to that of CD40 and CD40L in T cell–dependent antigen activation (*26, 27*). As such, BLyS, its receptor, or related antagonists may find medical utility in the treatment of B cell disorders associated with autoimmunity, neoplasia, or immunodeficiency syndromes.

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28. A full-length BLyS clone was identified, sequenced, and submitted to GenBank (accession number AF132600). BLyS was expressed in baculovirus and purified from Sf9 cell supernatant using a combination of ion exchange (poros HQ-50, poros HS-50, poros PI-50; PE Biosystems, Framingham, MA), size exclusion (Sephacryl S100 HR; Amersham Pharmacia Biotech, Piscataway, NJ), and hydrophobic interaction (Toyopearl Hexyl 650C; Tosohass, Montgomeryville, PA) columns. Purification of BLyS was monitored by SDS-PAGE analysis and the B cell costimulation assay. BLyS was formulated in 0.15 M NaCl and 50 mM sodium acetate at pH 6. Endotoxin concentrations were below the detection limit in the LAL assay (Associates of Cape Cod, Falmouth, MA). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
29. BLyS expression was assessed on the indicated cell types using BLyS-specific mAb 2E5 (IgG1) followed by phycoerythrin (PE)-conjugated F(ab')2 goat antibody to mouse IgG (CALTAG Laboratories, Burlingate, CA). Purified monocytes were cultured in tissue culture–treated plastic wells (Falcon #3043; Becton-Dickinson, Lincoln Park, NJ) for 3 days in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in the presence or absence of IFN-γ (100 U/ml). Comparable results were obtained with monocytes purified from three different donors in three independent experiments. BLyS binding was assessed using rBLyS biotinylated with a *N*-hydroxy-succinimidobiotin reagent (Pierce, Rockford, IL) and PE-conjugated streptavidin (Dako Corp., Glostrup, Denmark).
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32. BALB/cAnNCR mice (6 to 8 weeks old) from Charles River Laboratories were maintained according to recommended standards in microisolator cages with recycled paper bedding (Harlan Sprague-Dawley, Indianapolis, IN) and were provided with pelleted rodent diet (Harlan Sprague-Dawley) and bottled drinking water on an ad libitum basis. The animal protocols used in this study were reviewed and approved by the Human Genome Sciences Institutional Animal Care and Use Committee.
33. Human tonsillar B cells were purified by magnetic bead (MACS) depletion of $CD3^+$ cells. Purified cells were > 95% B cells as assessed by expression of CD19 and CD20. We placed rHuBLyS or the control protein rHuIL-2 into individual wells of a 96-well plate, to which we added $10^5$ B cells suspended in medium (RPMI-1640 containing 10% FBS, $5 \times 10^{-5}$ M 2ME, 100 U/ml penicillin, 100 μg/ml streptomycin, and $10^{-5}$ dilution of SAC or anti-IgM). Proliferation was quantitated by a 20-hour pulse (0.5 μCi/well) of [$^3$H]thymidine (6.7 Ci/mM) beginning 72 hours after factor addition. Analyses of both human and mouse B cell cultures indicated that the proliferative response was evident 24 hours after BLyS addition and progressively increased with a maximal response observed between 72 and 92 hours after culture initiation. The positive and negative controls were IL-2 and medium, respectively. SAC alone yielded background counts of 1427 ± 316. Values are reported as mean ± standard deviation of triplicate wells. Anti-IgM costimulation was performed as described for SAC with the exception that individual wells were precoated with 50 μl of a 10 μg/ml solution of anti-human IgM mAb (IgG1) for 12 hours at 4°C, after which wells were washed before addition of cells. An isotype-matched (IgG1) control antibody was included as a control for nonspecific Ig effects. Similar results were obtained using rBLyS purified from stable CHO transfectants and transiently transfected HEK-293T cells.
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# *hRAD30* Mutations in the Variant Form of Xeroderma Pigmentosum


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**Xeroderma pigmentosum (XP) is an autosomal recessive disease characterized by a high incidence of skin cancers. Yeast *RAD30* encodes a DNA polymerase involved in the error-free bypass of ultraviolet (UV) damage. Here it is shown that XP variant (XP-V) cell lines harbor nonsense or frameshift mutations in *hRAD30*, the human counterpart of yeast *RAD30*. Of the eight mutations identified, seven would result in a severely truncated hRad30 protein. These results indicate that defects in *hRAD30* cause XP-V, and they suggest that error-free replication of UV lesions by hRad30 plays an important role in minimizing the incidence of sunlight-induced skin cancers.**


