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Molecular cloning of cDNA encoding human DNA helicase Q1 which has homology to *Escherichia coli* Rec Q helicase and localization of the gene at chromosome 12p12

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ABSTRACT

A complementary DNA encoding DNA-dependent ATPase Q1 possessing DNA helicase activity, which is the major DNA-dependent ATPase in human cell extracts, was cloned from a cDNA library of human KB cells. The predicted amino acid sequence has seven consecutive motifs conserved in the RNA and DNA helicase super family and DNA helicase Q1 belongs to DEXH helicase family. A homology search indicated that helicase Q1 had 47% homology in the seven conserved regions with *Escherichia coli* RecQ protein. Three RNA bands of 4.0, 3.3, and 2.2 kilobases were detected in HeLa cells by Northern blotting. Analysis of the genomic DNA indicated the presence of a homologous gene in mouse cells. The DNA helicase Q1 gene was localized on the short arm of human chromosome 12 at 12p12.

INTRODUCTION

Enzymes having DNA-dependent ATPase and DNA helicase activities in prokaryotic cell systems play important roles in DNA repair (1). In the case of mammalian cells, human genes designated excision repair cross-complementing rodent repair deficiency (ERCC[1]) have been cloned, and the sequences of *ERCC-2* and *ERCC-3* have been shown to contain DNA/RNA helicase motifs (2,3). These gene products have DNA helicase activity and hence DNA-dependent ATPase activity (4,5).

Mutants of mammalian cells defective in DNA repair will serve as useful tools for the identification of enzymes and proteins involved in DNA repair and for the analysis of the molecular mechanism of the process. Cells from patients with xeroderma pigmentosum (XP) are defective in the incision step of nucleotide excision repair. XP is now categorized into seven complementation groups, A to G, and a variant (6). The defect in complementation group B cells has been corrected by microinjecting the complementary DNA of the *ERCC-3* gene (3). The *ERCC-2* gene corrected the nucleotide excision repair defect in XP group D cells (7).

Thus, we studied DNA-dependent ATPases in XP cells. Human cells contain at least five DNA-dependent ATPase activities that are separable by means of fast protein liquid chromatography (FPLC) Mono Q column chromatography. We found that the elution profile of one of the five DNA-dependent ATPases, ATPase Q1, from XP group C cells (XP-C) was altered compared with those of ATPase Q1 from human cells normal in repair and XP cells of complementation groups A to G except C. The elution profile of DNA-dependent ATPase Q1 was also altered in five cell lines derived from five patients belonging to complementation group C, indicating that the defect of XP-C cells in DNA repair is due to an alteration of DNA-dependent ATPase Q1 (8). We therefore purified DNA-dependent ATPase Q1 from HeLa cells (9). The microinjection of the purified ATPase Q1, however, neither restored unscheduled DNA synthesis in XP-C cells nor complemented extracts from XP-C cells for cell-free DNA repair synthesis. A cDNA clone that restores the ultraviolet

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sensitivity and unscheduled DNA synthesis of XP-C cells to normal levels, has no DNA helicase motif (10). These results indicate that ATPase Q1 itself is not the corresponding protein for the defect in DNA repair in XP-C cells.

In this study, we cloned the cDNA encoding DNA-dependent ATPase Q1 as the first step to understand the role of ATPase Q1 in DNA repair and to determine why ATPase Q1 is altered in XP-C cells.

MATERIALS AND METHODS

Materials

All restriction enzymes, T4 DNA ligase, T4 polynucleotide kinase, Klenow fragment, and unlabeled deoxyribonucleoside triphosphates were purchased from Takara Shuzo Co. Ltd (Kyoto, Japan). Enzyme reactions were performed as recommended by the suppliers.

Purification of DNA-dependent ATPase Q1 from HeLa cells

DNA-dependent ATPase Q1 was purified from 3×10^{10} HeLa cells as described (9).

Determination of partial amino acid sequences of purified ATPase Q1

Purified DNA-dependent ATPase Q1 (80 μg) was resolved by preparative SDS−PAGE, and the appropriate protein band in the gel slices was digested with 20 μg/ml lysylendopeptidase in 50 mM Tris−HCl, pH 9.0 and the digests were loaded onto a reverse phase HPLC column equilibrated with 5% acetonitrile and 0.1% trifluoroacetic acid. The peptide fragments were eluted with a linear gradient of acetonitrile from 5% to 30%. The amino acid sequences of the fractionated peptides were analyzed with an Applied Biosystems Model 470A automated gas-phase protein sequencer.

Synthesis of oligodeoxyribonucleotide probes

The oligodeoxyribonucleotides, GGI GTI GTI GCI CCI ACI CTI CCI (A/C)GI GAI GAT CTI GAI AA (41-mer) for probes was synthesized with an Applied Biosystems Model 391 DNA synthesizer. The primers for DNA sequencing were also synthesized, based on the sequences derived from cDNAs.

cDNA library and screening of cDNAs

A λgt10 cDNA library from human KB cells was prepared as described (11). More than 7.2×10^5 recombinant phage plaques transferred to duplicate nitrocellulose membrane were screened by plaque-hybridization. Prehybridization proceeded at 68°C for 5 h in a solution containing 6×SSC (1×SSC; 0.15 M NaCl, 0.015 M Na-citrate), 2×Denhardt's solution (1×Denhardt's solution; 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrolidone), and 50 μg/ml of heat-denatured salmon sperm DNA. Hybridization was performed overnight at 42°C in 3×Denhardt's solution containing 50 μg/ml of heat denatured salmon sperm DNA, the 41-mer probe labeled with ^{32}P by using T4 polynucleotide kinase, 4×SSC and 35% formamide. The membranes were washed once at room temperature and once at 68°C with 4×blot-wash buffer (1×blot-wash buffer; 1×SSC, 10 mM sodium phosphate, 0.025% SDS), once with 2×blot-wash buffer and once with 1×blot-wash buffer at 68°C for 10 min per wash, air-dried, and visualized by autoradiography using Kodak XAR film (Eastman Kodak, Rochester, NY). Positive plaques were further purified.

