (Fig. 8A). Cotransfection of small amounts of the fusion construct pGLAP2/11-226 resulted in GAL4-specific CAT activity. However, cotransfection of increased amounts of pGLAP2/11-226 did not progressively increase CAT activity but, rather, inhibited the GAL4-specific activity. These results indicate that amino acids 11–226 of AP-2 are capable of self-interference. To investigate whether the basic region of the DNA-binding domain of AP-2 is involved in self-interference, we deleted this region from the fusion construct pGLAP2/11-226. This GAL4 fusion construct pGLAP2/11-121 contains amino acids 11–121, within which is the activation domain of AP-2 but no sequences of the AP-2 DNA-binding domain. As shown in Figure 8A, overexpression of the activation domain of AP-2 in pGLAP2/11-121 is capable of self-interference. To investigate further, we transfected increasing amounts of GAL4–AP-2 fusion construct pGLAP2/11-226 into 9117 PA-1 cells and measured the level of trans-activation activity by endogenous AP-2. As shown in Figure 8B, pGLAP2/11-226 is effective in inhibiting the endogenous AP-2 activity. Note that the GAL4–AP-2 fusion protein cannot bind to other assays. The DNA-binding activities of PA-1 cell nuclear extracts were not significantly altered when the variant binding site was used as a competitor. The mobility of one of the shifts observed with PA-1 cell nuclear extracts comigrated with that of HeLa cells that are known to have AP-2 protein (Mitchell et al. 1987). The second shift observed with PA-1 cell nuclear extracts may represent modification of AP-2 protein in these cells. Both binding activities were elevated in N-ras PA-1 9113 cells (lanes 7) when compared with non-N-ras PA-1 6 (lanes 4) and 9117 (lanes 10) clone cells. Thus, N-ras-transformed PA-1 cells have protein capable of binding in a sequence-specific fashion to the AP-2 target site. Although the pattern of band shift is the same in clone 6, 9113, and 9117 cells, the sequence-specific DNA binding in 9113 N-ras PA-1 cells does not lead to productive activation of target genes, as indicated by transient transfection assays using model CAT reporter constructs. A third fast-migrating shift is observed only in N-ras PA-1 cell 9113. The possibility that this DNA–protein complex could represent an inhibitory form of AP-2-mediated trans-activation awaits further experimentation.

Figure 7. N-ras cells contain high levels of AP-2 protein that can bind to AP-2 target sequences. (A) Western blot analysis of PA-1 cell nuclear extracts. Experiments were performed as described in the legend to Fig. 5(A). The migration of AP-2 protein is indicated (arrowhead). (B) Electrophoretic mobility retardation assay of AP-2 in N-ras PA-1 cells. Experiments were carried out as described in Materials and methods. The protein–DNA complexes were resolved on a 4% polyacrylamide gel. (a) No competitor DNA; (b) 200-fold excess of wild-type competing oligonucleotide; (c) cold mutant oligonucleotide was included during binding reactions in the indicated lanes. Two AP-2 target sequence-specific shifts are marked by arrows. A third AP-2 target sequence-specific shift observed in N-ras PA-1 cells is marked by an asterisk (*).
needed for AP-2-mediated transcriptional activation. In AP-2 target sequences in the AP-2 reporter plasmid. For AP-2 target sequences in the AP-2 reporter plasmid. For

AP-2 expressor clone 1/GAL4-AP-2d and 1/GAL4-AP-2j cell lines contained proteins that could specifically bind to GAL4 target sequences (data not shown). Expression of GAL4–AP-2 in these cell lines inhibited the endogenous AP-2 trans-activation activity (data not shown) consistent with the transient transfection assays. Similar to N-ras PA-1 cells and the cell lines stably expressing the entire AP-2, the two GAL4–AP-2 cell lines are refractory to the growth inhibitory effect of RA (Fig. 5C) and exhibited anchorage-independent growth (Table 1). These studies confirm that the activation domain of AP-2 mediates transcriptional interference, which leads to transformation and differentiation resistance of PA-1 cells.

