Corrections and Retraction

CORRECTIONS

APPLIED MATHEMATICS, POPULATION BIOLOGY. For the article "Global asymptotic coherence in discrete dynamical systems," by David J. D. Earn and Simon A. Levin, which appeared in issue 11, March 14, 2006, of *Proc Natl Acad Sci USA* (103:3968–3971; first published March 7, 2006; 10.1073/pnas.0511000103), the authors note that on page 3971, inequality **25** holds only for particular classes of matrices $\tilde{\mathsf{M}}$, and strict inequality never holds (Theorem 5.6.9, page 297 of ref. 12). The authors are grateful to Jinhu Lü for recognizing this error. The argument given in the paper proves the following revised version of *Theorem 1* (page 3970).

Theorem 1. *Let X be a convex subset of a Banach space $\mathcal{B}$, and suppose the fundamental map $F: X \to X$ is differentiable at each $x \in X$. Suppose that $\|D_xF\|$ is bounded in X, and let $r = \sup_{x\in X} \|D_xF\|$. Suppose M is a stochastic $n \times n$ matrix, and define $\tilde{\mathsf{M}}$ as in Lemma 2. Let $|||\cdot|||$ be any matrix norm for which there exists a compatible monotone vector norm, and let $\mu = |||\tilde{\mathsf{M}}|||$. If $r\mu < 1$, then the full map $\vec{\boldsymbol{F}}: X^n \to X^n$, defined by $\vec{\boldsymbol{F}}(\vec{\boldsymbol{x}}) = \mathsf{M} \cdot F(\vec{\boldsymbol{x}})$, is globally asymptotically coherent, i.e., every initial state $\vec{\boldsymbol{x}}_0 \in X^n$ asymptotically approaches a coherent trajectory. If $r < 1$, then $\vec{\boldsymbol{F}}$ has a globally asymptotically stable fixed point.*

The authors note that all l_p norms are monotone, so the matrix norm $|||\cdot|||$ in the theorem can, for example, be taken to be any matrix norm induced by an l_p vector norm. The simplest examples are the maximum column sum and maximum row sum matrix norms, which are induced by the l_1 and l_∞ vector norms, respectively. The original statement of *Theorem 1* is valid for some classes of matrices (for example, if $\tilde{\mathsf{M}}$ is normal or triangular) but may not be true in the generality stated. In applications, the matrix M will almost always be primitive; if M is not primitive, then $\mu \geq 1$, in which case the theorem has nontrivial content only in the situation where $r < 1$.

The authors also note the following typographical errors, which do not affect the conclusions of the article. On page 3968, Eq. **7** should read: "$\mathsf{M} \cdot \boldsymbol{e} = \boldsymbol{e}$." On page 3969, Eq. **14** should read:

$$\mathsf{M} = \begin{pmatrix} m_1 & 1 - m_1 \\ 1 - m_2 & m_2 \end{pmatrix}, \qquad [14]$$

and on page 3970, left column, first full paragraph, "unless $m_1 = m_2 = 0 \ldots$ or $m_1 = m_2 = 1$" should read: "unless $m_1 = m_2 = 1 \ldots$ or $m_1 = m_2 = 0$." On page 3971, in Eq. **24d**, there should be no primes (e.g., "$x_1' - x_n'$" should read: "$x_1 - x_n$").

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CHEMISTRY. For the article "Dewetting-induced collapse of hydrophobic particles," by X. Huang, C. J. Margulis, and B. J. Berne, which appeared in issue 21, October 14, 2003, of *Proc Natl Acad Sci USA* (100:11953–11958; first published September 24, 2003; 10.1073/pnas.1934837100), the authors note that on page 11953, right column, eighth line from the bottom, "$\varepsilon = 592.5$ cal/mol" should read: "$4\varepsilon = 592.5$ cal/mol." This error does not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0609680103

MICROBIOLOGY. For the article "Evolution of sensory complexity recorded in a myxobacterial genome," by B. S. Goldman, W. C. Nierman, D. Kaiser, S. C. Slater, A. S. Durkin, J. Eisen, C. M. Ronning, W. B. Barbazuk, M. Blanchard, C. Field, C. Halling, G. Hinkle, O. Iartchuk, H. S. Kim, C. Mackenzie, R. Madupu, N. Miller, A. Shvartsbeyn, S. A. Sullivan, M. Vaudin, R. Wiegand, and H. B. Kaplan, which appeared in issue 41, October 10, 2006, of *Proc Natl Acad Sci USA* (103:15200–15205; first published October 2, 2006; 10.1073/pnas.0607335103), the author name J. Eisen should have appeared as J. A. Eisen. The online version has been corrected. The corrected author line appears below.

B. S. Goldman, W. C. Nierman, D. Kaiser, S. C. Slater, A. S. Durkin, J. A. Eisen, C. M. Ronning, W. B. Barbazuk, M. Blanchard, C. Field, C. Halling, G. Hinkle, O. Iartchuk, H. S. Kim, C. Mackenzie, R. Madupu, N. Miller, A. Shvartsbeyn, S. A. Sullivan, M. Vaudin, R. Wiegand, and H. B. Kaplan

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BIOCHEMISTRY. For the article "Enzyme–microbe synergy during cellulose hydrolysis by *Clostridium thermocellum*," by Yanpin Lu, Yi-Heng Percival Zhang, and Lee R. Lynd, which appeared in issue 44, October 31, 2006, of *Proc Natl Acad Sci USA* (103:16165–16169; first published October 23, 2006; 10.1073/pnas.0605381103), the authors note that on page 16167, at the top of the right column, the references to steady states 1 and 2 are switched, as may be seen from inspection of Table 1. The corrected text should read: "In continuous culture, a $DS_{EM}^{E_T}$ value of 2.72 is obtained based on microbial and SSF steady states 2, for which ≈75% of the feed cellulose was hydrolyzed. For microbial and SSF steady states 1, for which ≈66% hydrolysis was achieved, $DS_{EM}^{E_T} = 4.70$. Values for enzyme–microbe synergy on a pellet cellulase basis, $DS_{EM}^{E_P}$, are quite similar to values observed in continuous culture: 3.05 for microbial and SSF steady states 2 and 4.61 for microbial and SSF steady states 1." This error does not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0609576103

MEDICAL SCIENCES. For the article "Human cancers express a mutator phenotype," by Jason H. Bielas, Keith R. Loeb, Brian P. Rubin, Lawrence D. True, and Lawrence A. Loeb, which appeared in issue 48, November 28, 2006, of *Proc Natl Acad Sci USA* (103:18238–18242; first published November 15, 2006; 10.1073/pnas.0607057103), several references to nucleotide instability (NIN) should have appeared as point mutation instability (PIN). On page 18238, in the key terms, "nucleotide instability (NIN)" should be replaced with "point mutation instability (PIN)." On page 18239, in the last sentence of the first paragraph of the *Discussion*, "nucleotide instability or NIN" should read: "point mutation instability or PIN." Last, on page 18239, in the last sentence of the second paragraph of the *Discussion*, "an increase in NIN" should read: "an increase in PIN." The online version has been corrected. These errors do not affect the conclusions of the article.

www.pnas.org/cgi/doi/10.1073/pnas.0610370103

RETRACTION

GENETICS. For the article "A common mutational pattern in Cockayne syndrome patients from xeroderma pigmentosum group G: Implications for a second XPG function," by Thierry Nouspikel, Philippe Lalle, Steven A. Leadon, Priscilla K. Cooper, and Stuart G. Clarkson, which appeared in issue 7, April 1, 1997, of *Proc Natl Acad Sci USA* (94:3116–3121), the editors wish to note that Steven Anthony Leadon has submitted a letter to PNAS that states, "I have recently had the opportunity to review some of the raw data used for Figure 6 in this paper in the above-referenced publication and it is clear that the data as reported in this figure cannot be relied upon. Therefore, I request that you retract Figure 6 of this paper." Fig. 6 is hereby retracted.