Xeroderma pigmentosum (XP) patients are hypersensitive to sunlight, and they suffer from a high incidence of skin cancers. Cells from seven different XP complementation groups (A to G) are defective in nucleotide excision repair (*1*), whereas cells from the variant form of XP (XP-V) excise UV photoproducts at a normal rate (*2*). XP-V cells, however, are much slower than normal cells in replicating DNA containing UV photoproducts (*3*), and XP-V cell-free extracts are deficient in bypass replication of a *cis-syn* thymine-thymine (T-T) dimer (*4*). XP-V cells are hypermutable with UV light and exhibit an unusual mutational spectrum (*5*). The *RAD30* gene of *Saccharomyces cerevisiae* functions in error-free bypass of UV lesions (*6*, *7*). RAD30 encodes a DNA polymerase, Polη, which can efficiently replicate past a *cis-syn* T-T dimer in template DNA, and Rad30 inserts two adenines across from the dimer (*8*). Here, we determine if the human homolog of yeast *RAD30* is responsible for XP-V.

A human cDNA clone, H96386 [345 base pairs (bp)], that encodes a peptide with homology to the $NH_2$-terminus of yeast Rad30 protein was used to screen a spleen cDNA library (*9*), and a single clone that contains a 3-kb cDNA insert was isolated. Sequence analysis (*10*) of this cDNA (*11*) (GenBank accession number AF158185) indicated that the protein encoded by the human gene displays significant homology to the *S. cerevisiae* Rad30 protein (Fig. 1A). We have named the human gene *hRAD30*. Excluding the intervening region from amino acid residues 452 to 606 in hRad30, the human and yeast Rad30 proteins share 23% identical and 53% conserved residues, and the two proteins have several highly conserved motifs throughout their length (Fig. 1A).


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The yeast *REV1* gene encodes a deoxycytidyl transferase (*12*) that is homologous with Rad30 (*6*, *13*) (Fig. 1B). However, yeast Rev1 shares only 13% identical and 32% conserved residues with hRad30, and many of the conserved motifs in the COOH-terminal portion of yeast and human Rad30 are absent from Rev1 (Fig. 1B). For example, the Rad30 motif EH(M/A)DYH(F/L)AL is absent from Rev1 (Fig. 1B). In addition, the $NH_2$- and COOH-terminal sequences of Rev1 are not found in yeast or human Rad30 (Fig. 1B). Thus, hRad30 is the human counterpart of yeast Rad30.

To determine whether defects in *hRAD30* are responsible for XP-V, we sequenced (*10*) *hRAD30* cDNA from eight different human XP-V cell lines and from five other control cell lines (*14*). RNA isolated from these cell lines was used to generate *hRAD30* cDNA, which was then amplified by polymerase chain reaction (PCR) (*10*). In the five control cell lines, which include a normal lymphoblast cell line, a HeLa cell line, and three cell lines derived from XPA, XPB, and XPC patients, we found no sequence changes in *hRAD30*. By contrast, eight distinct mutations were identified (Table 1) in the eight XP-V cell lines. The XP1CH and XP2CH cell lines are derived from two XP-V–affected sibs who are offspring of a first cousin marriage. Both have a C to T transition at nucleotide (nt) 376 (Fig. 2A) which creates a TAA nonsense codon that would result in a truncated protein of 125 amino acids. The XP115LO cell line, also derived from an individual who is an offspring of a first cousin marriage, carries a C to T transition at nt 1117 (Fig. 2B) that creates a TAA stop codon at position 372. The XP30RO cell line has a deletion of 13 bp (Fig. 2C) that would truncate hRad30 at position 35. The XP5MA cell line has a deletion of 104 bp (Fig. 2D) that would truncate hRad30 at position 220. In each of these five XP-V cell lines, only the respective mutant allele was observed. The XP6DU cell line has two different mutant alleles of *hRAD30*. One of these would result in the termination of hRad30 at position 69, and the other would result in the deletion of the Leu-75 residue from the protein. In cell line XPPHBE, one allele has a frameshift mutation that would terminate hRad30 at position 359. We have been unable to identify a mutation within the second *hRAD30* allele in XPPHBE; however, this allele is expressed at about 20% the level of the mutant form (*15*). In cell line XP1SF, one *hRAD30* allele has a C to T transition mutation that generates a TAG stop codon, resulting in a truncated protein of only 22 amino acids. In this cell line also, we have been unable to identify a mutation within the second *hRAD30* allele, but here too the wild-type allele is expressed at a lower level (~10%) than the mutant allele (*15*).