The activation domain of AP-2 causes inhibition of its activity when overexpressed. This effect may be attributable to sequestering a coactivator. Our preliminary experiments suggested that high levels of AP-2 can inhibit the transcriptional activity mediated by a viral activator VP16 (data not shown) indicating that the activators AP-2 and VP16 utilize a common coactivator. Transcriptional activity mediated by the adenovirus major late promoter was not affected by the levels of AP-2 (data not shown), suggesting that the general transcriptional machinery was not affected by AP-2 overexpression. It is interesting to note that AP-2 overexpression can result in inhibition of both AP-2 and VP16 even though the activation domain of AP-2 is rich in proline and glutamine residues (Williams et al. 1991a) and the activation domain of VP16 is rich in acidic residues. RA coordinately induces high levels of AP-2 expression and activity in PA-1 cells (see above) indicating that the general transcription machinery is not affected by AP-2 overexpression. We speculate that RA modulates the coactivator or alters other mechanisms that result in increased AP-2 activity.

**Discussion**

Ras proteins play an important role in response to multiple extracellular stimuli in mammalian cells. The phenotypic effects of an activated ras gene vary from transformation of fibroblasts to differentiation of neural cells (Bollag and McCormick 1991). The differentiation of F9 embryonal carcinoma cells into endoderm-like cells (a response normally elicited by RA and cAMP) can be induced by ras in a c-jun-dependent manner (Yamaguchi-Iwai et al. 1990). In contrast to these differentiation-promoting responses, activated ras genes inhibit the differentiation of skeletal myoblast cells (Olson et al. 1987). However, the targets for downstream signal(s) from Ras proteins that effect these changes are not clearly understood. In this report we have found strong evidence to implicate the transcription factor AP-2 in the N-ras pathway of signal transduction. Previously, we have shown that N-ras-induced transformation did not alter...
the expression of c-myc, N-myc, c-fos, c-myb, or junB genes, whose products are involved in transcriptional activation (Chiao et al. 1991). N-ras oncogene-mediated transformation increases the expression of AP-2 with a concomitant inhibition of its activity. We speculate that inhibition of the transcription activity of AP-2 is a necessary event for N-ras oncogene-induced cellular transformation of PA-1 cells for the following reasons. Normal AP-2 function appears to be required for proper negative control of cell growth. RA-induced cell growth arrest and differentiation of human teratocarcinoma cells N Tera 2 and PA-1 result in increased expression of AP-2 mRNA, with a concomitant increase in AP-2 activity [Williams et al. 1988; Lüscher et al. 1989; Tainsky et al. 1991, Fig. 5]. SV40 large T antigen, a cell growth-promoting factor, inhibits the activity of AP-2 by directly binding to AP-2 and thereby preventing the formation of an AP-2/DNA complex [Mitchell et al. 1987]. T antigen also interacts with transcription factors p53 and retinoblastoma gene product. The global changes in gene expression associated with large T antigen-induced cellular transformation may involve the sequestration of these transcription factors and AP-2.

The mechanism by which the N-ras oncogene induces the expression of AP-2 remains to be understood, but it probably involves downstream changes in signal transduction mechanisms. Expression of the N-ras oncogene is not sufficient for transformation. The full transforming effect of this oncogene in the absence of the suppressor function is necessary to alter AP-2 gene regulation [Fig. 1]. PA-1 cells appear to have an intracellular signal transduction pathway used by growth factors and ras-transforming oncogenes. Changes within this pathway make nontumorigenic PA-1 cells susceptible to N-ras oncogene transformation and responsive to multiple growth factors (Chiao et al. 1991). Our results implicate AP-2 in this signal transduction pathway. Numerous other reports suggest the involvement of PKC in ras-induced responses [Bollag and McCormick 1991], and the PKC pathway was shown to affect AP-2 activity [Lüscher et al. 1989]. Modulation of the PKC pathway by an activated Ras protein may provide a clue for AP-2 gene regulation. The AP-2 gene may also have a ras-responsive enhancer element similar to ones identified in the promoters of murine retrotransposon NVL3 and transforming growth factor β-1 [Owen et al. 1990]. Alternatively, AP-2 mRNA stability may be increased in N-ras-transformed PA-1 cells.