Leadon's request for retraction of Fig. 6 is part of a Voluntary Exclusion Agreement Leadon entered into with the U.S. Department of Health and Human Services (HHS) through the Public Health Service and the Office of Research Integrity in the case of *Steven Anthony Leadon, University of North Carolina.* The specific terms of the Agreement between Leadon and HHS are published in the Notice of Findings of Scientific Misconduct from HHS [71 *Federal Register* 110 (June 8, 2006/Notices), pp 33308–33309].

The editors also wish to note that the other authors of the PNAS article (Thierry Nouspikel, Philippe Lalle, Priscilla K. Cooper, and Stuart G. Clarkson) and the communicating member (Philip C. Hanawalt) have submitted the following statement to PNAS: "Figs. 1 through 5 in the PNAS paper document experiments performed by Thierry Nouspikel and Philippe Lalle in Stuart Clarkson's laboratory in Geneva, in which it was established that XP-G patients with severe early onset Cockayne syndrome (CS) produce truncated and unstable XPG proteins but that a pair of mildly affected XP-G siblings without symptoms of CS are able to synthesize a full-length product from one allele with a missense mutation. The conclusion was that XPG must have a second function in addition to its role as a structure-specific nuclease in nucleotide excision repair. The validity of that conclusion is not challenged by the retraction of Fig. 6, and the abstract stands correct. The conclusions of the paper have been confirmed independently by a number of laboratories [e.g., Shiomi *et al.* (2004) *Mol Cell Biol* 24:3712–3719; Tian *et al.* (2004) *Mol Cell Biol* 24:2237–2242; Zafeiriou *et al.* (2001) *Pediatr Res* 49:407–412; Emmert *et al.* (2002) *J Invest Dermatol* 118:972–982]."

Solomon H. Snyder, Senior Editor, PNAS

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Genetics

A common mutational pattern in Cockayne syndrome patients from xeroderma pigmentosum group G: Implications for a second XPG function

(transcription-coupled repair/nucleotide excision repair/UV irradiation)

THIERRY NOUSPIKEL*, PHILIPPE LALLE*, STEVEN A. LEADON†, PRISCILLA K. COOPER‡, AND STUART G. CLARKSON*§

*Department of Genetics and Microbiology, University Medical Centre (CMU), 9 Avenue de Champel, 1211 Geneva 4, Switzerland; †Department of Radiation Oncology, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7512; and ‡Life Sciences Division, Building 934, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Communicated by Philip C. Hanawalt, Stanford University, Stanford, CA, January 14, 1997 (received for review December 6, 1996)

ABSTRACT **Xeroderma pigmentosum (XP) patients have defects in nucleotide excision repair (NER), the versatile repair pathway that removes UV-induced damage and other bulky DNA adducts. Patients with Cockayne syndrome (CS), another rare sun-sensitive disorder, are specifically defective in the preferential removal of damage from the transcribed strand of active genes, a process known as transcription-coupled repair. These two disorders are usually clinically and genetically distinct, but complementation analyses have assigned a few CS patients to the rare XP groups B, D, or G. The *XPG* gene encodes a structure-specific endonuclease that nicks damaged DNA 3′ to the lesion during NER. Here we show that three XPG/CS patients had mutations that would produce severely truncated XPG proteins. In contrast, two sibling XPG patients without CS are able to make full-length XPG, but with a missense mutation that inactivates its function in NER. These results suggest that XPG/CS mutations abolish interactions required for a second important XPG function and that it is the loss of this second function that leads to the CS clinical phenotype.**

Nucleotide excision repair (NER) is the process by which UV light damage and other bulky lesions are removed from DNA. It is a universal process that works on the same principles from bacteria to man. The lesion is recognized, nicks are made in the damaged strand on either side of the lesion, the oligonucleotide containing the lesion is removed, and the resulting gap is filled and sealed (reviewed in ref. 1). In human cells, the process requires ≈30 polypeptides (2, 3). Defects in these proteins can lead to some severe clinical phenotypes, notably xeroderma pigmentosum (XP) and Cockayne syndrome (CS).

XP is an autosomal recessive disease that usually involves deficiencies in early steps of NER, due to mutations in any one of seven different genes (*XPA–XPG*), which correspond to XP complementation groups A–G. Defects in an eighth gene, *XPV* (for XP variant), account for the remaining 20% or so of XP cases; here the defects appear to be in a postreplication repair process rather than NER (reviewed in ref. 1). Typically, XP patients exhibit acute sun sensitivity, dramatic skin changes, including high incidence of cancers in sunlight-exposed areas, and frequently also progressive neurological degeneration.

CS is another rare sun-sensitive disorder with a recessive inheritance pattern. It is characterized by severe growth defects with cachexia, neuronal demyelination, mental retardation, microcephaly, skeletal and retinal abnormalities, and dental caries, but no cancer predisposition. Over 140 CS cases have been reported (4) that fall into five complementation groups. Most patients exhibit only CS symptoms and they belong to groups CS-A and CS-B. The hallmark of cells from these CS patients is their inability to preferentially repair DNA damage in the transcribed strand of active genes (5–7), a process known as strand-specific repair or transcription-coupled repair (TCR; reviewed in refs. 8–10).

The *CSA* gene codes for a protein belonging to the WD-repeat family (11), whose members have regulatory rather than catalytic roles in many cellular functions, including cell division, signal transduction, mRNA modification, and transcription (reviewed in ref. 12). The product of the *CSB* gene (13) contains a region of multiple ATPase/putative helicase motifs characteristic of the expanding and diverse SNF2 protein family. Members of this family can have roles in chromatin remodeling or maintenance, activation or repression of transcription, and DNA recombination or repair (reviewed in ref. 14). How CSA and CSB function in TCR is presently unknown.

In very rare cases, complementation analyses have assigned some CS patients to XP groups B, D, or G; some of these XP/CS patients also present an XP phenotype. The *XPB* (15) and *XPD* (16, 17) genes code for DNA helicases with DNA-dependent ATPase activity that are components of TFIIH. This multisubunit factor is required both for transcription initiation by RNA polymerase II and also for NER (18–22), in which it presumably is involved in opening the DNA helix in the vicinity of the lesion.