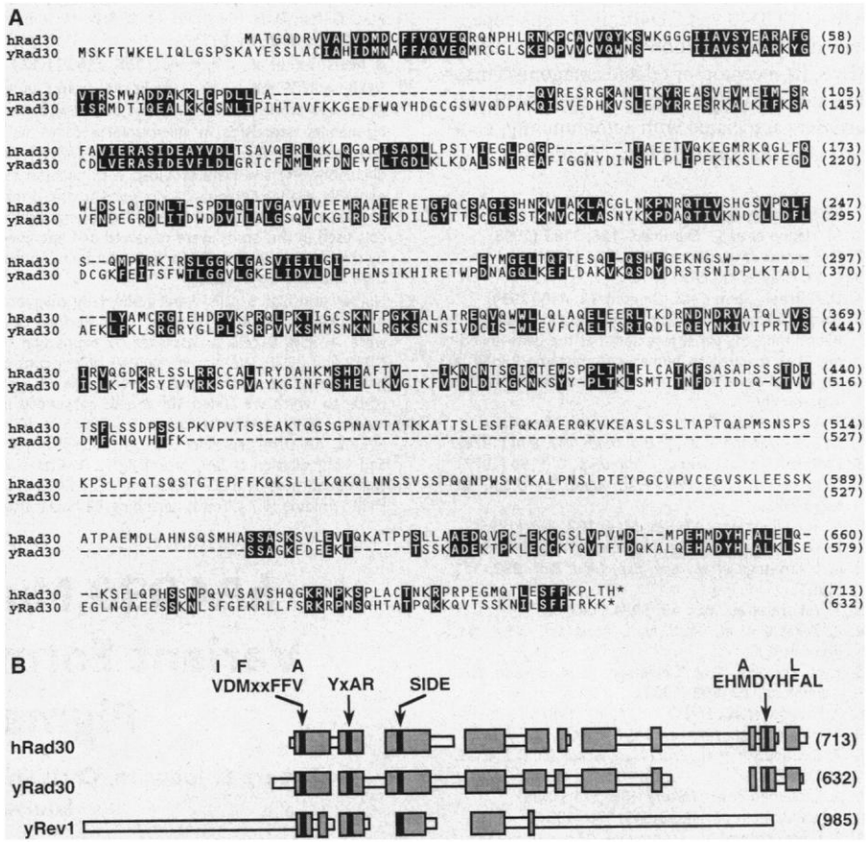


**Fig. 1.** (**A**) Alignment of human Rad30 (hRad30) and *S. cerevisiae* Rad30 (yRad30) protein sequences. Identical and highly conserved residues are highlighted. Numbers in parentheses indicate amino acid positions; asterisks indicate stop codons. (**B**) Schematic representation of homology between human Rad30, *S. cerevisiae* Rad30, and *S. cerevisiae* Rev1 (yRev1). Unshaded boxes represent nonhomologous sequences and shaded boxes represent homologous sequences. Positions of highly conserved motifs are indicated. Gaps have been introduced for optimal alignment. Protein lengths are indicated by numbers on the right. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

In six of the cell lines (XP1CH, XP2CH, XP115LO, XP30RO, XP5MA, and XP6DU), we observed only the mutant form or forms of the *hRAD30* allele. Of the six different mutations present in these cell lines, five would result in premature termination of translation. The longest of these truncated proteins would contain only 372 amino acids and thus would retain only about 50% of the protein. Because the human and yeast Rad30 proteins share regions of homology beyond residue 372 of hRad30 (Fig. 1A), this hRad30 truncation is likely to result in the loss of function. The other four truncated proteins would contain only 35 to 220 amino acids of hRad30, and it is likely that they inactivate hRad30 function. Yeast Rad30 deleted beyond residue 340 has no DNA polymerase activity, and this mutation does not complement the UV sensitivity of the *rad30*Δ strain (*16*). One mutant allele of *hRAD30* is deleted for the Leu-75 codon. This residue is identical in the yeast and human proteins, and it lies in a conserved region of the protein; thus, it may also affect hRad30 function. Two of the cells lines (XPPHBE and XP1SF) have a mutation in only one of the two chromosomes, and these mutations also would produce severely truncated proteins. However, in both these cell lines, the allele with no mutational alteration is expressed at a reduced level. Also, we have not excluded the possibility that these apparently wild-type alleles harbor a mutation in the 5′- or 3′-flanking regions that have not been sequenced.