Although the AP-2 mRNA is induced to a higher level, the trans-activation activity of AP-2 is inhibited in N-ras-transformed PA-1 cells. Similarly, AP-2 overexpression in the absence of a N-ras oncogene also causes inhibition of AP-2 trans-activation activity revealing a self-interference effect of AP-2. A likely explanation for this inhibition is that AP-2 activity results from a complex set of proteins and that there are cofactors necessary for the AP-2 trans-activation activity, some of which are in limiting amounts. Excess AP-2 molecules may interact with one or more of these putative cofactors, making them unavailable for the AP-2 molecules bound to the target site. In support of this, AP-2 and the fusion protein containing GAL4 DNA-binding domain and AP-2 activation domain can inhibit each other at high levels of expression, although both molecules bind to different target DNA sequences. Transcriptional interference activities have been described in which activation domains of two transcription factors share one common target protein that is present in limited amounts [Prashne 1988]. Overexpression of one of the transcription factors interacting with the common target protein renders it unavailable for the second transcription factor. Transcriptional interference by AP-2 is different because at high expression levels it is also autoinhibitory. Such autointerference has been observed previously with GAL4–VP16 [Kelleher et al. 1990]. The autointerference of AP-2 sheds light on the physiological consequences of overexpression of transcription factors, which appears to lead to cellular transformation as suggested by the cell lines stably overexpressing AP-2. Studies with cell lines expressing GAL4–AP-2 fusion protein indicate that the activation domain of AP-2 mediates such transcriptional interference, which confers oncogenic potential to cells. Our data are similar to numerous studies in which oncogenes such as fos, jun, myc, myb, rel, and others that are transcription factors that can transform normal cells. AP-2 induces transformation via a novel mechanism of transcriptional self-interference.

It is intriguing that the self-interference of AP-2 does not occur as part of the normal signal transduction pathway induced by RA. In differentiation-competent PA-1 cells, RA treatment, which induces levels of AP-2 expression equivalent to that detected in N-ras-transformed cells, fails to cause self-interference. RA may also induce the factors necessary for AP-2 transcriptional activity or it may invoke altogether a different mechanism to accomplish an induced AP-2 activity.

In summary, this study is the first report on the physiological effects of transcriptional interference in transformation. The N-ras oncogene causes overexpression of AP-2 and increases the level of AP-2 protein. This increase in AP-2 results in self-interference mediated by its activation domain. The activation domain of AP-2 probably sequesters a necessary coactivator, resulting in reduced AP-2 transcriptional activity. Such reduction in AP-2 activity promotes differentiation resistance and transformation of PA-1 cells very similar to the effect of N-ras oncogene on PA-1 cells, and this correlation strongly suggests that AP-2 plays a crucial role in the downstream signal transduction of N-ras oncogene.

**Materials and methods**

**Cell culture**

PA-1 cells were grown in modified Eagle’s medium with Earl’s salts (GIBCO Laboratories, Gaithersberg, MD) supplemented with 7% fetal bovine serum (Hazelton Biologics, Lenexa, KS) and antibiotics at 37°C in 5% CO₂–95% air. Growth analysis of RA-treated PA-1 cells was done by seeding 2×10⁴ cells into 60 mm dishes in duplicate and treating them with 10⁻⁵M RA for 10 days. To determine anchorage-independent growth, 1×10⁴ cells
were dispersed in 0.35% agarose and the suspension was spread over a layer of 0.7% agarose. After 10 days of incubation, colonies >45 μm in diameter were counted.

