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The Upstream Sequence −537 to −278 Is Necessary for Transcription of the Human Nucleolar Antigen p120 Gene

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Two *cis*-acting elements in the p120 gene play important roles in transcription; the region from −537 to −278 is necessary for initiation of transcription, and the region from −1426 to −1223 is necessary for efficient transcription. The distal element(s) which lies upstream of −278 is required for initiation of transcription.

The p120 antigen reported and identified by Freeman et al. (10) is one of the most interesting among the proliferating-cell nucleolar antigens (1, 2, 4, 6, 7, 10, 11, 22–24). It was readily detectable by indirect immunofluorescence in most malignant tumor specimens and tumor cell lines tested (10). Recently, Hazlewood et al. (16) reported that the human tumor cell lines examined contained 15 to 60 times more p120 mRNA than does human term placenta. The p120 antigen is present in neoplastic HeLa and HL-60 cells but not in growth-arrested HeLa cells. However, the p120 antigen was detectable immediately following serum refeeding (10). Another important observation was that the p120 antigen was not detectable in retinoic acid-treated HL-60 cells following morphological differentiation (10). These results indicate that the p120 antigen appears early in the G_1 phase of the cell cycle and is associated with proliferation. Ochs et al. (20) reported that the p120 antigen was localized to the microfibrillar structure of the nucleolus and suggested that the p120 antigen might be a component of the nucleolar matrix of the highly pleomorphic and functionally hyperactive nucleoli of tumor cells (3, 5).

Recently, Fonagy et al. (9) determined the p120 cDNA sequence and R. G. Larson (unpublished data) analyzed the genomic structure of the p120 gene. The protein sequence (deduced from the cDNA sequence) contains four consecutive major domains: a basic domain; an acidic domain; a hydrophobic, methionine-rich domain; and a domain rich in cysteine and proline residues.

Since protein p120 is found in various types of tumor cells, studies of its role in proliferation and tumorigenesis, along with studies of its regulation in tumor cells, are in progress. The purpose of this study is to define *cis*-acting elements of the p120 gene in order to develop information on the regulation of this gene during the early G_1 phase of the cell cycle.

To define the necessary 5′-flanking sequence of the p120 gene, a hybrid gene, −2532/+98 CAT, was constructed by introducing the fragment −2532 to +98 (numbering is with reference to ATG) into the *Sma*I site of pΔCAT. (The chloramphenicol acetyltransferase gene, including the simian virus 40 splice and the polyadenylation signal, was introduced into the *Bam*HI site of the pUC 19 vector.) This hybrid construct was then transfected into HeLa cells by the calcium phosphate precipitation method (14). Transfections were done with pRSV CAT (13) and pΔCAT as positive and negative controls, respectively. Equimolar (equivalent to 10 μg of pΔCAT) amounts of all test plasmids were cotransfected along with 2 μg of phMThGH (human growth hormone under the control of the metallothionein IIA promoter [12]) as an internal control. CAT assays (13) after transfection and then 40 h of incubation showed that the sequence from −2532 to +98 is able to drive transcription at about 25% of the efficiency of pRSV CAT (result of four independent experiments; data not shown).

Exonuclease III digestion was done to make deletions from the 5′ end (−2532) of the p120 promoter. Deletion results (Table 1) show that the efficiency of transcription remains the same for deletions up to −2192 (p−2192/+98 CAT) when compared with that of the −2532/+98 CAT construct. Further deletions toward the 3′ end resulted in a gradual decrease in transcriptional activity. For example, the −1559/+98 construct retained about 75% activity, the −1240/+98 construct retained about 69% activity, the −574/+98 construct retained about 29% activity, and the −280/+98 construct retained only 2% activity. Accordingly, sequences upstream of −280 were required for the initiation of transcription and for efficient transcription of the p120 gene.

