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Genetics

# Characterization of a splicing mutation in group A xeroderma pigmentosum

**(RNA processing/restriction fragment length polymorphism)**

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**ABSTRACT** **The molecular basis of group A xeroderma pigmentosum (XP) was investigated by comparison of the nucleotide sequences of multiple clones of the XP group A complementing gene (*XPAC*) from a patient with group A XP with that of a normal gene. The clones showed a G → C substitution at the 3′ splice acceptor site of intron 3, which altered the obligatory AG acceptor dinucleotide to AC. Nucleotide sequencing of cDNAs amplified by the polymerase chain reaction revealed that this single base substitution abolishes the canonical 3′ splice site, thus creating two abnormally spliced mRNA forms. The larger form is identical with normal mRNA except for a dinucleotide deletion at the 5′ end of exon 4. This deletion results in a frameshift with premature translation termination in exon 4. The smaller form has a deletion of the entire exon 3 and the dinucleotide at the 5′ end of exon 4. The result of a transfection study provided additional evidence that this single base substitution is the disease-causing mutation. This single base substitution creates a new cleavage site for the restriction nuclease *Alw*NI. Analysis of *Alw*NI restriction fragment length polymorphism showed a high frequency of this mutation in Japanese patients with group A XP: 16 of 21 unrelated Japanese patients were homozygous and 4 were heterozygous for this mutation. However, 11 Caucasians and 2 Blacks with group A XP did not have this mutant allele. The polymorphic *Alw*NI restriction fragments are concluded to be useful for diagnosis of group A XP in Japanese subjects, including prenatal cases and carriers.**

Xeroderma pigmentosum (XP) is an autosomal recessive human genetic disorder manifested as extreme sensitivity to sunlight resulting in a very high incidence of skin cancer, and frequent neurological abnormalities. XP occurs at a frequency of one to four per million live births and its carrier frequency is estimated as 0.2–0.4% in the general population (1). Cells from XP patients are hypersensitive to the lethal effect of UV radiation because they have a defective DNA repair system (2). Nine complementation groups have been identified to date (3). XP cells in complementation groups A through H have defects in the excision repair system (4). The XP variant was first thought to have impaired postreplication repair (4), but recent studies have suggested that it also has a defect in excision repair (5, 6). The molecular basis of XP complementation groups has not been characterized: neither the genes nor the gene products have been identified. In a previous study, we cloned a mouse DNA repair gene that complements the defect of group A XP and named it the *XPAC* gene (7). Recently, we cloned a human *XPAC* cDNA that encodes 273 amino acid residues (21) and a human *XPAC* gene that is about 25 kilobases (kb) long and is split into six exons (I.S., K. Iwai, K.T., and Y.O., unpublished data). In this study, we report the characterization of a splicing mutation in the *XPAC* gene from a Japanese patient with group A XP who had typical symptoms. We have detected a high frequency of this mutation in Japanese patients with group A XP.

## MATERIALS AND METHODS

**Cells.** Forty-two XP cell strains and 4 cell strains that have normal excision repair were examined (Table 1). Primary skin fibroblasts and peripheral blood lymphocytes from patients with XP were transformed by simian virus 40 (SV40) or Epstein–Barr virus. Several SV40-transformed fibroblast cell lines were provided by the following individuals: Masayoshi Namba [Okayama University, Okayama (WI38VA13; normal)]; Hiraku Takebe and Mitsuo Ikenaga [Kyoto University, Kyoto (XP2OSSV and XP2YOSV; group A and group F]; Yoshisada Fujiwara [Kobe University, Kobe (XP24-KOSV; group E)]; Dirk Bootsma and Jan H. J. Hoeijimakers [Erasmus University, Rotterdam (XP12ROSV and XP8-LOSV; group A)]; and B. Klein [State University Leiden, The Netherlands (XP3BRSV; group G)]. XP35OS (group A), XP4OS (group A), XP39OS (group A), XPEMB-1 (group A), XP15OS (group A), and XP2SA (variant) were provided by the Japanese Cancer Research Resources Bank, Tokyo. The following cells were purchased from NIGMS Human Genetic Mutant Cell Repository (Camden, NJ): GM130 (normal), GM131 (normal), XP12BE (group A), GM82 (group A), GM544B (group A), GM710A (group A), GM1630 (group A), GM2009 (group A), GM2033 (group A), GM2062 (group A), GM2090 (group A), GM2990 (group A), GM2994 (group A), GM 5292 (group A), GM2252 (group B), GM2246 (group C), GM2486 (group D), and GM3248 (group H). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) or RPMI 1640 medium containing 5% or 10% fetal bovine serum.