In contrast, the product of the *XPG* gene (23–25) has no documented role in transcription. It is a founding member of the RAD2/XPG family (23–26) that comprises two related groups of nucleases (reviewed in refs. 27 and 28). One group includes the *Schizosaccharomyces pombe* rad2 protein, its human homolog DNase IV (also called MF-1 or FEN-1), and RAD27, the product of the YKL510 open reading frame of *Saccharomyces cerevisiae*. These ≈43-kDa enzymes resemble the 5′ nuclease domains of bacterial DNA polymerases and are able to cleave DNA flap structures to remove unpaired 5′ regions. The second group comprises much larger proteins of >120 kDa and includes human XPG and its yeast homologs, the *Saccharomyces cerevisiae* RAD2 and *Schizosaccharomyces pombe* rad13 proteins. Both XPG and RAD2 are structure-specific endonucleases that nick damaged DNA 3′ to the lesion in an early step of NER (29–32).

To try to understand how defects in a nuclease could give rise to two very different clinical phenotypes, we examined the

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Abbreviations: NER, nucleotide excision repair; XP, xeroderma pigmentosum; CS, Cockayne syndrome; TCR, transcription-coupled repair; RT-PCR, reverse transcription-coupled PCR; CPD, cyclobutane pyrimidine dimer.

§To whom reprint requests should be addressed. e-mail: Stuart.Clarkson@medecine.unige.ch.

XPG gene in the first three documented cases of combined XP-G/CS (33–35). Here we report an unexpected common mutational pattern in these three patients that is distinct from that found in two sibling XP-G patients (36) with a very mild XP phenotype and no CS symptoms. We suggest that this pattern has implications for an important second XPG function and for the CS clinical phenotype.

MATERIALS AND METHODS

Cell Culture. Primary fibroblasts were cultured at 37°C in the presence of 5% CO_2 in minimal essential medium (Seromed Biochrom, Berlin) supplemented with 2 mM glutamine, heat-inactivated 15% fetal calf serum (Inotech, Dottikon, Switzerland), 100 units/ml penicillin, and 10 μg/ml streptomycin. Fibroblasts were passed twice a week irrespective of their confluency. Lymphoblast cell lines were grown in suspension at 37°C in the presence of 5% CO_2 in RPMI 1640 medium (Seromed) supplemented with 10% fetal calf serum.

PCR Cloning. Poly(A)$^+$ RNA was isolated from 24 225-cm^2 flasks of XPCS1LV fibroblasts with a FastTrack kit (Invitrogen). Poly(A)$^+$ RNA (850 ng) primed with 50 ng of random hexamers was reverse-transcribed with Superscript II (GIBCO) and amplified with an Expand Long-template PCR kit (Boehringer Mannheim) using primers 5′-TCTCGGCCGCTCTTAGGACGCAGCCGC and 5′-TTTATGCATCTTTGCGACAAATTCATTACAAATGG. PCR conditions were: 2 min at 94°C; then 8 cycles of 15 sec at 94°C, 30 sec at 68°C (this temperature was dropped 1°C with each cycle), and 3 min at 68°C; and then 22 cycles of 15 sec at 94°C, 30 sec at 61°C, and 3 min at 68°C (15 sec was added per cycle for the last 8 cycles). XPCS2LV fibroblasts from 5 162-cm^2 flasks were lysed in guanidinium isothiocyanate, and RNA was purified by cesium chloride step-gradient centrifugation. RNA (5 μg) was primed with 50 ng of p(dT)$_{12–18}$ (Pharmacia) and reverse-transcribed with Superscript II. PCR amplification was performed with the same primers as above, using TaKaRa LA-PCR kit (Takara Shuzo, Kyoto) with the following conditions: 4 min at 94°C; then 10 cycles of 30 sec at 94°C, 1 min at 68°C (temperature was dropped 1°C with each cycle), 3.5 min at 72°C; and then 20 cycles of 30 sec at 94°C, 1 min at 58°C, and 3.5 min at 72°C. The same procedure was repeated with fibroblasts from patient 94RD27, except that amplification was performed with High Fidelity PCR kit (Boehringer Mannheim). The purified PCR products were digested with *Eag*I and *Nsi*I and subcloned into pBluescript II-SK$^+$. Positive clones were selected by hybridization and were sequenced with a deaza-G/A T7 sequencing kit (Pharmacia). For XPCS2LV, alleles with a C-T polymorphism at position 335 were distinguished by *Sty*I digestion (37) and were cycle-sequenced with an Applied Biosystems automatic sequencer.

Oligonucleotide Typing. Reverse transcription-coupled PCR (RT-PCR) products were amplified using *Taq* polymerase (Perkin–Elmer) and primers 5′-GATTTAATTTCCATTCCAAAGGCC and 5′-GTGGCTCACCATCCACGTCG. Due to an intron located between positions 2151 and 2152, genomic DNA and pre-mRNA were amplified with an intron-specific 5′ primer 5′-TCCAGAAAGCTCTTGATGATTGC. The denatured PCR products were slot-blotted onto a positively charged nylon membrane (Boehringer Mannheim) and were hybridized in TMAC buffer (3 M tetramethylammonium chloride/50 mM Tris·HCl, pH 8/2 mM EDTA/5× Denhardt's solution/0.1% SDS) with [γ-^{32}P]ATP-labeled primers 5′-ATCACACTTTGCAC (WT) or 5′-AATCACACTTGCAC (ΔA) for 3 hr at 36°C, and then they were washed twice for 10 min in 6× standard saline citrate and twice for 15 min in TMAC buffer at 40°C.

Restriction Analysis. A 613-bp PCR fragment was amplified from genomic DNA from XPCS2LV and XPCS1LV, using *Taq* polymerase and primers 5′-TGTCCAAAAGGAAATGAATCAGC and 5′-AGCAGGGCAGCTTGCATAGC. The purified fragments were then digested with *Fok*I and analyzed on a 5% acrylamide gel stained with ethidium bromide. Similarly, a 100-bp PCR fragment was amplified from genomic DNA or reverse-transcribed RNA from 94RD27 and a control patient, using primers 5′-GACCTAATCCTCATGACACC and 5′-GGTTTGAGGTAGGCC. The products were digested with *Nci*I and analyzed on a 6% acrylamide gel.

Western Blot Analysis. Cells were washed in PBS and proteins were solubilized for 30 min at 4°C in lysis buffer (50 mM Tris·HCl, pH 7.5/250 mM NaCl/1 mM EDTA/0.1% Triton X-100) containing protease inhibitors (2 μg/ml aprotinin/2 μg/ml leupeptin/1 μg/ml pepstatin/0.56 mM phenylmethylsulfonyl fluoride). Lysates were cleared by centrifugation at 13,800 × g for 20 min at 4°C. Equivalent cell extracts (150 μg of protein) were boiled for 5 min in SDS gel loading buffer, electrophoresed on 5% polyacrylamide gels, then transferred to Immobilon-PVDF membrane (Millipore) using a semidry transfer apparatus (LKB). Membranes were blocked for 1 hr in 3% skim milk in TBS-T (100 mM Tris·HCl/0.9% NaCl/0.05% Tween 20) and incubated overnight at 4°C with the appropriate antibody in 1% skim milk in TBS-T. Mouse monoclonal antibodies to XPG (1:300; a gift of E. Evans and R. D. Wood, ICRF Clare Hall Laboratories, South Mimms, U.K.) and TAF$_{II}$130 (1:500; Santa Cruz Biotechnology) and rabbit polyclonal antibody to XPB p89 (1:500; Santa Cruz Biotechnology) were used. Blots were washed three times for 15 min each in TBS-T and incubated 1 hr with a peroxidase-conjugated anti-mouse or anti-rabbit IgG antibody (1:3000 in 1% skim milk in TBS-T; Amersham). After three washes of 15 min each in TBS-T, signals were detected using enhanced chemiluminescence (ECL); membranes were stripped and reprobed according to the manufacturer's recommendations (Amersham).