**Northern blot analysis**

Total RNA was isolated by guanidium isothiocyanate and CsCl-gradient centrifugation and enriched for poly(A)+ RNA by two consecutive cycles of chromatography on oligo(dT)-cellulose columns using standard techniques (Sambrook et al. 1989). Five micrograms of poly(A)+-selected RNA was electrophoresed over a layer of 0.7% agarose. After 10 days of incubation, colo-

**Transcript transfections of PA-1 cells and CAT assays**

AP-2 response element sequences from the distal basal level element of human metallothionin gene II A corresponding to nucleotides −188 to −161 were oligomerized, and a reporter construct 3X AP-2RE#{sub}m=ttk CAT was made by cloning three response elements adjacent to the herpes simplex virus tk promoter in the vector pBLCAT2 (Luckow and Schütz 1987). Transient transfection of PA-1 cells was performed using calcium phosphate precipitation as described by Graham and Van der Eb (1973). Generally, 4 μg of plasmid was used in transfections. When varying amounts of DNA were used in transfections, all amounts were equalized by the addition of pBluescript DNA. CAT activity was measured by the conversion of [14C]chloramphenicol to mono- and diacetyl chloramphenicol, essentially as described by Gorman et al. (1982). Briefly, 48 hr after transfection, cells were harvested and lysed in 100 mM Tris buffer at pH 7.8 by three cycles of freezing and thawing. About 8 μg of total cell extract was heated for 10 min at 65°C and mixed with 0.8 mM acetyl CoA and 0.05 mM [14C]chloramphenicol in 100 μl of Tris buffer at pH 7.8 and incubated at 37°C. Acetylated forms of chloramphenicol were partitioned by extracting with ethyl acetate and separated by thin layer chromatography. The percentage conversion of acetylated forms of chloramphenicol was calculated by measuring radioactivity for 30 min in a Betascope blot analyzer (Betagen, Framingham, MA).

**Expression vectors**

AP-2 cDNA of ~1.9 kb isolated from 6928 PA-1 cells (Buettner et al. 1993) was cloned in proper orientation in the EcoRI site of plasmid pSG5 (Stratagene, La Jolla, CA) to generate pSAP2. SV40 early promoter and β-globin intron sequences that enable efficient expression of cloned genes are located before the EcoRI site in pSG5. The SalI fragment of pSAP2 that includes the entire AP-2 expression cassette was isolated, filled in with Klenow polymerase, and ligated with pSV2-neo that was cut with EcoRI and filled in with Klenow polymerase. This expression plasmid pNAP2 enabled the establishment of stable transfectants of PA-1 cells. In vitro transcription and translation experiments using the expression plasmids pSAP2 and pNAP2 yielded a 52-kD protein that could bind to the AP-2 target sequences in mobility retardation assays. The pSAP2 frameshift mutation was constructed as follows. Expression plasmid pSAP2 was cut after codon 20 at BamHI site (nucleotide 58) (nucleotides and codons are numbered from the start codon ATG), filled in with Klenow fragment of DNA polymerase I, and religated. This construct pSAP2/FS21 has four additional nucleotides inserted after codon 20, resulting in a frameshift mutation. The GAL4 DNA-binding domain and AP-2 activation domain containing fusion construct pGLAP2/11-226 was made by inserting a RsaI fragment of AP-2 filled in with the Klenow enzyme (from nucleotide 1019 to 576) into EcoRI-cut and mung bean nuclelease-blunted pSG424 (Sadowski et al. 1988). pGLAP2/11-226 was cut with BamHI (at nucleotide 363 of AP-2) and SacI and religated to create pGLAP2/11-121. All these constructs were verified for their nucleotide sequence and reading frame by double-stranded DNA sequence analysis. A 600-bp N-ras cDNA carrying an oncogenic mutation at codon 12 was isolated from PA-1 cells (Tainsky et al. 1984) and cloned into the HindIII site of pβactin-16 (Fregien and Davidson 1986) in the sense orientation to construct pNras and in the anti-sense orientation to create pNrasAS.

