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Transfected Human β-Polymerase Promoter Contains a *ras*-Responsive Element

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β-Polymerase is a vertebrate cellular DNA polymerase involved in gap-filling synthesis during some types of genomic DNA repair. We report that a cloned human β-polymerase promoter in a transient expression assay is activated by $p21^{v\text{-}ras^H}$ expression in NIH 3T3 cells. A decanucleotide palindromic element, GTGACGTCAC, at positions −49 to −40 in the promoter is required for this *ras*-mediated stimulation.

DNA β-polymerase (β-pol) is a "housekeeping" enzyme with extensive primary structure conservation among vertebrate homologs. The enzyme has been implicated in short-patch DNA repair after some types of DNA damage and is generally regarded as one of the DNA repair polymerases of vertebrate cells. The enzyme is specified by a single-copy gene on chromosome 8 in humans (21) and mice (S. H. Wilson, *in* P. Strauss and S. Wilson, ed., *The Eukaryotic Nucleus*, in press), and levels of β-pol mRNA and enzymatic activity remain essentially constant as a function of the cell cycle and of the growth phase in cultured human cells (35). However, some human cell lines, such as the teratocarcinoma cell line NTera2D, contain higher levels of β-pol mRNA than do other cell lines (35, 36; Wilson, in press), and some rodent tissues, such as testis and brain tissues, contain much higher levels of β-pol mRNA than do other tissues (Wilson, in press). In addition to this tissue- and cell-specific expression, β-pol mRNA levels increase shortly after treatment of CHO cells with certain DNA-damaging agents, including *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (10). To gain insight into mechanisms of β-pol gene expression, several groups of investigators have cloned segments of mammalian β-pol genes and mapped functional promoter sequences 5′ of the major transcription start site (29, 34; Wilson, in press). Widen et al. (29), for example, found that the 114-base-pair sequence 5′ of the major transcription start site in the human β-pol gene can serve as a promoter in directing transient expression of the bacterial chloramphenicol acetyltransferase (CAT) gene in HeLa cells. In addition, this fusion gene is transcriptionally activated by cotransfection with an expression plasmid for the adenovirus E1A and E1B proteins (29) and also is transcriptionally activated by MNNG treatment of cells (P. Kedar and S. H. Wilson, submitted for publication). The mechanism of this promoter activation in each case is not clear, but the promoter could be activated through alterations in the state of phosphorylation of proteins that regulate transcription (1, 23, 27, 28).

From their peripheral location in the cell, *ras* proteins are believed to help mediate transmission of growth signals from the membrane to the nucleus via activation of downstream messengers, leading to alterations in gene expression (2, 25). Recent studies have shown that *ras*-induced activation of protein kinase C (pKC) is one important distal messenger pathway for *ras* transformation (13–19, 28), among other pathways. While pKC activity may represent a critical component of *ras* transformation, activation of pKC does not completely mimic the transforming activity of *ras*. This observation suggests that pKC-independent pathways may participate in *ras* transformation. Consistent with this hypothesis, it has recently been shown that *ras* could alter *myc* expression, even in cells depleted of pKC (19). Another potentially informative approach for detecting other downstream signals is to determine if promoters that are pKC independent are responsive to *ras*. The β-pol promoter appears to be an example, as it is not responsive in transient expression assays to pKC induction by phorbol ester treatment of cells and protein binding to elements in the β-pol promoter is not inhibited by an oligonucleotide containing an AP-1-binding site, i.e., a pKC-responsive element (8).

We found that normal NIH 3T3 cells transfected (12) with pβP8 express CAT activity, but at relatively low levels. By contrast, pβP8 transfection of *ras*-transformed NIH 3T3 cells (13-3-B4) results in a much higher level of CAT expression. As expected, expression in both cell lines was dependent on the amount of pβP8 used (Fig. 1). These and the other transfections to be described were conducted along with a small amount of internal plasmid control (29), so that any differences in transfection efficiency were eliminated by normalizing CAT activities to the β-galactosidase activity of the control plasmid. In the experiment whose results are shown in Fig. 1a, relative β-galactosidase expression levels per dish were 0.04 and 0.19 for NIH 3T3 and 13-3-B4 cells, respectively; therefore, the absolute activity of the β-pol fusion gene per dish was higher than the values shown in Fig. 1a. For comparison, additional mouse NIH 3T3 cell lines representing examples of other transformation phenotypes were tested. These lines were (i) SRD, which expresses the v-*src* oncogene product; (ii) SPONT, a spontaneous transformant of NIH 3T3 cells; and (iii) EJ, a line expressing an independently derived version of the ras^H gene. The results are shown in Fig. 1b. EJ cells, which express the highly transforming $c\text{-}ras^{H\text{-}Val\text{-}12}$ gene, showed results similar to those obtained with the $v\text{-}ras^H$ gene-expressing cells, 13-3-B4. On the other hand, transfection of SRD or SPONT cells resulted in the same level of β-pol promoter activity as was found with normal NIH 3T3 cells. These results indicate that activation of the promoter fusion gene is seen with both *ras*-transformed lines but that it is not a general phenomenon of transformed cells.