TCR of UV Damage. Strand-specific removal of cyclobutane pyrimidine dimers (CPDs) was assayed by the loss of T4 endonuclease V sites (5) from the active metallothionein IA (*MTIA*) gene using RNA probes (7, 38). Confluent fibroblast cultures from patients XP125LO and XPCS1LV and a normal individual (GM38; NIGMS Human Genetic Mutant Cell Repository, Camden, NJ) were washed with PBS, irradiated with 10 J/m^2 254-nm UV, and then harvested immediately or allowed to repair for 3 or 6 hr after readdition of medium. Cells were lysed with 0.5% SDS in 10 mM Tris/10 mM EDTA, DNA was purified and digested with *Eco*RI, and duplicate 10-μg samples from each time point were incubated at 37°C for 15 min with T4 endonuclease V (a gift of I. Mellon, University of Kentucky) or with buffer alone (10 mM Tris·HCl, pH 8/100 mM NaCl/10 mM EDTA/10% ethylene glycol). After electrophoresis through 0.7% alkaline agarose gels, DNA samples were transferred to a GeneScreen Plus (NEN) membrane and hybridized with strand-specific RNA probes prepared from plasmid pZMTIIA (38). The amount of hybridization to a 10-kb fragment containing the active *MTIA* gene was quantitated by scanning densitometry. The percent of fragments containing no dimers (the zero class) is equal to the ratio of the amount of full-length fragments in the enzyme-treated and untreated samples. The average number of dimers (enzyme-sensitive sites) per fragment was calculated from the zero class using the Poisson expression.

RESULTS

XPCS1LV. Patient XPCS1LV, a girl with psychomotor retardation and microcephaly who died at 6.5 years, was severely sunlight-sensitive, with several pigmented cutaneous spots (33, 34). Poly(A)$^+$ RNA from a culture of her primary fibroblasts was reverse-transcribed and the ≈4-kb *XPG* cDNA (23) was amplified by PCR. The products were cloned in a plasmid vector and several clones were sequenced. All were found to lack a single nucleotide within an AAA triplet at nucleotides 2170–2172, which results in a TGA stop codon after amino acid 659 (Fig. 1*A*; numbering based on EMBL and Swiss-Prot database accession nos. X69978 and P28715, respectively). Such a deletion is characteristic of a slippage error during DNA replication. Its existence

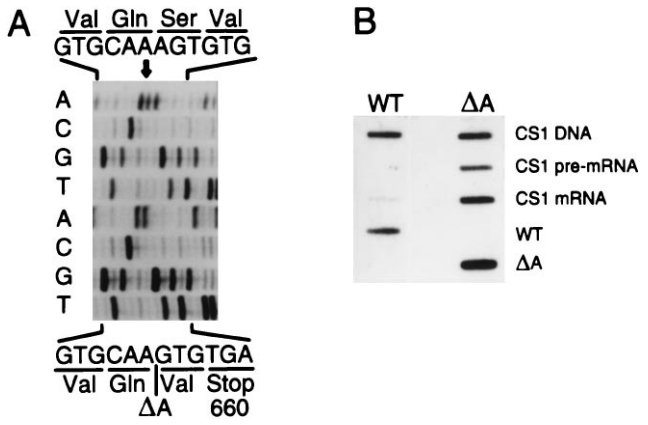

FIG. 1. Mutations in the *XPG* gene of patient XPCS1LV. (*A*) Sequence of wild-type (top 4 rows) and XPCS1LV (bottom 4 rows) RT-PCR products showing the single nucleotide deletion within the AAA triplet at nucleotides 2170–2172. (*B*) Slot-blot hybridization of PCR products with wild-type (WT) or mutation-specific (ΔA) oligonucleotide probes. The wild-type sequence is present in genomic DNA from XPCS1LV, but not in pre-mRNA or mRNA. cDNA clones WT and ΔA are included as controls.

was independently confirmed by oligonucleotide typing, which also revealed the presence of the wild-type *XPG* sequence in genomic DNA, but not in pre-mRNA or mRNA (Fig. 1*B*). We conclude that the only expressed *XPG* allele in patient XPCS1LV is the one encoding a truncated protein. This would undoubtedly compromise function, because a less severe truncation of XPG to 959 aa inactivates NER *in vivo* (37).

XPCS2LV. A similar procedure was applied to fibroblasts from patient XPCS2LV, a male with extreme microcephaly, dysmorphism, and sun-sensitive skin with several pigmented spots, who died at age 20 months (33, 34). Surprisingly, one *XPG* allele carries the same single nucleotide deletion as XPCS1LV (Fig. 2 *A* and *B*). Although both Flemish, these two patients are not known to be related. This shared deletion may reflect a DNA polymerase slippage hot spot or, more likely, a relatively recent common ancestor, because both patients also possessed a very rare HLA DRB1-DRB3 haplotypic combination (data not shown).

The second *XPG* allele contains a C → T transition at nucleotide 984 (Fig. 2*C*), which converts Arg-263 into a TGA stop codon, resulting in an even more severely truncated protein. This transition is located within a CpG dinucleotide and so may have resulted from deamination of a 5-methylcytosine. Its existence was independently confirmed by the loss of a *Fok*I restriction site from a relevant RT-PCR product (data not shown) and from half of the PCR products from the same region of genomic DNA (Fig. 2*D*). We conclude that XPCS2LV is a compound heterozygote for these two mutations that result in truncated XPG proteins.

94RD27. Patient 94RD27, a male born to healthy first cousin Moroccan parents, had extremely severe early-onset CS and died at 7 months (35). His fibroblasts express only one form of *XPG* that lacks the T at position 2972 (Fig. 3*A*). This deletion causes a frameshift after amino acid 925; another 55 aa unrelated to XPG would be added before the next in-frame stop codon. This deletion creates a new *Nci*I restriction site and, in accordance with the history of consanguinuity, the genomic DNA is not heterozygous at this site (Fig. 3*B*). We conclude that 94RD27 is homozygous for this single-base deletion that also results in a truncated XPG protein.