**Western blot analysis**

Nuclear extracts were made from PA-1 cells as described by Dignam et al. (1983), and 6 μg protein was subjected to 12% SDS-PAGE (Laemmli 1970) on a 10% polyacrylamide membrane (Amersham Corp., Arlington Heights, Ill). The Western blot was blocked with 50 mM Tris-HCl (pH 7.4) containing 0.9% NaCl, 3% bovine serum albumin, and 0.05% Tween 20 for 12 hr and incubated with AP-2 (C-18), a rabbit polyclonal antibody made against a synthetic peptide corresponding to AP-2 carboxy-terminal amino acids 420-437 (Santa Cruz Biotechnology, CA). The AP-2-antibody interaction was visualized by probing with a 125I-labeled goat anti-rabbit antibody (ICN Biomedicals, Inc., Irvine, CA) and subsequent exposure to Kodak X-Omat X-ray film at −70°C.

**Mobility retardation assay**

The 34-bp double-stranded oligonucleotide sequence 5'-AG-GAACTGACCGCCGCGCCGTGGTCAGAG-3' identical to the sequence of the distal basal level element of hMtllA gene (nucleotides −188 to −161) contains an AP-2 response element (as underlined). 32P-End-labeled oligonucleotide (2×10⁶) was mixed with 3 μg of crude nuclear extracts prepared from PA-1 cell extracts (described by Pollock and Treisman 1990) in 50 mM HEPES at pH 7.9, 6 mM MgCl₂, 50 mM KCl, 2.5 mM DTT, 100 μg/ml of BSA, 0.1% NP-40, and 0.5 μg of salmon sperm DNA, incubated at 30°C for 30 min and electrophoresed on 4% polyacrylamide gel. A mutant oligonucleotide that has two alterations in the AP-2 response element (5'-AGGAACCTGAC- CGACCGGTCCGCCGTGGTCAGAG-3') was used as a competit-

**Acknowledgments**

We thank Dr. M. Van Dyke for his help with mobility retardation assays and critical comments on this paper, L. Wildrick for editorial assistance, and T. Busch for photographic assistance. We acknowledge the generous gifts of plasmid constructs from Dr. M. Ptashne for pSG424 and G5EBCAT and Dr. M. Karin for 5× TRE_{ol}–tkCAT. This work was supported by the National Cancer Institute (grant CA42810) to M.A.T and by the National
Kannan et al.

Institutes for Health [core center grant 16672] to the M.D. Anderson Cancer Center.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

Bauer, R., A. Imhof, A. Pscherer, M. Moser, S. Seegers, M. Ker-scher, M.A. Tainsky, F. Hofstaeedter, and R. Buettner. 1994. The genomic structure of the human AP-2 transcription factor. *Nucleic Acids Res.* 22: 1413–1420.

Bollag, G. and F. McCormick. 1991. Regulators and effectors of ras proteins. *Annu. Rev. Cell Biol.* 7: 601–632.

Bos, J.L. 1989. ras Genes in human cancer: A review. *Cancer Res.* 49: 4682–4689.

Buettner, R., S.O. Yim, Y.S. Hong, E. Boncinelli, and M.A. Tainsky. 1991. Alteration of homeobox gene expression by N-ras transformation of PA-1 human teratocarcinoma cells. *Mol. Cell. Biol.* 1: 3573–3583.

Buettner, R., P. Kannan, A. Imhof, R. Bauer, S.O. Yim, R. Glock-shuber, M.W. Van Dyke, and M.A. Tainsky. 1993. An alternatively spliced mRNA from the AP-2 gene encodes a negative regulator of transcriptional activation by AP-2. *Mol. Cell. Biol.* 13: 4174–4185.

Carpenter, G. 1987. Receptors for epidermal growth factors and other polypeptide mitogens. *Annu. Rev. Biochem.* 56: 881–914.

Chiao, P.J., P. Kannan, S.O. Yim, D.B. Krizman, T.A. Wu, G.E. Gallick, and M.A. Tainsky. 1991. Susceptibility to ras onco-gene transformation is coregulated with signal transduction through growth factor receptors. *Oncogene* 6: 713–720.

Chiu, R., M. Imagawa, R.J. Imbra, J.R. Bockover, and M. Karin. 1987. Multiple cis- and trans-acting elements mediate the transcriptional response to phorbol esters. *Nature* 329: 648–651.

Dignam, J.D., R.M. Lebovitz, and R. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* 11: 1475–1489.