To further explore the possibility that a highly transforming *ras* gene product is able to activate transcription of the β-pol promoter fusion gene, we conducted cotransfection

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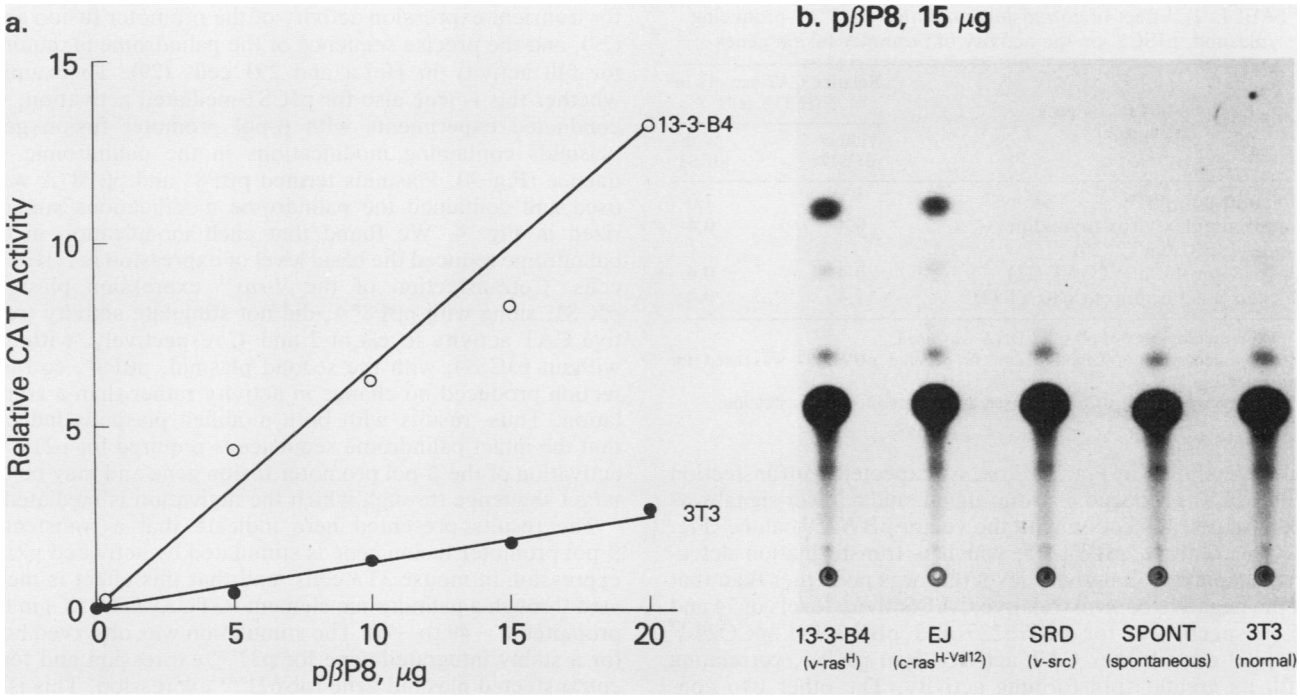

FIG. 1. CAT activity after transfection of β-pol fusion gene pβP8 into two cell lines. (a) pβP8, in the indicated quantities, was transfected into NIH 3T3 and 13-3-B4 cells (4), and equal amounts of cell extract protein eventually were used for measurement of CAT activity (29); results are expressed relative to β-galactosidase activity of the same extract. (b) Autoradiogram illustrating results of transient expression of CAT activity with 15 μg of pβP8 and different cell lines, as indicated. The upper two rows of spots are ^{14}C-labeled acetylated derivatives of chloramphenicol resolved by thin-layer chromatography on a silica gel plate. The cell description is shown in parentheses; the cell lines used were SRD, NIH 3T3 cells transformed by v-*src* (5), NIH EJ cells transformed by the activated c-*ras*^{H} of the EJ bladder carcinoma cell line (7), and spontaneously transformed NIH 3T3 cells (SPONT) (all from A. M. Diamond, University of Chicago).