XPG Protein Expression. Fig. 4 summarizes our results for these three XP-G/CS patients and compares them with the mutations found earlier (37) in the mildly affected XP-G patient XP125LO (36) and in her brother, XP124LO, who carries the same two single point mutations in *XPG* (data not

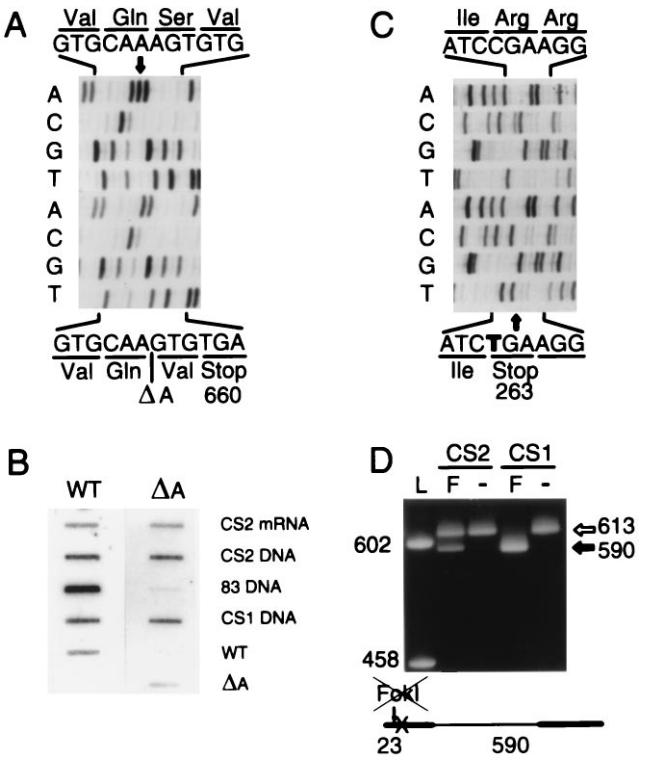

FIG. 2. Mutations in the *XPG* gene of patient XPCS2LV. (*A* and *C*) Sequence of wild-type (top) and XPCS2LV (bottom) RT-PCR products showing the same A deletion as in XPCS1LV in one allele (*A*) and the C → T transition at position 984 in the second allele (*C*). (*B*) Slot-blot hybridization of PCR products with wild-type (WT) or mutation-specific (ΔA) oligonucleotide probes. The wild-type and mutant sequences are present in both genomic DNA and mRNA from XPCS2LV. Genomic DNA from XPCS1LV and XP125LO (83 DNA) and cDNA clones WT and ΔA are included as controls. (*D*) *Fok*I restriction analysis of genomic PCR products from XPCS2LV (CS2) and XPCS1LV (CS1). L, *Hae*III digest of pBluescript II-SK$^+$ DNA, with fragment lengths in bp; F, *Fok*I digests; −, undigested products. The C → T transition destroys a *Fok*I site (GGATG[N]$_9$/and/[N]$_{13}$CATCC) in one allele of XPCS2LV.

shown). The one on the paternal allele generates a premature stop codon at position 960 (Fig. 4, mutation 4), whereas the

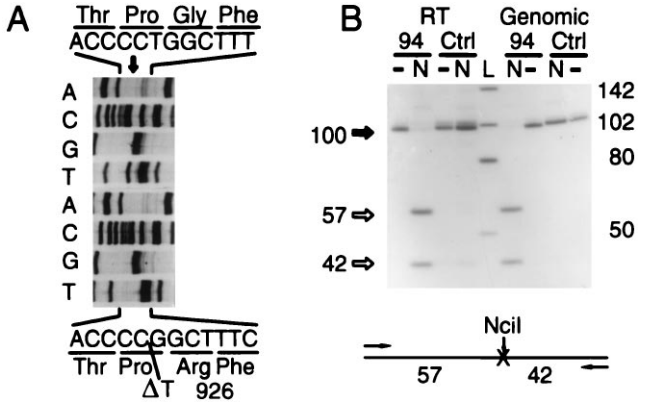

FIG. 3. Mutations in the *XPG* gene of patient 94RD27. (*A*) Sequence of wild-type (top) and 94RD27 (bottom) RT-PCR products showing the T deletion at position 2972. (*B*) *Nci*I restriction analysis of RT-PCR and genomic PCR products from RD9427 (lanes 94) and a wild-type control (Ctrl). −, Undigested products; N, *Nci*I digests; L, *Hae*III digest of pBluescript II-SK$^+$ DNA, with fragment lengths in bp. The T deletion creates a new *Nci*I site (CC/SGG) that is absent from control RNA and DNA. Note that the uncut 94RD27 genomic PCR product is slightly shorter than the control and that it is fully cut by *Nci*I to yield 57-bp and 42-bp fragments.

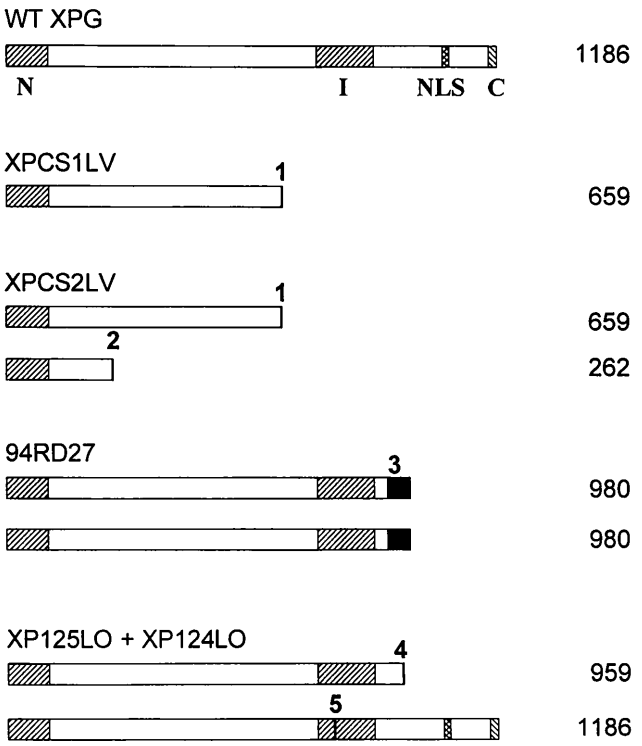

FIG. 4. Alignment of wild-type XPG with those predicted in the three XP-G/CS patients and the two sibling non-CS XP-G patients. The number of amino acids in each protein is listed on the right. Hatched areas indicate: N and I, conserved regions; NLS, putative bipartite nuclear localization signal; C, basic domain. Mutations: 1, Single A deletion within nucleotides 2170–2172; 2, nonsense C → T transition at nucleotide 984; 3, single T deletion at position 2972 (black area indicates the frameshift between amino acids 926 and 980); 4, nonsense G → T transversion at nucleotide 3075; and 5, missense C → T transition at nucleotide 2572.

maternal allele carries an Ala → Val missense mutation at position 792 (Fig. 4, mutation 5).

As expected, no full-length XPG is detectable in fibroblast extracts from the three XP-G/CS patients by immunoblotting with a polyclonal antibody raised against the N terminus of XPG (39); these Western blots also fail to detect bands corresponding to the truncated proteins (data not shown), suggesting that the latter may be unstable. In contrast, full-length XPG is detectable in extracts from the sibling XP-G patients. This is most clearly seen with lymphoblast extracts probed with a monoclonal antibody to XPG (Fig. 5).