The fact that seven of the eight mutations identified in XP-V cell lines result in severely truncated proteins indicates that mutations in *hRAD30* are responsible for XP-V. Our results also suggest that loss of hRad30 function is not lethal. XP-V cells are hypermutable with UV light, and they are less likely than normal cells to incorporate deoxyadenosine 5′-monophosphate opposite photoproducts involving thymine (*5*). These observations support the notion that hRad30 functions in error-free replication

**Table 1.** Mutations identified in XP-variant cell lines.

| Cell line | Repository number | Ancestry | Mutational alteration (type) | Effect on hRad30 protein |
|---|---|---|---|---|
| XP1CH | GM03055 | Russian-Armenian | C→T at +376 (nonsense) | Premature termination at $Val^{125}$ |
| XP2CH | GM03053 | Russian-Armenian | C→T at +376 (nonsense) | Premature termination at $Val^{125}$ |
| XP115LO | GM02359A | Iranian | C→T at +1117 (nonsense) | Premature termination at $Val^{372}$ |
| XP30RO | GM03617 | Lebanese | 13-bp deletion from +104 to +116 (frameshift) | Frameshift at $Ala^{35}$* |
| XP5MA | GM03379 | German | 104-bp deletion from +661 to +764 (frameshift) | Frameshift at $Lys^{220}$† |
| XP6DU | GM03618 | Scottish | −1 G at +207 (frameshift) | Frameshift at $Lys^{69}$‡ |
| | | | 3-bp deletion from +222 to +224 (inframe deletion) | Deletion of $Leu^{75}$ |
| XPPHBE | GM02449C | | +1 G at +1078 (frameshift) | Frameshift at $Asn^{359}$§ |
| XP1SF | GM06090 | Chinese | C→T at + 67 (nonsense) | Premature termination at $Glu^{22}$ |

*After the frameshift, the alternate reading frame encodes seven unrelated amino acids before terminating. †After the frameshift, the alternate reading frame encodes one proline residue before terminating. ‡After the frameshift, the alternate reading frame encodes 29 unrelated amino acids before terminating. §After the frameshift, the alternate reading frame encodes 31 unrelated amino acids before terminating.

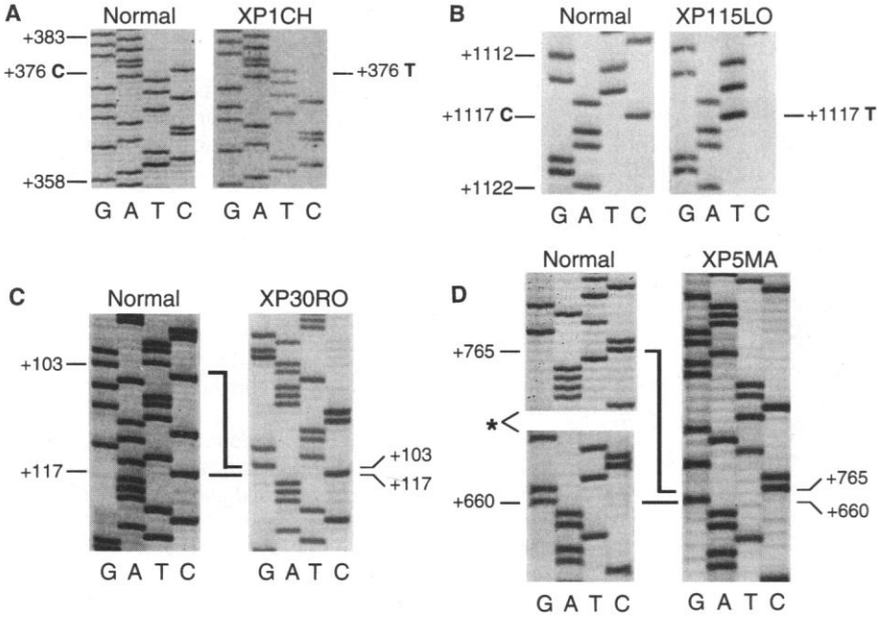


**Fig. 2.** Detection of *hRAD30* mutations in XP-V cell lines. Numbers indicate nucleotide position in relation to +1 of the ATG initiation codon. The positions of mutational alterations are indicated. (**A**) The C→T mutation at nt +376 in XP1CH cell line. (**B**) The C→T mutation at nt +1117 in XP115LO cell line. (**C**) The 13-bp deletion from nt +104 to +116 in XP30RO cell line. (**D**) The 104-bp deletion from nt +661 to +764 in XP5MA cell line. The asterisk indicates the missing 92 nt of the normal *hRAD30* sequence. The orientation of the *hRAD30* sequence in (A) and (D) is different from that in (B) and (C).

of DNA containing a T-T dimer in a manner similar to the yeast Rad30 protein, and its inactivation leads to UV hypermutability and to increased incidence of sunlight-induced skin cancers in XP-V patients. Increased bypass of DNA lesions by the mutagenic DNA polymerase, Polζ, would be the cause of UV hypermutability in XP-V cells. Less drastic mutations in *hRAD30* that do not elicit the clinical form of XP-V may contribute to skin cancers in the general population.