**Extractions of DNA and RNA.** Total genomic DNA was extracted from the cultured cells as described by Maniatis *et al.* (8). Total RNA was purified as described (9) and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography (10).

**Filter Hybridization.** Restriction enzyme-digested DNA and poly(A)$^+$ RNA were partitioned in agarose gel, transferred to a nitrocellulose sheet (Schleicher & Schuell) or a nylon membrane (Hybond-N, Amersham), and hybridized to random-primer-extended probes (Amersham) as recommended by the suppliers.

**Isolation and Sequencing of a Mutant Genomic *XPAC* Gene.** A phage DNA library of XP2OSSV cells was constructed as

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Abbreviations: XP, xeroderma pigmentosum; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

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Table 1. *Alw*NI restriction fragment length polymorphism (RFLP) in 42 patients with XP and 4 normal controls

| Subject | Genotype* | Subject | Genotype* |
|---|---|---|---|
| Normal control | | Group A XP | |
| WI38VA13 | +/+ | Caucasian | |
| HeLa | +/+ | GM82 | +/+ |
| GM130 | +/+ | GM544B | +/+ |
| GM131 | +/+ | GM710A | +/+ |
| Group A XP | | GM1630 | +/+ |
| Japanese | | GM2009 | +/+ |
| XP2OS | −/− | GM2062 | +/+ |
| XP6TO | −/− | GM2990 | +/+ |
| XP7TO | −/− | GM2994 | +/+ |
| XP31TO | −/− | XP12RO | +/+ |
| XP32TO | −/− | XP12BE | +/+ |
| XP35TO | −/− | XP8LO | +/+ |
| XP54TO | −/− | Black | |
| XP78TO | −/− | GM2090 | +/+ |
| XP84TO | −/− | GM2033 | +/+ |
| XP87TO | −/− | Group B XP | |
| XP96TO | −/− | GM2252 | +/+ |
| XP3OS | −/− | Group C XP | |
| XP10OS | −/− | GM2246 | +/+ |
| XP15OS | −/− | Group D XP | |
| XP35OS | −/− | GM2486 | +/+ |
| XPEMB-1 | −/− | Group E XP | |
| XP67TO | +/− | XP24KO | +/+ |
| XP75TO | +/− | Group F XP | |
| XP27OS | +/− | XP2YO | +/+ |
| GM5292 | +/− | Group G XP | |
| XP39OS | +/+ | XP3BR | +/+ |
| | | Group H XP | |
| | | GM3248 | +/+ |
| | | Variant XP | |
| | | XP2SA | +/+ |

*A plus sign denotes the presence of a 5.2-kb *Alw*NI band, and a minus sign the presence of a 0.7-kb *Alw*NI band.

described (8) using EMBL3 phage vector. Phage plaques were screened with the $^{32}$P-labeled human *XPAC* cDNA probe, and several corresponding genomic clones were isolated. For identification of the mutation causing XP, regions containing exons, including the flanking introns and 5′ flanking region, were subcloned into the vector M13. Single-stranded M13 templates were sequenced by the dideoxy chain-termination method (11).