Dugaczyk, A., J.A. Haron, E.M. Stone, O.E. Dennison, K.N. Rothblum, and R.J. Schwartz. 1983. Cloning and sequencing of a deoxyribonucleic acid copy of glyceraldehyde-3-phosphate dehydrogenase messenger ribonucleic acid isolated from chicken muscle. *Biochemistry* 22:1605–1613.

Egan, S.E., B.W. Giddings, M.W. Brooks, L. Buday, A.M. Size-land, and R.A. Weinberg. 1993. Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. *Nature* 366: 45–51.

Evans, R.M. 1988. The steroid and thyroid hormone receptor super family. *Science* 240: 879–895.

Fregien, N. and N. Davidson. 1986. Activating elements in the promoter region of the chicken β-actin gene. *Gene* 48: 1–11.

Gibbs, J.B. and M.S. Marshall. 1989. The ras oncogene - an important regulatory element in lower eucaryotic organisms. *Microbiol. Rev.* 53: 171–185.

Gorman, C.M., L.F. Moffat, and B.H. Howard. 1982. Recombi-nant genomes which express chloramphenicol acetyltrans-ferase in mammalian cells. *Mol. Cell. Biol.* 2: 1044–1053.

Graham, F.L. and A.J. van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52: 456–467.

Haslinger, A. and M. Karin. 1985. Upstream promoter element of the human metallothionin-IIA gene can act like an en-hancer element. *Proc. Natl. Acad. Sci.* 82: 8572–8576.

Hyman, S.E., M. Comb, J. Pearlberg, and H.M. Goodman. 1989. An AP-2 element acts synergistically with the cyclic AMP and phorbol ester-inducible enhancer of the human proenkephalin gene. *Mol. Cell. Biol.* 9: 321–324.

Imagawa, M., R. Chiu, and M. Karin. 1987. Transcription factor AP-2 mediates induction by two different signal-transduction pathways: Protein kinase C and cAMP. *Cell* 51: 251–260.

Kelleher, R.J. III., P.M. Flanagan, and R.D. Kornberg. 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. *Cell* 61: 1209–1215.

Krizman, D.B., B.C. Giovanella, and M.A. Tainsky. 1990. Susceptibility for N-ras-mediated transformation requires loss of tumor suppressor activity. *Somat. Cell. Mol. Genet.* 16: 115–127.

Laemmli, U.K. 1970. Cleaveage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.

Leask, A., C. Byrne, and E. Fuchs. 1991. Transcriptional factor AP2 and its role in epidermal-specific gene expression. *Proc. Natl. Acad. Sci.* 88: 7948–7952.

Lee, W., A. Haslinger, M. Karin, and R. Tjian. 1987. Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionin gene and SV40. *Nature* 325: 368–372.

Luckow, B. and G. Schütz. 1987. CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. *Nucleic Acids Res.* 15: 5490.

Lüscher, B., P.J. Mitchell, T. Williams, and R. Tjian. 1989. Regulation of transcription factor AP-2 by the morphogen retinoic acid and by second messengers. *Genes & Dev.* 3: 1507–1517.

Medema, R.H. and J.L. Bos. 1993. The role of p21ras in receptor tyrosine kinase signaling. *CRC Crit. Rev. Oncogene* 4: 615–661.

Mitchell, P.J., C. Wang, and R. Tjian. 1987. Positive and negative regulation of transcription in vitro: Enhancer-binding protein AP-2 is inhibited by SV40 T antigen. *Cell* 50: 847–861.

Miyamoto, M., T. Fujita, Y. Kimura, M. Maruyama, H. Harada, Y. Sudo, T. Miyata, and T. Taniguchi. 1988. Regulated expression of a gene encoding a nuclear factor, IRF-1, that specifically binds to IFN-β gene regulatory elements. *Cell* 54: 503–513.

Montminy, M.R. and L.M. Bilezikjian. 1987. Binding of a nucleo-protein to the cyclic AMP responsive element of the somatomatotin gene. *Nature* 328: 175–178.