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9. The *hRAD30* clone was derived from a high-throughput cDNA screening service (Genome Systems, St. Louis, MO). Oligonucleotides N4920 (5′-CCCGGTACTTGGTGAGGTTAGCTTTCCCACGGG-3′) and N4921 (5′-ATAATTGCAGTGAGTTATGAAGCTCGTGCATTTGGAG-3′), which amplify nucleotides +139 to +280 of the *hRAD30* gene, were derived from the H96386 cDNA sequence and used to generate a 142-bp PCR fragment. This fragment was used as a probe to screen a human spleen cDNA library. One clone, 21749, was found to carry a 3.0-kb insert in vector pcDNA2.1 (Invitrogen).
10. RNA was isolated from various cell lines by use of the Tri-Reagent protocol from Molecular Research Center (Cincinnati, OH). hRad30 cDNA was generated from total human RNA by use of the Superscript One Step reverse transcriptase (RT)–PCR system (Gibco-BRL) and amplified in three overlapping fragments. Fragment 1 was amplified with oligonucleotides RT5′ (5′-GGGAATAAATCTCGCTCGAAACTCACTGGACCGG-3′) and N4920 (*9*); this fragment encompasses nt −152 to +280 of hRAD30. Fragment 2 was generated with oligonucleotides N4921 (*9*) and N4918 (5′-CTTTTGCCTTGATGAGATACGGCAGAAACAACCAGGG-3′); this fragment encompasses nt +139 to +2048 of hRAD30. Fragment 3 encompasses nt +1738 to +2267 and was amplified with oligonucleotides N4919 (5′-GGGGTGTCGAAGCTAGAAGAATCCTCTAAAGCAACTCC-3′) and RT3′ (5′-TTATTTTTTGTATTAAAAATTTCATAATTCCCTTTCTCAG-3′). Amplified cDNAs from cell lines and clone 21749 (*9*) were sequenced with the Thermo Sequenase kit (Amersham Pharmacia Biotech). In addition to the oligonucleotides used for RT-PCR, we used the following oligonucleotides as primers to sequence the *hRAD30* gene: 5′ Seq (5′-AACTTCTTAGCATCATCTGCCCAC-3′), N5035 (5′-GCCTATCTCGGCAGACTTGTTGCC-3′), N5321 (5′-GAAATGAGAGCAGCCATAGAGAGGG-3′), N5086 (5′-GAGATCCTAGGGATAGAATACATGGG-3′), N5322 (5′-GAGCATCATAGCGGGTAAGGGC-3′), N5087 (5′-CTGGCACCTTTGGCAGAGAACTTGGG-3′), 1565AS (5′-CTCAGTTCCTGTACTTTGACTGG-3′), N5036 (5′-GGGCCAAATCCATCTCTGCAGG-3′), and 3′ Seq (5′-GCAGAAATCCTTTTTGCAGCCCC-3′). Samples were run on 6 to 8% tris-taurine-EDTA polyacrylamide gels containing 8 M urea. Gels were fixed in 10% acetic acid, 10% methanol solution and dried before autoradiography. Each of the mutations in the XP-V cell lines were independently amplified and sequenced three or four times.
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14. Cell lines were obtained from the Coriell Cell Line Repository (CCR, Camden, NJ). Lymphoblast cell lines were normal (GM00892B), XPA (GM02250D), XPB (GM02252A), XPC (GM02246C), and XPPHBE (GM02449C). Fibroblast cell lines were XP1CH (GM03055), XP2CH (GM03053), XP115LO (GM02359A), XP30RO (GM03617), XP5MA (GM03379), XP6DU (GM03618), and XP1SF (GM06090).
15. To quantitate the mutant and wild-type hRad30 transcripts in the XP-V cell lines XPPHBE (GM02449C) and XP1SF (GM06090), we cloned the amplified cDNAs encompassing the respective mutations from each cell line into pUC19-based vectors and sequenced 20 independent clones from each cell line.
16. C. M. Kondratick, M. T. Washington, R. E. Johnson, S. Prakash, L. Prakash, unpublished observations.


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