Figure 1A shows the strategy of construction of internal deletion mutants of the −2532/+98 CAT construct. Transfection of these constructs into HeLa cells, followed by CAT analyses, suggests that there are three important regions between −1426 and +98 (Fig. 1). Deletion of the sequence −278 to −1 from the p−2532/+98 CAT construct totally abolished the promoter activity (CAT activity is the same as that of pΔCAT). This region contains a TATAA box element, and the transcription initiation sites lie within this region (see Fig. 3). Deletion of the sequence −537 to −278 from the p−2532/+98 CAT construct also abolished transcription, showing that this region is essential. A GC-rich region, GGCGGGGCCGCG (−422 to −411), is found within the sequence from −537 to −278 and may be a site for Sp1 transcription factor binding. Sp1 transcription factor is known to play an important role in the transcription of a variety of genes (8, 17, 19). The third important region is between −1426 and −1223 (see CAT analyses of p−902/−537, p−1223/−933, and p−1426/−537 in Fig. 1A). The region from −1426 to −1223 contributes about 50% of the overall transcription efficiency.

To further define the *cis*-acting regions −537 to −278 and −1426 to −1223, these sequences were introduced upstream, in both orientations, into the *Xba*1 site of the vector-polylinker sequence upstream of the thymidine kinase (TK) promoter CAT construct (pTK CAT). pTK CAT contains the CAT coding sequence under the control of the herpesvirus TK promoter (−109 to +51) cloned into the

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TABLE 1. Identification of *cis*-acting elements in p120 gene transcription

CAT construct[a]	Relative transcription[b]
−2532/+98	100
−2192/+98	98.7 ± 31.4
−2032/+98	72.6 ± 13.8
−1803/+98	87.1 ± 17.9
−1559/+98	75.5 ± 8.2
−1451/+98	67.6 ± 37
−1240/+98	69.0 ± 18.9
−1035/+98	49.2 ± 22.1
−804/+98	33.6 ± 8.6
−574/+98	29.3 ± 3.2
−280/+98	1.9 ± 0.9
−200/+98	2.6 ± 1.4

[a] CAT constructs were cotransfected with human growth hormone expression vector, phMThGH, as an internal control. CAT assays were corrected for transfection efficiency.

[b] Means ± standard deviations of four independent experiments.

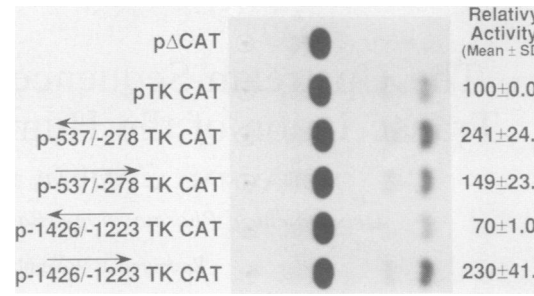

FIG. 2. Effect of *cis*-acting elements −537 to −278 and −1426 to −1223 on the transcription of heterologous TK promoter. The *cis*-acting regions were placed, in both orientations, upstream of pTK CAT. →, Orientation of the inserted *cis*-acting regions. Transcription efficiencies of these hybrid promoters were analyzed by CAT assay following transfection of these constructs into HeLa cells. Relative activity values are means ± standard deviations (SD) of four independent experiments.

*Bam*HI site of the pUC 19 vector. The region from −537 to −278 was able to stimulate transcription of the TK promoter independent of the orientation of the sequence (Fig. 2). The region from −1426 to −1223 increased transcription of the TK promoter only when the sequence was in the regular orientation (Fig. 2).