Removal of UV-Induced Damage. Despite the presence of full-length XPG, XP125LO lymphoblasts have very low levels of unscheduled DNA synthesis after UV treatment (23), suggesting that the maternal Ala-792 → Val missense mutation severely affects global NER. This assay is too insensitive, however, to preclude the possibility that the full-length mutant protein may have residual NER capacity, in particular, residual transcription-coupled NER activity. To address the latter issue, we measured removal of CPDs from both strands of the active *MTIA* gene in UV-irradiated fibroblasts from a normal individual and from patients XP125LO and XPCS1LV. No lesion removal was detected from either strand during 6 hr of repair following 10 J/m^2 of UV in either XP125LO or the XP-G/CS strain (Fig. 6). In contrast, the normal fibroblasts removed dimers rapidly and strand-selectively, with 69% removed from the transcribed strand in 6 hr, compared with 30% from the nontranscribed strand. We conclude that these XP-G and XP-G/CS cells are completely defective in both global NER and transcription-coupled NER of UV damage.

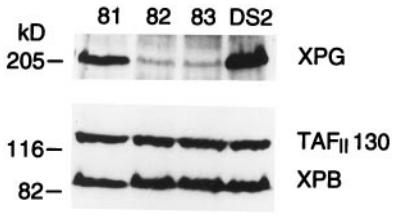

FIG. 5. Western blot analysis of lymphoblast extracts probed with antibodies to XPG, TAF$_{II}$130, and XPB. The lymphoblast lines XPG82 (lane 82) and XPG83 (lane 83) are derived from patients XP124LO and XP125LO, respectively; XPG81 (lane 81) is from their obligate heterozygote mother. DS2 is a wild-type lymphoblast line (40). Note that the XPG protein, which has a predicted molecular mass of 133 kDa, is present in the two XP-G patients, but in reduced amounts, and that it migrates very close to the 205-kDa myosin marker.

DISCUSSION

Our results confirm the complementation analyses that implicate *XPG* in certain forms of CS (34, 35), and they extend these analyses by identifying three causative mutations in this gene. The three patients analyzed here presented unusually severe early-onset CS, and they died in infancy or early childhood. All three XP-G/CS patients had few clinical manifestations of XP, perhaps because of limited sun exposure and their early death. Their striking common pattern was an inability to produce full-length XPG. Recently, a fourth XP-G/CS patient was reported (XP20BE), a boy with severe CS symptoms and XP-type pigmentation, but no skin cancers, who also died in early childhood (41). A clear prediction from our results is that he, and any future XP-G/CS patients, would have mutations that prevent the production of full-length XPG protein.

The paternal allele of the mildly affected XP-G siblings would also be expected to generate a truncated version of XPG, similar to that produced in XP-G/CS patient 94RD27 (Fig. 4). However, the XP-G siblings can make full-length XPG (Fig. 5), thanks to the maternal allele that carries an Ala-792 → Val missense mutation. Several lines of evidence indicate that this single amino acid change abolishes NER activity. XP125LO lymphoblast cells exhibit only background levels of unscheduled DNA synthesis (23). UV resistance is not restored to these cells by expression of a full-length *XPG* cDNA containing the missense mutation (37). The location of this mutation in a highly conserved region suggests that it perturbs the endonuclease active site (23, 37). The crystal structure of bacteriophage T4 RNase H, another member of the RAD2/XPG protein family, also indicates that the affected amino acid lies within the active site, adjacent to a glutamate needed for magnesium binding and catalytic activity (42). Finally, and most directly, lymphoblast extracts from patient XP125LO are incapable of making the normal NER 3′ incision on a cisplatin-containing substrate (43), and fibroblasts from the same patient are unable to remove UV-induced pyrimidine dimers from either strand of an active gene (Fig. 6).

We conclude that the clinical presentation of CS in patients with *XPG* mutations is unrelated to the incision function of XPG in NER. Rather, we propose that the XP-G/CS phenotype results from disruption of protein–protein or protein–DNA interactions that are important for a second XPG function. XPG and its homologs are distinguishable from the shorter proteins in the RAD2/XPG family by the large distance between two regions of sequence similarity (23–27). If these N and I regions (Fig. 4) are juxtaposed in XPG, as they are in T4 RNase H (42), clearly there is scope for the residues between them to adopt a conformation suitable for a second function. What might this function be, and how is it related to CS?

As outlined earlier, XPG is one of five defective gene products implicated in this clinical phenotype. Two others, the XPB and XPD ATPases/helicases, are subunits of the basal transcription factor TFIIH (18–22). This finding led to the

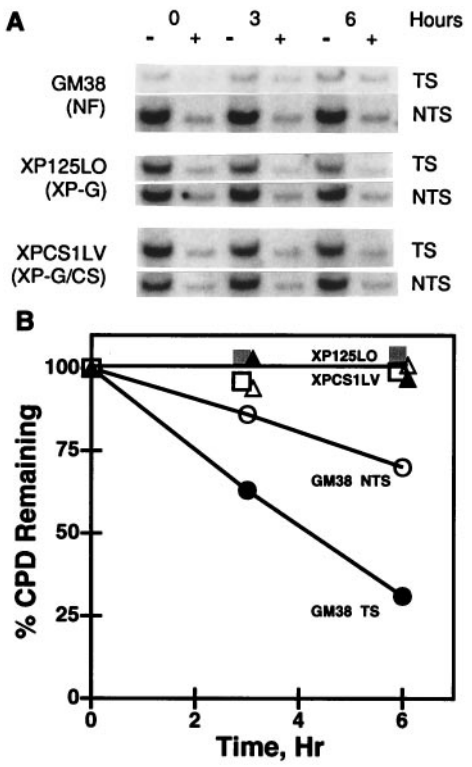

FIG. 6. Strand-selective repair of CPDs in the *MTIA* gene. (*A*) Human primary fibroblasts were exposed to 10 J/m^2 UV and were allowed to repair for the indicated times. *Eco*RI-digested genomic DNA was treated (+) or not (−) with T4 endonuclease V, electrophoresed, blotted, and hybridized with RNA probes that distinguish the transcribed (TS) and nontranscribed (NTS) strands of the *MTIA* gene. (*B*) Repair values calculated from scanning densitometry measurements of the hybridization signals in the autoradiogram. The mean of all six 0 time values, 0.71 ± 0.03 (SD) CPD per 10 kb, was set equal to 100%. Filled symbols represent TS; open symbols represent NTS. Circles, normal fibroblasts, GM38; squares, XP-G fibroblasts XP125LO; and triangles, XP-G/CS fibroblasts, XPCS1LV.

suggestion that certain mutations in *XPB* and *XPD* may affect only NER and thereby give rise to XP, whereas other kinds of mutations in these genes, and indeed in genes for other TFIIH subunits, may have additional subtle effects on transcription that result in the more complex and pleiotropic features of CS and other "transcription syndromes" (44, 45). Consistent with this hypothesis, the *XPD* mutations in the two known XP-D/CS patients are distinct from those found in patients with XP-D alone (46, 47). The *XPB* frameshift mutation found in the first XP-B/CS patient results in a severe NER defect but also a modest decrease in transcriptional activity *in vitro* (48). Although experimental evidence is lacking, the sequence motifs found within CSA and CSB (11, 13) may also hint at their possible involvement in transcription.