**Amplification of *XPAC* cDNAs of XP2OS and Sequences of Exon 4-Containing Flanking Introns by the Polymerase Chain Reaction (PCR).** The first strand of the cDNA of XP2OS was generated by reverse transcription of mRNA. The oligonucleotide primers, designed to contain an *Eco*RI restriction site at their 5′ end, were primer C1 from exon 1, 5′-GGGAATTCACGGCGGCTGCGGCTACTGG-3′, and primer C2 from exon 6, 5′-GGGAATTCAAGGACCAATCTAAATTTCC-3′. The primers were synthesized in an Applied Biosystems 380A DNA synthesizer by the methoxy phosphoramidite method and purified with oligonucleotide purification cartridges (Applied Biosystems) by the procedures recommended by the suppliers. Amplifications with *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin–Elmer/Cetus) were performed in an automatic thermocycler (PTC-100, MJ Research, Watertown, MA) as described (12). An aliquot of the PCR product was analyzed by gel electrophoresis, ethidium bromide staining, and Southern blot hybridization with the $^{32}$P-labeled human *XPAC* cDNA probe. Two amplified fragments were detected by this analysis. The fragments were subcloned into the vector M13 and 10 independent subclones per fragment were sequenced. The oligonucleotide primers complementary to the sequences flanking exon 4, containing an *Eco*RI restriction site, were primer 41, 5′-GGGAATTCTTGCTGGGCTATTTGCAAAC-3′, and primer 42, 5′-GGGAATTCGCCAAACCAATTATGACTAG-3′ (see Fig. 6*C*). Amplifications were performed as described (12).

**DNA Transfection.** Samples of $10^6$ XP2OSSV cells were seeded into 100-mm Petri dishes (Corning 25020) in DMEM containing 10% fetal bovine serum. The next day, the cells were cotransfected with DNAs (5 μg each) from phage clones and pSV2neo (2 μg) by the calcium phosphate precipitation method (13). After incubation for 2–3 weeks in selection medium (14), the pSV2neo-transformed XP colonies formed were UV-irradiated (4 J/m$^2$) (Toshiba germicidal lamp; 1.2 J/m$^2$ per sec) three times at intervals of 4 days.

## RESULTS

**Identification of a Gene Mutation in XP2OS.** Northern blot analysis showed that the *XPAC* mRNA of XP2OS is smaller than that of normal controls and is markedly reduced in quantity (Fig. 1). Analysis of the *Eco*RI and *Bam*HI restriction patterns of isolated genomic clones of XP2OSSV cells revealed no detectable differences from the wild-type locus (data not shown). These results suggested that the gene mutation in XP2OS is a nonsense mutation or a splicing abnormality. Nucleotide sequencing of multiple genomic clones of XP2OS showed that the mutant gene sequence is identical to that of the normal *XPAC* gene except for a G → C substitution at the 3′ splice acceptor site of intron 3, altering the obligatory AG acceptor dinucleotide to AC (Fig. 2). This single base substitution (GAGATGCTG → CAGATGCTG) creates a new cleavage site for the restriction endonuclease *Alw*NI.

The genomic DNA of XP2OSSV cells digested with *Alw*NI was examined by Southern blot hybridization using the *Pst* I–*Rsa* I fragment of the *XPAC* cDNA as a probe (Fig. 3*B*). Cells that were homozygous for the absence of this mutation gave a 5.2-kb band that hybridized with the cDNA probe. The presence of the new *Alw*NI site resulted in two fragments (4.5 kb and 0.7 kb), of which only the 0.7-kb fragment was detected with the cDNA probe. In XP2OS, the 5.2-kb band was absent and was replaced by a 0.7-kb band (Fig. 3*A*). This result indicates that the patient is homozygous for this mutation.