experiments with normal NIH 3T3 cells and a plasmid retroviral vector (termed pJCS1) capable of transiently expressing the v-*ras*^{H} gene (30) and, as a negative control, with a plasmid vector alone (pBW1594). pJCS1 induced activity of the β-pol fusion gene plasmid (pβP8), whereas control plasmid pBW1594 did not (Fig. 2). To examine the question of promoter specificity for pJCS1 stimulation, we conducted similar cotransfection experiments with CAT fusion genes containing the simian virus 40 promoter pSV2CAT, the herpes simplex virus thymidine kinase promoter pB2CAT2, or the chicken β-actin promoter βprom p8CAT (Table 1). Cotransfection with pJCS1 had little or no effect on the thymidine kinase fusion gene or the simian virus 40 fusion gene and inhibited activity of the chicken β-actin fusion gene. Thus, activation by expression of the v-*ras*^{H} gene, similar to activation seen with the β-pol promoter, is not a general property of promoter fusion genes.

A cotransfection experiment was conducted to evaluate the effects of various modifications in the v-*ras*^{H} gene and/or expression construct (Fig. 3). These modifications and plasmids were: (i) a defective point-mutated p21$^{v\text{-}ras^{H}}$ gene (encoding Ser-186 in place of the normal Cys-186) otherwise identical with pJCS1, termed pBW1225; (ii) a Harvey murine sarcoma virus *ras* DNA variant termed pCO24-I′ that is more active biologically than wild-type Harvey murine sarcoma virus as a result of noncoding substitution (166 bp of the c-*ras* 5′ noncoding sequence substituted for 148 bp of the usual v-*ras*^{H} 5′ noncoding sequence); (iii) a v-*ras*^{H} gene promoted by a Friend murine leukemia virus long terminal repeat, termed pBW1670; and (iv) a biologically active v-*ras*^{H} insertion-deletion mutant termed pBW739. The re-

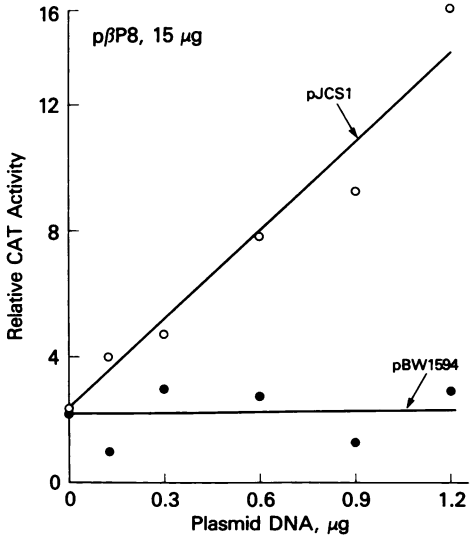

FIG. 2. Cotransfection of NIH 3T3 cells with 15 μg of pβP8 and different amounts of the p21$^{v\text{-}ras^{H}}$-expressing plasmid (pJCS1) or the vector alone (pBW1594), as indicated. After thin-layer chromatography, spots with acetylated derivatives of chloramphenicol were cut from the plate and counted. The negative control plasmid pBW1594, which was constructed by Berthe M. Willumsen, lacks the *Bam*HI fragment containing all v-*ras*^{H} coding sequences of pJCS1.

TABLE 1. Effect of cotransfection with a p21$^{v\text{-}ras^H}$-producing plasmid, pJSC1, on the activity of promoter fusion genes

Promoter and fusion gene[a] (reference)	Relative CAT activity in NIH 3T3 cells	
	Without pJCS1[b]	With pJCS1[c]
Human β-pol; pβP8	2.1	7.0
Herpes simplex virus thymidine kinase; pBLCAT2 (20)	0.4	0.4
Simian virus 40; pSV2CAT (11)	0.4	0.4
Chicken β-actin; βprom p8CAT (3)	1.4	0.4

[a] Three micrograms of plasmid DNA was used.
[b] One microgram of negative control plasmid pBW1594 was used for cotransfection.
[c] One microgram of pJCS1 was used as indicated for cotransfection.

sults are shown in Fig. 3. First, as expected, cotransfection with pJCS1 produced a strong signal, and a lower signal was seen for cotransfection with the vector pBW1594 alone (Fig. 3). By contrast, pBW1225, which is transformation defective, displayed an activity level that was no higher than that of the negative control (relative CAT activity levels of 14 and 15, respectively, for pBW1225 and pBW1594). pCO24-I′ induced even higher CAT activity than pJCS1, correlating with its greater transforming activity. The other two constructs produced results similar to those obtained with pJCS1. These transfection results, therefore, appear to parallel the relative transforming activity levels of the various plasmids (24, 26, 31).