It is more difficult to apply a transcription hypothesis to the XP-G form of CS, because XPG is not an integral component of TFIIH and it has no known function in transcription. However, XPG copurifies with TFIIH under some chromatographic conditions (49), and XPG has been reported to interact with several TFIIH subunits (50). Most recently, evidence has been presented for a tight association between RAD2 and yeast TFIIH (51). It is conceivable that certain mutations within XPG and RAD2 could perturb interactions with TFIIH and cause subtle defects in transcription. However, the nature of the XP-G/CS mutations reported here, and the failure to detect the resulting truncated XPG proteins, suggest that XPG would be nonfunctional and incapable of interacting with TFIIH in these rare patients. The mutations identified in CS-A (11) and CS-B (9) patients would also be expected to result in total loss of function. Similarly, disruption mutants of *RAD2* and of the yeast homologs of *CSA* and *CSB* (*RAD28* and *RAD26*, respectively) do not affect viability (52–54). Thus, in contrast to XPB and XPD, the other three proteins implicated in CS and their yeast counterparts are not essential for RNA polymerase II transcription.

In seeking a molecular explanation of CS, we suggest that attention be focused on the DNA repair defect that is specific to CS-A and CS-B patients, namely, their inability to carry out TCR. A causal relationship between CS and the NER version of TCR is difficult to imagine because most XP groups are not associated with CS. In particular, many Japanese XP-A patients with essentially no NER activity present a very severe XP phenotype but no CS symptoms (55). However, in normal human cells, TCR corrects not only UV-induced DNA damage but also lesions induced by ionizing radiation; the latter process requires CSB but not XPA (7). Since damage from ionizing radiation under biological conditions is largely caused by hydroxyl radicals and other reactive oxygen species, its spectrum is very similar to that of spontaneous oxidative damage generated during normal cellular metabolism (56, 57). Transcriptionally active, rapidly proliferating cells would be most exposed to such natural metabolic damage, and hence would be particularly at risk if TCR were impaired or nonfunctional. Thus, rather than being due to a transcriptional defect, the CS clinical phenotype may be related to a deficiency in TCR of endogenous DNA damage (7, 8), perhaps during critical time windows in early development.

Recently we have provided evidence that XPG is required for TCR of such damage (58), and we suggest that this represents the second XPG function. Cells from the three XP-G/CS patients characterized here are unable to preferentially remove thymine glycols from transcribed strands of active genes whereas this TCR activity occurs at normal levels in cells from XPG patient XP125LO and from XP-A and XP-F patients (58). These results strongly suggest that TCR of oxidative damage is independent of the XPG incision function in NER, and even of NER altogether. Instead, this activity presumably is initiated by glycosylases and AP endonuclease in a base excision repair pathway (reviewed in ref. 1). We suspect that this pathway requires at least a transient interaction between XPG and TFIIH, and perhaps other proteins, to displace RNA polymerase II stalled at a lesion (51, 59). Rather than having a catalytic role, XPG may have an assembly function in this pathway, similar to its suggested role in helping to establish the NER preincision complex (3).

Mutations in three of the five genes implicated in CS have been shown to result in a partial (*CSA*) or complete (*CSB* and *XPG*) deficiency in TCR of oxidative base damage (7, 58). It would seem reasonable to expect similar defects in cells from XP-B/CS and XP-D/CS patients. XP-G/CS cells exhibit an additional defect, a decreased rate of removal of oxidative damage from the genome as a whole (58). This may contribute to the unusually severe phenotype presented by the XP-G/CS patients, and it may also hint at an additional XPG function in a base excision repair pathway that is not coupled to transcription. In conclusion, a positive and perhaps causal relationship exists between an inability to produce full-length XPG, an inability to remove oxidative base damage from active genes, and the appearance of the devastating CS clinical phenotype.