To elucidate the abnormal RNA splicing of XP2OS, we analyzed the amplified mutant cDNA sequences (Fig. 4*A*). This analysis revealed that a cryptic 3′ splice acceptor site is activated in exon 4, that both the 3′ splice acceptor site of

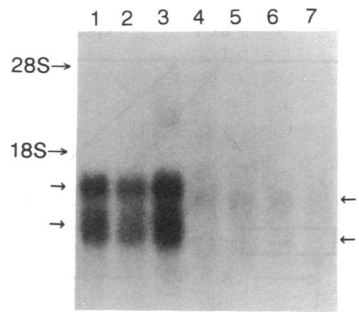



FIG. 1. Northern blot analysis of poly(A)$^+$ RNA (10 μg per lane) from patients with group A XP (lanes 4–7) and normal controls (lanes 1–3) with the *Pst* I–*Rsa* I fragment of the human *XPAC* cDNA as a probe. Lanes: 1, WI38VA13; 2, GM130; 3, GM131; 4, XP2OS; 5, XP78TO; 6, XP84TO; 7, XP96TO. Two bands in normal controls are due to alternative polyadenylylation (21). The *XPAC* mRNAs of group A XP cells were smaller than that of normal controls, and amounts were markedly reduced. We thought that two weak bands in group A XP cells corresponded to the *XPAC*defII mRNA. The bands representing the *XPAC*defI mRNA were not detected. Positions of 28S and 18S rRNA are shown.

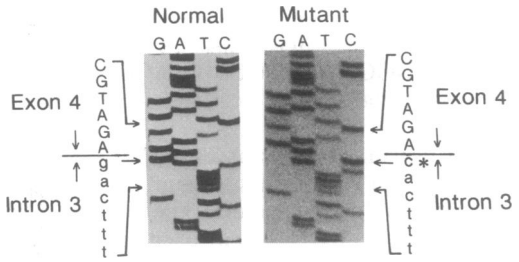



FIG. 2. DNA sequence analysis of the 3′ splice acceptor site of intron 3. This comparison of the normal and mutant (XP2OS) sequences at the intron/exon border shows a single base substitution (asterisk) in the mutant.

intron 2 and the 5′ splice donor site of intron 3 are often abolished, and that as a result two *XPAC* mRNA forms (*XPAC*defI and *XPAC*defII mRNA) are generated. The *XPAC*defI mRNA is identical with the normal *XPAC* mRNA except for a dinucleotide deletion at the 5′ end of exon 4. However, this deletion results in a frameshift and a new stop codon in exon 4. The *XPAC*defII mRNA has a deletion of the entire exon 3 and the dinucleotide of the 5′ end of exon 4 (Fig. 4*B*). These results strongly suggest that this single base substitution is the disease-causing mutation.

**Cotransfection of Normal and Mutant *XPAC* Genes into XP2OSSV Cells.** Cloned normal (EMBL3/IS-7 and EMBL3/IS-30) and mutant (EMBL3/2OS-3 and EMBL3/2OS-1)

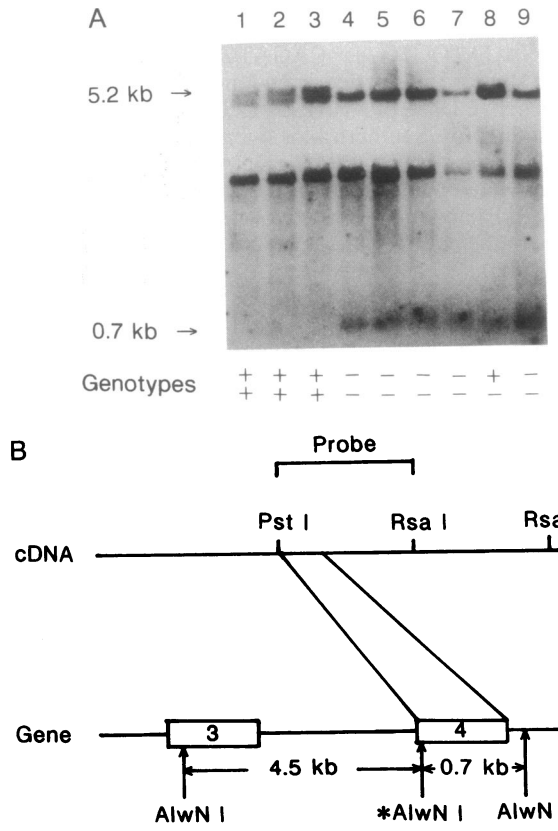
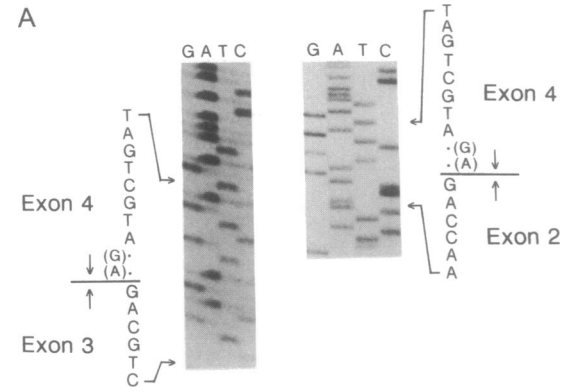