The decanucleotide palindromic element spanning residues −49 to −40 of the β-pol promoter is an important region for transient expression activity of the promoter fusion gene (29), and the precise sequence of the palindrome is required for full activity in HeLa and 293 cells (29). To examine whether this is true also for pJCS1-mediated activation, we conducted experiments with β-pol promoter fusion gene plasmids containing modifications in the palindromic sequence (Fig. 4). Plasmids termed pβP8* and pβP8*A were used and contained the palindrome modifications summarized in Fig. 4. We found that each modification in the palindrome reduced the basal level of expression in NIH 3T3 cells. Cotransfection of the v-*ras*^{H} expression plasmid, pJCS1, along with pβP8*A, did not stimulate activity (relative CAT activity levels of 2 and 4, respectively, with and without pJCS1); with the second plasmid, pβP8*, cotransfection produced no change in activity rather than a stimulation. Thus, results with both modified plasmids indicate that the intact palindrome sequence is required for p21$^{v\text{-}ras^H}$ activation of the β-pol promoter fusion gene and may be the target sequence through which the activation is mediated.

The results presented here indicate that a transfected β-pol promoter fusion gene is stimulated by activated p21^{ras} expression in mouse 3T3 cells, and that this effect is mediated through a palindromic element (GTGACGTCAC) in the promoter at −49 to −40. The stimulation was observed both for a stably integrated gene for p21^{ras} expression and for a cotransfected plasmid gene for p21^{ras} expression. This is of interest in understanding both general mechanisms of promoter responsiveness to ^{ras} and the mechanism of the β-pol promoter, particularly in view of the fact that the β-pol promoter-CAT fusion gene used here is strongly up regulated by another viral oncogene transactivator, the adenovirus E1A and E1B proteins (29). It is reasonable to suspect