Primer extension (18) was used to locate the transcription initiation site of the p120 gene. Two micrograms of poly(A)$^+$ RNA was hybridized with an end-labeled complementary primer (5′ CCTTTCGGCCTGGCCCCCGCTTCTCC 3′, +26 nucleotides downstream of ATG), primer extension was done by using 40 U of avian myeloblastosis virus reverse transcriptase for 90 min at 42°C, and analysis was done on a sequencing gel. The primer extension results suggest multiple transcription initiation at −21 (nucleotide A), −30 (nucleotide C), and −43 (nucleotide A) upstream of ATG (Fig. 3). These transcription initiation sites are +35, +48, and +57 downstream of the TATAA box (−78 to −82), respectively. Transcription from the CAT constructs may be from the same initiation sites, since deletion of this region abolishes transcription completely (see result of pΔ −278/−1 in Fig. 1). Multiple transcription initiation sites have been reported for other genes (8, 15, 21).

This study on *cis*-acting elements of the p120 gene will facilitate further studies related to the signal(s) or the *trans*-acting factor(s) which controls the expression of this gene in the early G_1 phase of the cell cycle.

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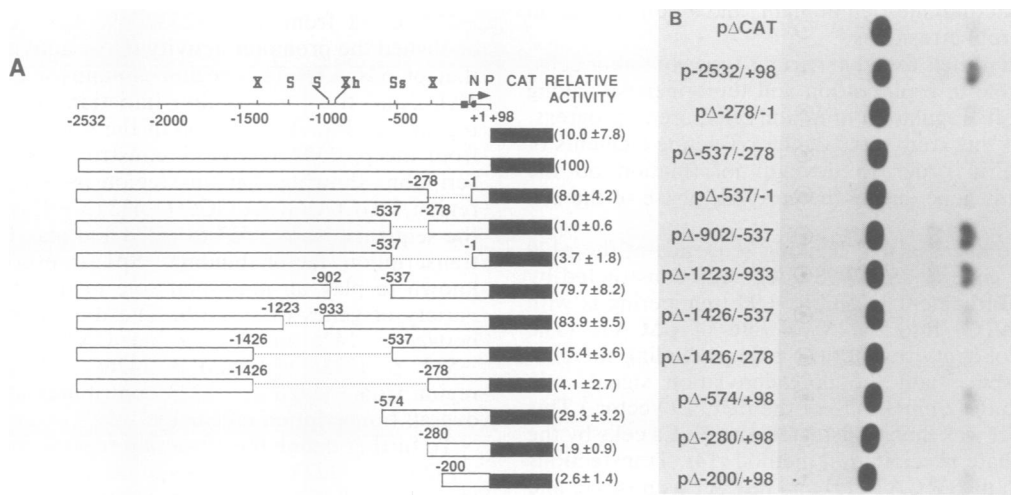

FIG. 1. (A) Effect of internal deletions on transcription efficiency. Internal deletion mutants and a few 5′ deletion mutants of the −2532/+98 CAT construct are presented schematically. The relative transcription efficiencies obtained from CAT assays are shown in parentheses (means ± standard deviations of four independent experiments). Symbols: □, p120 promoter; ■, CAT coding sequence; ■, TATAA box; ●, ATG. X, *Xba*I; S, *Sma*I; Xh, *Xho*I; N, *Nco*I; P, *Pst*I. (B) Representative CAT assay done with the internal deletion mutants and some of the 5′ deletion mutants shown in panel A.

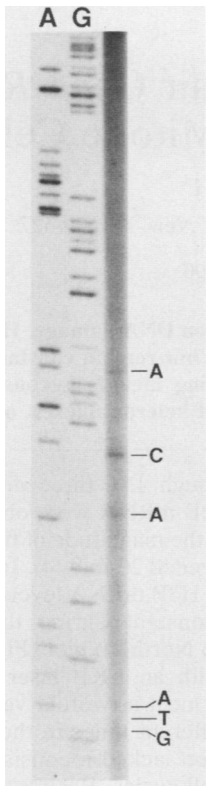

FIG. 3. Transcription initiation site(s) of the p120 gene. Transcription initiation sites were mapped by primer extension. Lanes A and G, Sequence reactions with the same primer that was used for primer extension; right lane, primer-extended products.

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