FIG. 3. (*A*) Southern blot analysis of *Alw*NI digests of genomic DNA (10 μg per lane) from patients with group A XP (lanes 4–9) and normal controls (lanes 1–3) with the *Pst* I–*Rsa* I fragment of the *XPAC* cDNA as a probe. Lanes: 1, WI38VA13; 2, GM130; 3, GM131; 4, XP2OS; 5, XP35TO; 6, XP54TO; 7, XP78TO; 8, XP67TO; 9, XP84TO. The genotype of each sample is shown below the autoradiogram; a plus sign denotes the presence of the 5.2-kb band, and a minus sign the presence of the 0.7-kb band. (*B*) Diagram of the locations of the cDNA probe and the new *Alw*NI site (asterisk) generated by the single base substitution.



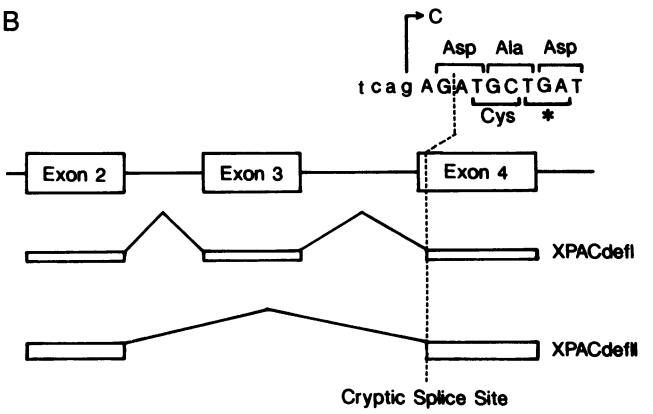




FIG. 4. (*A*) Amplified cDNA sequences derived from XP2OS. Two cDNA forms were obtained. One has a dinucleotide deletion at the 5′ end of exon 4 (*Left*). The other has a deletion of the entire exon 3 and the dinucleotide at the 5′ end of exon 4 (*Right*). The dots indicate the position of the deletion. (*B*) Processing of mutant transcripts. A part of the *XPAC* gene is shown at the top, with exons represented by open boxes and introns by thin lines. The two mRNAs produced by the mutant gene are shown below; exons are shown as open boxes, with splices indicated by bent lines. The nucleotide sequence of the 3′ splice acceptor site of intron 3 is shown at the top. The location of the mutation is shown by an arrow, and that of the 3′ cryptic splice site by a vertical dashed line. The abnormal splicing results in a frameshift and a new stop codon (asterisk) in *XPAC*defI mRNA.