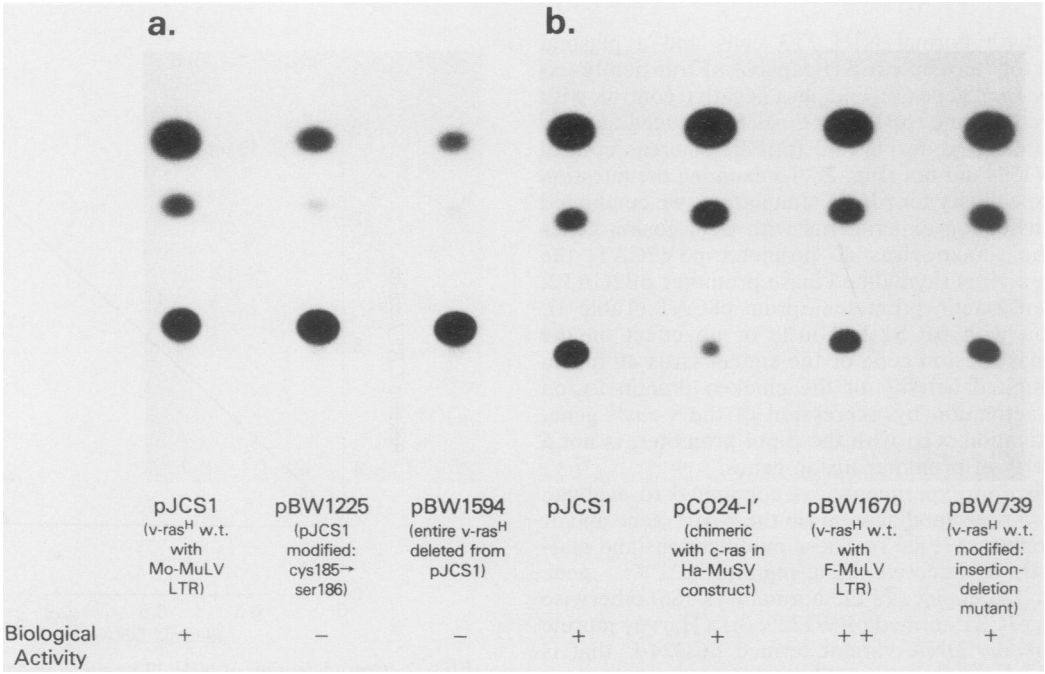

FIG. 3. Autoradiograms illustrating results of transient expression of CAT activity after cotransfection of NIH 3T3 cells with the β-pol promoter fusion gene pβP8 and 1 μg of the p21$^{v\text{-}ras^H}$ protein expression construct, as indicated. pJCS1 is the usual construct for p21$^{v\text{-}ras^H}$ expression. The other plasmids are described in the text and by annotation in the figure; Biological Activity refers to transforming activity of the *ras* gene or plasmid (9, 24, 26, 30–33). The upper two rows of spots are acetylated derivatives of chloramphenicol and were cut and counted. Abbreviations: w.t., wild type; Mo-MuLV, Moloney murine leukemia virus; LTR, long terminal repeat; Ha-MuSV, Harvey murine sarcoma virus; F-MuLV, Friend murine leukemia virus. Symbols: −, no transforming activity observed; +, transforming activity observed (number of plus signs indicates level of activity).

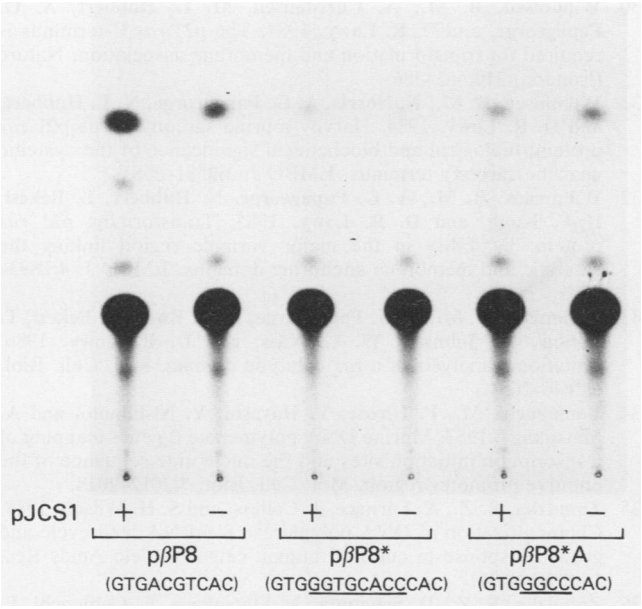

FIG. 4. Autoradiogram showing transient expression of CAT activity by wild-type (pβP8) and two modified β-pol fusion genes (22) cotransfected with pJCS1 in NIH 3T3 cells. The NIH 3T3 cells were transfected with 15 μg of pβP8, pβP8*, or pβP8*A as described elsewhere (29). The sequence of the decanucleotide palindrome at residues −49 to −40 in the human β-pol promoter is shown in parentheses, and altered residues in the two modified plasmids are underlined. Cotransfection with pJCS1 had no effect on the basal activity of either modified fusion gene, as determined by cutting and counting the acetylated derivatives. Symbols: +, cotransfection with pJCS1; −, no cotransfection with pJCS1.

that the mechanism of the activation is secondary to alterations in the state of phosphorylation of cellular transcription factors rather than direct binding by the oncogene protein to the β-pol promoter. It is possible that proteins involved in the signal transduction system, such as $p21^{v\text{-}ras^H}$, exert regulation over a variety of DNA metabolism enzymes through phosphorylation or dephosphorylation of proteins that bind to critical promoter sequence elements, such as the GTGACGTCAC palindrome of the β-pol promoter.

Promoters for several other genes, when tested as fusion genes, are stimulated by activated $p21^{ras}$ expression. These promoters also are stimulated by treatment of cells with the phorbol ester tumor promoter agent 12-*O*-tetradecanoylphorbol-13-acetate (TPA), but not by treatment with cyclic AMP, and this distinction has provided a general descriptive classification for several mammalian genes (e.g., reference 6). In contrast, with the human β-pol promoter there is no indication that the cloned promoter fusion gene or the endogenous gene is stimulated by TPA (Kedar and Wilson, submitted); this is the case despite the presence in the β-pol promoter of the critical palindromic element GTGACGTCAC, which has sequence similarity with the TPA-responsive element of other genes (29). On the premise that the TPA response is mediated by protein AP-1, Englander and Wilson (8) found that nuclear extract protein binding to the β-pol palindromic element is not inhibited by an oligonucleotide corresponding to an AP-1-binding element. Taken together, these results suggest that the β-pol promoter may fall into a class of promoters that are not TPA responsive but are *ras* responsive.

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