Olson, E.N., G. Spizz, and M.A. Tainsky. 1987. The oncogenic forms of N-ras or H-ras prevent skeletal myoblast differentiation. *Mol. Cell. Biol.* 7: 2104–2111.

Owen, R.D. and M.C. Ostrowski. 1990. Transcriptional activation of a conserved sequence element by ras does not require c-fos or c-jun. *Proc. Natl. Acad. Sci.* 87: 3866–3870.

Owen, R.D., D.M. Bortner, and M.C. Ostrowski. 1990. ras oncogene activation of a VL30 transcriptional element is linked to transformation. *Mol. Cell. Biol.* 10: 1–9.

Pollock, R. and R. Treisman. 1990. A sensitive method for the determination of protein-DNA binding specificities. *Nucleic Acids Res.* 18: 6197–6204.

Ptashne, M. 1988. How eukaryotic transcriptional activators work. *Nature* 335: 683–689.

Sadowski, L., J. Ma, S. Trizenberg, and M. Ptashne. 1988. GAL4VP16 is an unusually potent transcriptional activator. *Natura* 335: 563–564.
N-ras oncogene modulates transcription factor AP-2

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Santos, E. and A.R. Nebra. 1989. Structural and functional properties of ras proteins. FASEB J. 3: 2151–2163.

Sen, R. and D. Baltimore. 1986. Inducibility of κ immunoglobulin enhancer-binding protein NF-kB by a post-translational mechanism. Cell 47: 921–928.

Tainsky, M.A., C.S. Cooper, B.C. Giovanella, and G.F. Vande Woude. 1984. An activated N-ras gene: Detected in late but not early passage human PA-1 teratocarcinoma cells. Science 225: 643–645.

Tainsky, M.A., D.B. Krizman, P.J. Chiao, S.O. Yim, and B.C. Giovanella. 1988. PA-1, a human model for multistage carcinogenesis: Oncogenes and other factors. Anticancer Res. 8: 899–914.

Tainsky, M.A., S.O. Yim, D.B. Krizman, P. Kannan, P.J. Chiao, T. Mukhopadhyay, and R. Buettner. 1991. Modulation of differentiation in PA-1 human teratocarcinoma cells after N-ras oncogene-induced tumorigenicity. Oncogene 6: 1575–1582.

Treisman, R. 1986. Identification of a protein-binding site that mediates transcriptional response of the c-fos gene serum factors. Cell 46: 567–574.

Weinberg, R.A. 1992. The integration of molecular genetics into cancer management. Cancer 70: 1653–1658.

Williams, T. and R. Tjian. 1991a. Analysis of the DNA-binding and activation properties of the human transcription factor AP-2. Genes & Dev. 5: 670–682.

——. 1991b. Characterization of a dimerization motif in AP-2 and its function in heterologous DNA-binding proteins. Science 251: 1067–1071.

Williams, T., A. Admon, B. Luscher, and R. Tjian. 1988. Cloning and expression of AP-2, a cell-type-specific transcription factor that activates inducible enhancer elements. Genes & Dev. 2: 1557–1569.

Yamaguchi-Iwai, Y., M. Sataka, Y. Murakami, M. Sakai, M. Muramatsu, and Y. Ito. 1990 Differentiation of F9 embryonal carcinoma cells induced by the c-jun activated c-Ha-ras oncogenes. Proc. Natl. Acad. Sci. 87: 8670–8674.

Yamamoto, D.R. 1985. Steroid receptor regulated transcription of specific genes and gene networks. Annu. Rev. Genet. 19: 209–252.

Zeuthen, J., J.D.R. Norgaard, P. Avner, M. Fellows, J. Wartiovaara, A. Vaheri, R. Rosen, and B.C. Giovanella. 1980. Characterization of a human teratocarcinoma derived cell line. Int. J. Cancer 25: 19–32.
N-ras oncogene causes AP-2 transcriptional self-interference, which leads to transformation.

P Kannan, R Buettner, P J Chiao, et al.

*Genes Dev.* 1994, 8:
Access the most recent version at doi:10.1101/gad.8.11.1258