*XPAC* genes were cotransfected in XP2OSSV cells. Phage clone EMBL3/IS-7 contains the 5′ flanking region and exons 1–3. EMBL3/IS-30 contains exons 4–6. The two clones overlap at the 3′ end of intron 3. EMBL3/2OS-3, which contains the 5′ flanking region and exons 1–3, overlaps EMBL3/IS-30 at the 3′ end of intron 3. EMBL3/2OS-1, which contains exons 4–6, overlaps with EMBL3/IS-7 at the

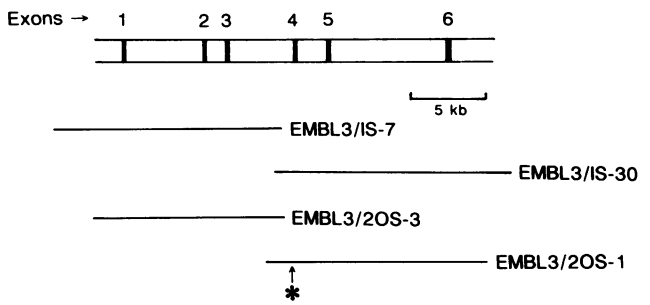



FIG. 5. Physical map of the *XPAC* gene. At the top the entire human *XPAC* gene is shown with exons 1–6 denoted by vertical bars. The normal and mutant phage clones used for the transfection study are shown below. Asterisk indicates the position of the detected point mutation.

Table 2. Cotransfection of normal and mutant *XPAC* genes into XP2OSSV cells

| Donor DNA | No. of colonies per eight dishes | |
|---|---|---|
| | UV-resistant | G418-resistant |
| pSV2neo + EMBL3/IS-7 + EMBL3/IS-30 | 36 | 1410 |
| pSV2neo + EMBL3/2OS-3 + EMBL3/IS-30 | 21 | 867 |
| pSV2neo + EMBL3/IS-7 + EMBL3/2OS-1 | 0 | 1207 |
| pSV2neo (5 μg) | 0 | 2775 |

Two micrograms of pSV2neo and 5 μg of each EMBL3 clone were used, unless otherwise indicated.

3′ end of intron 3 (Fig. 5). Each phage clone (5 μg) was cotransfected with plasmid pSV2neo (2 μg) into $10^6$ XP2OSSV cells (Table 2). Cotransfections of EMBL3/IS-7 and EMBL3/IS-30 and of EMBL3/2OS-3 and EMBL3/IS-30 conferred UV resistance on XP2OSSV cells. On the other hand, no UV-resistant XP colonies were obtained by the cotransfection of EMBL3/IS-7 and EMBL3/2OS-1. This result provides additional evidence that this single base substitution is the disease-causing mutation.

***Alw*NI Endonuclease Restriction Fragment Length Polymorphism (RFLP) in XP.** To determine the frequency of this splicing mutation in XP, we examined *Alw*NI RFLP by Southern blot hybridization of DNA from 34 patients with group A XP (21 Japanese, 11 Caucasians, 2 Blacks), 8 with other groups of XP, and 4 normal controls. Of the 21 unrelated Japanese patients with group A XP, 16 were homozygous and 4 were heterozygous for this mutation. None of the 11 Caucasians or the 2 Blacks with group A XP had this mutant allele. This mutation was also absent from 4 normal controls and 8 patients with other groups of XP (Table 1). The endonuclease *Alw*NI may not cleave the recognition sequence of genomic DNA due to methylation of the recognition site, and such cases would give a 5.2-kb *Alw*NI band. To test this possibility, we examined *Alw*NI RFLP by using the PCR. The nucleotide sequences of exon 4 including the flanking introns were amplified by the PCR, and the amplified DNA was digested with *Alw*NI and run in polyacrylamide gel. DNA from subjects who were homozygous for the absence of this mutation gave a 328-base-pair (bp) band. The presence of the new *Alw*NI site generated two fragments (244 bp and 84 bp) (Fig. 6 *A* and *C*). The results of *Alw*NI RFLP analysis using the PCR were identical with those obtained by Southern blot hybridization. Using the PCR, we also examined *Alw*NI RFLP in the family of XP84TO, a patient who was homozygous for this mutation. The DNAs of both parents gave bands of 328, 244, and 84 bp (Fig. 6*B*), indicating that the parents are heterozygous for this mutation and are obligate carriers of the group A XP trait.