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1. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (Am. Soc. Microbiol., Washington, DC).
2. Aboussekhra, A., Biggerstaff, M., Shivji, M. K. K., Vilpo, J. A., Moncollin, V., Podust, V. N., Protic, M., Hübscher, U., Egly, J.-M. & Wood, R. D. (1995) *Cell* **80,** 859–868.
3. Mu, D., Hsu, D. S. & Sancar, A. (1996) *J. Biol. Chem.* **271,** 8285–8294.
4. Nance, M. A. & Berry, S. A. (1992) *Am. J. Med. Genet.* **42,** 68–84.
5. Mellon, I., Spivak, G. & Hanawalt, P. C. (1987) *Cell* **51,** 241–249.
6. van Hoffen, A., Natarajan, A. T., Mayne, L. V., van Zeeland, A. A., Mullenders, L. H. F. & Venema, J. (1993) *Nucleic Acids Res.* **21,** 5890–5895.
7. Leadon, S. A. & Cooper, P. K. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10499–10503.
8. Hanawalt, P. C. (1994) *Science* **266,** 1957–1958.
9. Lehmann, A. R. (1995) *Trends Biochem. Sci.* **20,** 402–405.
10. Friedberg, E. C. (1996) *Annu. Rev. Biochem.* **65,** 15–42.
11. Henning, K. A., Li, L., Iyer, N., McDaniel, L. D., Reagan, M. S., Legerski, R., Schultz, R. A., Stefanini, M., Lehmann, A. R., Mayne, L. V. & Friedberg, E. C. (1995) *Cell* **82,** 555–564.
12. Neer, E. J., Schmidt, C. J., Nambudripad, R. & Smith, T. F. (1994) *Nature (London)* **371,** 297–300.
13. Troelstra, C., van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D. & Hoeijmakers, J. H. J. (1992) *Cell* **71,** 939–953.
14. Eisen, J. A., Sweder, K. S. & Hanawalt, P. C. (1995) *Nucleic Acids Res.* **23,** 2715–2723.
15. Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. & Hoeijmakers, J. H. J. (1990) *Cell* **62,** 777–791.
16. Weber, C. A., Salazar, E. P., Stewart, S. A. & Thompson, L. H. (1990) *EMBO J.* **9,** 1437–1447.
17. Flejter, W. L., McDaniel, L. D., Johns, D., Friedberg, E. C. & Schultz, R. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 261–265.
18. Schaeffer, L., Roy, R., Humbert, S., Moncollin, V., Vermeulen, W., Hoeijmakers, J. H. J., Chambon, P. & Egly, J.-M. (1993) *Science* **260,** 58–63.
19. Feaver, W. J., Svejstrup, J. Q., Bardwell, L., Bardwell, A. J., Buratowski, S., Gulyas, K. D., Donahue, T. F., Friedberg, E. C. & Kornberg, R. D. (1993) *Cell* **75,** 1379–1387.
20. Van Vuuren, A. J., Vermeulen, W., Ma, L., Weeda, G., Appeldoorn, E., Jaspers, N. G. J., van der Eb, A. J., Bootsma, D., Hoeijmakers, J. H. J., Humbert, S., Schaeffer, L. & Egly, J.-M. (1994) *EMBO J.* **13,** 1645–1653.
21. Schaeffer, L., Moncollin, V., Roy, R., Staub, A., Mezzina, M., Sarasin, A., Weeda, G., Hoeijmakers, J. H. J. & Egly, J.-M. (1994) *EMBO J.* **13,** 2388–2392.
22. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J.-C., Zawel, L., Ahn, K., Sancar, A. & Reinberg, D. (1994) *Nature (London)* **368,** 769–772.
23. Scherly, D., Nouspikel, T., Corlet, J., Ucla, C., Bairoch, A. & Clarkson, S. G. (1993) *Nature (London)* **363,** 182–185.
24. MacInnes, M. A., Dickson, J. A., Hernandez, R. R., Learmonth, D., Lin, G. Y., Mudgett, J. S., Park, M. S., Schauer, S., Reynolds, R. J., Strniste, G. F. & Yu, J. Y. (1993) *Mol. Cell. Biol.* **13,** 6393–6402.
25. Shiomi, T., Harada, Y., Saito, T., Shiomi, N., Okuno, Y. & Yamaizumi, M. (1994) *Mutat. Res.* **314,** 167–175.
26. Murray, J. M., Tavassoli, M., Al-Harity, R., Sheldrick, K. S., Lehmann, A. R., Carr, A. M. & Watts, F. Z. (1994) *Mol. Cell. Biol.* **14,** 4878–4888.
27. Harrington, J. J. & Lieber, M. R. (1994) *Genes Dev.* **8,** 1344–1355.
28. Robins, P., Pappin, D. J. C., Wood, R. D. & Lindahl, T. (1994) *J. Biol. Chem.* **269,** 28535–28538.
29. O'Donovan, A., Davies, A. A., Moggs, J. G., West, S. C. & Wood, R. D. (1994) *Nature (London)* **371,** 432–435.
30. Matsunaga, T., Mu, D., Park, C.-H., Reardon, J. T. & Sancar, A. (1995) *J. Biol. Chem.* **270,** 20862–20869.
31. Habraken, Y., Sung, P., Prakash, L. & Prakash, S. (1995) *J. Biol. Chem.* **270,** 30194–30198.
32. Moggs, J. G., Yarema, K. J., Essigmann, J. M. & Wood, R. D. (1996) *J. Biol. Chem.* **271,** 7177–7186.
33. Jaeken, J., Klocker, H., Schwaiger, H., Bellmann, R., Hirsch-Kauffmann, M. & Schweiger, M. (1989) *Hum. Genet.* **83,** 339–346.
34. Vermeulen, W., Jaeken, J., Jaspers, N. G. J., Bootsma, D. & Hoeijmakers, J. H. J. (1993) *Am. J. Hum. Genet.* **53,** 185–192.
35. Hamel, B. C. J., Raams, A., Schuitema-Dijkstra, A. R., Simons, P., van der Burgt, I., Jaspers, N. G. J. & Kleijer, W. J. (1996) *J. Med. Genet.* **33,** 607–610.
36. Norris, P. G., Hawk, J. L. M., Avery, J. A. & Giannelli, F. (1987) *Br. J. Dermatol.* **116,** 861–866.
37. Nouspikel, T. & Clarkson, S. G. (1994) *Hum. Mol. Genet.* **3,** 963–967.
38. Leadon, S. A. & Lawrence, D. A. (1991) *Mutat. Res.* **255,** 67–78.
39. O'Donovan, A., Scherly, D., Clarkson, S. G. & Wood, R. D. (1994) *J. Biol. Chem.* **269,** 15965–15968.
40. Lalle, P., Moyret-Lalle, C., Wang, Q., Vialle, J.-M., Navarro, C., Bressac-de Paillerets, B., Magaud, J.-P. & Ozturk, M. (1995) *Oncogene* **10,** 2447–2454.
41. Moriwaki, S.-I., Stefanini, M., Lehmann, A. R., Hoeijmakers, J. H. J., Robbins, J. H., Rapin, I., Botta, E., Tanganelli, B., Vermeulen, W., Broughton, B. C. & Kraemer, K. H. (1996) *J. Invest. Dermatol.* **107,** 647–653.
42. Mueser, T. C., Nossal, N. G. & Hyde, C. C. (1996) *Cell* **85,** 1101–1112.
43. Sijbers, A. M., de Laat, W. L., Ariza, R. R., Biggerstaff, M., Wei, Y.-F., Moggs, J. G., Carter, K. C., Shell, B. K., Evans, E., de Jong, M. C., Rademakers, S., de Rooij, J., Jaspers, N. G. J., Hoeijmakers, J. H. J. & Wood, R. D. (1996) *Cell* **86,** 811–822.
44. Bootsma, D. & Hoeijmakers, J. H. J. (1993) *Nature (London)* **363,** 114–115.
45. Vermeulen, W., van Vuuren, A. J., Chipoulet, M., Schaeffer, L., Appeldoorn, E., Weeda, G., Jaspers, N. G. J., Priestley, A., Arlett, C. F., Lehmann, A. R., Stefanini, M., Mezzina, M., Sarasin, A., Bootsma, D., Egly, J.-M. & Hoeijmakers, J. H. J. (1994) *Cold Spring Harbor Symp. Quant. Biol.* **59,** 317–329.
46. Broughton, B. C., Thompson, A. F., Harcourt, S. A., Vermeulen, W., Hoeijmakers, J. H. J., Botta, E., Stefanini, M., King, M. D., Weber, C. A., Cole, J., Arlett, C. F. & Lehmann, A. R. (1995) *Am. J. Hum. Genet.* **56,** 167–174.
47. Takayama, K., Salazar, E. P., Lehmann, A. R., Stefanini, M., Thompson, L. H. & Weber, C. A. (1995) *Cancer Res.* **55,** 5656–5663.
48. Hwang, J. R., Moncollin, V., Vermeulen, W., Seroz, T., van Vuuren, H., Hoeijmakers, J. H. J. & Egly, J.-M. (1996) *J. Biol. Chem.* **271,** 15898–15904.
49. Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T. & Sancar, A. (1995) *J. Biol. Chem.* **270,** 2415–2418.
50. Iyer, N., Reagan, M. S., Wu, K.-J., Canagarajah, B. & Friedberg, E. C. (1996) *Biochemistry* **35,** 2157–2167.
51. Habraken, Y., Sung, P., Prakash, S. & Prakash, L. (1996) *Proc. Natl. Acad. Sci USA* **93,** 10718–10722.
52. Higgins, D. R., Prakash, L., Reynolds, P. & Prakash, S. (1984) *Gene* **30,** 121–128.
53. Bhatia, P. K., Verhage, R. A., Brouwer, J. & Friedberg, E. C. (1996) *J. Bacteriol.* **178,** 5977–5988.
54. van Gool, A. J., Verhage, R., Swagemakers, S. M. A., van de Putte, P., Brouwer, J., Troelstra, C., Bootsma, D. & Hoeijmakers, J. H. J. (1994) *EMBO J.* **13,** 5361–5369.
55. Satokata, I., Tanaka, K., Miura, N., Miyamoto, I., Satoh, Y., Kondo, S. & Okada, Y. (1990) *Proc. Natl. Acad. Sci USA* **87,** 9908–9912.
56. Ward, J. F. (1988) *Prog. Nucleic Acid Res. Mol. Biol.* **35,** 95–125.
57. Riley, P. A. (1994) *Int. J. Radiat. Biol.* **65,** 27–33.
58. Cooper, P. K., Nouspikel, T., Clarkson, S. G. & Leadon, S. A. (1997) *Science* **275,** 990–993.
59. Donahue, B. A., Yin, S., Taylor, J. S., Reines, D. & Hanawalt, P. C. (1994) *Proc. Natl. Acad. Sci USA* **91,** 8502–8506.