## DISCUSSION

All intronic sequences invariably contain GT and AG nucleotides at their 5′ and 3′ ends, respectively (15). These conserved sequences at the 5′ and 3′ splice sites are thought to be necessary for RNA splicing (16). The branch-point consensus sequence (YNYTRAY) between 18 and 40 nucleotides upstream of the 3′ splice site in all eukaryotic introns is also thought to be important in RNA splicing (17). In this study, we found a G → C substitution in the 3′ splice acceptor site of intron 3. Nucleotide sequencing of the mutant *XPAC* cDNA showed that two abnormally spliced mRNAs (*XPAC*defI and *XPAC*defII) were generated. Other mRNA forms might be generated; however, their amount would be too small to be detected by our method. This result suggests that this mutation causes aberrant RNA splicing. Similar single base substitutions in the 3′ splice acceptor site and consequent abnormal RNA splicing have been reported in β-thalassemia (18) and familial apolipoprotein E deficiency (19). *XPAC*defI mRNA has a dinucleotide deletion at the 5′ end of exon 4 resulting in a frameshift and a new stop codon in exon 4. This change predicts the synthesis of a truncated protein containing only the first 130 of the 273 amino acids of the normal *XPAC* protein. *XPAC*defII mRNA has a deletion of the entire exon 3 and the dinucleotide at the 5′ end of exon 4. This deletion results in loss of 36 amino acid residues. Splicing abnormalities and premature translation termination are considered to cause instability of mRNA (20). In our study, the amount of *XPAC* mRNA was abnormally low in patients with group A XP who were homozygous for this mutation. Furthermore, a transfection study indicated that this mutation is responsible for the lack of activity of the XPAC protein. In summary, these results strongly suggest that this mutation causes abnormal RNA splicing, resulting in instability of the mRNA and loss of activity of the XPAC protein. Therefore, we conclude that the single base substi-

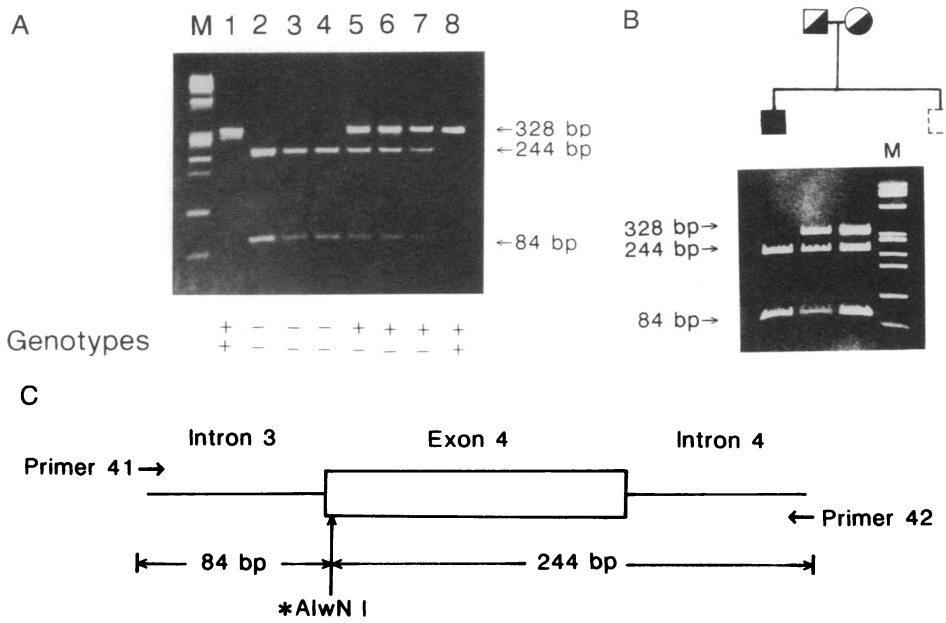



FIG. 6. (*A*) *Alw*NI RFLP analysis of amplified DNAs. Aliquots of PCR products were digested with *Alw*NI and then analyzed by 8% polyacrylamide gel electrophoresis. Lanes: 1, WI38VA13; 2, XP2OS; 3, XP84TO; 4, XP10OS; 5, XP67TO; 6, XP75TO; 7, XP27OS; 8, XP12BE. The marker lane (M) contained a *Hae* III digest of φX174 DNA. The genotypes of *Alw*NI RFLP are shown below the photograph; a plus sign denotes the presence of the 328-bp band, and a minus sign the presence of the 244-bp and 84-bp bands. (*B*) Pedigree of XP84TO analyzed by *Alw*NI polymorphism in amplified DNAs. The marker lane (M) contained a *Hae* III digest of φX174 DNA. (*C*) Diagram of the positions of PCR primers and the location of the new *Alw*NI site (asterisk) generated by the single base substitution.

tution in the 3′ splice acceptor site of intron 3 is the disease-causing mutation.

This single base substitution creates a new cleavage site for the restriction endonuclease *Alw*NI. Analysis of *Alw*NI RFLP showed a high frequency of this mutation in Japanese patients with group A XP: 16 of 21 unrelated Japanese patients with group A XP were homozygous and 4 were heterozygous for this mutation. None of the 11 Caucasians or the 2 Blacks with group A XP examined had this mutant allele. This result suggests that the mutations in almost all of Japanese patients with group A XP have a common origin in preceding generations. Thus, *Alw*NI RFLP should be a very useful genetic marker for diagnosis of group A XP in Japanese subjects, including prenatal cases and carriers.

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1. Peterson, M. C., Gentner, N. E., Middlestadt, M. V. & Weinfeld, M. (1984) *J. Cell. Physiol.* **3,** 45–62.
2. Cleaver, J. E. (1968) *Nature (London)* **218,** 652–656.
3. Fischer, E., Keijzer, W., Thielman, H. W., Popanda, O., Bohnert, E., Edler, E. G., Jung, E. G. & Bootsma, D. (1985) *Mutat. Res.* **145,** 217–225.
4. Friedberg, E. C. (1984) *DNA Repair* (Freeman, New York), pp. 505–525.
5. Kondo, S., Sato, Y. & Kuroki, T. (1987) *Mutat. Res.* **183,** 95–101.
6. Wood, R. D., Robbins, P. & Lindahl, T. (1988) *Cell* **53,** 97–106.
7. Tanaka, K., Satokata, I., Ogita, Z., Uchida, T. & Okada, Y. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5512–5516.
8. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
9. Chirgwin, J. M., Przybyla, A. F., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
10. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
11. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
12. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
13. Corsaro, C. M. & Pearson, M. L. (1981) *Somatic Cell Mol. Genet.* **7,** 603–616.
14. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2072–2076.
15. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50,** 349–383.
16. Treisman, R., Orkin, S. H. & Maniatis, T. (1983) *Nature (London)* **302,** 591–596.
17. Reed, R. & Maniatis, T. (1985) *Cell* **41,** 95–105.
18. Antonarakis, S. E., Orkin, S. H., Cheng, T., Scott, A. F., Sexton, J. P., Trusko, S. P., Charache, S. & Kazazian, H. H., Jr. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1154–1158.
19. Cladaras, C., Hadzopoulou, C. M., Felber, B. K., Pavlakis, G. & Zannis, V. I. (1987) *J. Biol. Chem.* **262,** 2310–2315.
20. Spritz, R. A. & Forget, B. D. (1983) *Am. J. Hum. Genet.* **35,** 333–361.
21. Tanaka, K., Miura, N., Satokata, I., Miyamoto, I., Yoshida, M. C., Satoh, Y., Kondo, S., Yasui, A., Okayama, H. & Okada, Y. (1990) *Nature (London)* **348,** 73–76.