Subcloning and sequencing of the cDNA inserts

The cDNA inserts of positive clones were inserted into the pUC19 vector. Based upon restriction maps of the cDNA inserts, several restriction-endonuclease fragments were isolated and subcloned into the appropriate multicloning sites of pUC19. The inserts were automatically analyzed by a DNA sequencer (ABI 373A). The DNA sequences and protein structures were analyzed using the software Gene Works by IntelliGenetics, Inc. (CA, USA).

Northern blotting

Total RNA fractions were prepared from human HeLa cells or mouse FM3A cells by homogenization with guanidine thiocyanate followed by centrifugation through a cesium chloride cushion. Poly $(A)^+$ RNA was purified using a batch oligo (dT)-latex bead procedure. RNA samples were fractionated by electrophoresis in 1.2% agarose gels containing formaldehyde and blotted onto nylon membranes (Hybond-N, Amersham) by capillary blotting. Prehybridization proceeded as described under 'cDNA library and screening of cDNAs'. Hybridization was performed overnight at 42°C in 4×SSC containing 2×Denhardt's solution, 50 μg/ml heat-denatured salmon sperm DNA, 50% formamide, and ^{32}P-labeled probe (probe 1 in Fig. 2.). The membrane was washed once at room temperature, twice at 68°C with 2×blot-wash buffer, once at 68°C with 1×blot-wash buffer, once with 0.5×blot-wash buffer, and once with 0.2×blot-wash buffer for 5 min per wash, air-dried and autoradiographed.

Genomic Southern analysis

Human genomic DNA and mouse genomic DNA was isolated from HeLa cells and FM3A cells, respectively, digested to completion with *Eco*RI, *Bam*HI, or *Hind*III, fractionated by electrophoresis and transferred to a nylon membrane. Prehybridization was carried out as described under 'cDNA library and screening of cDNAs'. Hybridization was performed at 42°C overnight under conditions of high or low stringency. The medium for high stringency consisted of 4×SSC, 2×Denhardt's solution, 50 μg/ml heat-denatured salmon sperm DNA, 50% formamide , and the ^{32}P-labeled probe (probe 1, 2, or 3 in Fig. 2). That for low stringency was 6×SSC, 2×Denhardt's, 50 μg/ml heat-denatured salmon sperm DNA, 20% formamide, and the ^{32}P-labeled probe. The membranes were washed as described under 'Northern Blotting'.

Assay of DNA-dependent ATPase and DNA helicase activities

DNA-dependent ATPase and DNA helicase activities were assayed as described previously (12,13).

Isolation of human genomic cosmid clones

A cosmid library was constructed from the genomic DNA of the human B-lymphoblastoid cell line, AKIBA, which was partially digested with *Sau*3AI and then ligated to the cosmid vector pWE15 [a gift from Dr H.Inoko (14)]. The library was screened using ^{32}P-labeled 2.0 kb *Sma*I−*Eco*RV fragment of the cDNA (probe 1 in Fig. 2) as the probe by colony hybridization, as described (15). The probe was prepared by random primer labeling (16). Two independent clones, cHQ1-#16 and cHQ1-#17, were isolated and characterized by Southern blotting. These clones shared the 9.4 and 3.8 kb *Eco*RI fragments, and the 9.4 kb contains part of the ATPase Q1 gene judging from the result of hybridization with probe 1 of the cDNA (Fig. 5A, lane 1).

Polymerase chain reaction (PCR)

PCR was performed to chromosome map the human ATPase Q1 gene using a DNA panel of human/rodent somatic cell hybrid cells as described (17). A pair of oligonucleotide primers were constructed from the 3'-untranslated sequence of the cDNA using a 394 DNA/RNA synthesizer (Applied Biosystems, Inc.). The sequences of the primers used were as follows: 5'-AAA TAT TTG AGA ATA AGT TCA TAC-3' and 5'-AAA GAT TGG ACA TCC CTG ATC TAC-3'. These sequences correspond to nucleotide numbers 2196–2219 and 2343–2366 of the cDNA. The size of amplified DNA is 171 bp. These primers were used as expressed sequence tags [EST, (18)] for human ATPase Q1 gene.

The panels of human/rodent somatic cell hybrid (NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel #2 and BIOSMAP™ Somatic Cell Hybrid PCRable™ DNAs) were purchased from Coriell Cell Repositories (Camden, NJ) and BIOS Co.(New Haven, CT), respectively.

Fluorescence *in situ* hybridization (FISH)

R-banding for direct mapping (19) and a cooled CCD digital imaging system were used to chromosome map the human ATPase Q1 gene. Probe labeling and *in situ* hybridization were done as described (20). R- or G-banded metaphase chromosomes were prepared according to Takahashi *et al.* (21). The results of the FISH experiments were imaged using a Zeiss Axioskop epifluorescence microscope equipped with a CCD camera (Photometrics PXL 1400). The microscope was equipped with precision filters specific for each fluorophore. Images were saved as grayscale data files for acquisition, using the software IPLab™ (Signal Analytics Co.). Merging and pseudocoloring was accomplished using Adobe Photoshop™ 2.5J (Adobe Systems Inc.) on a Macintosh Quadra 840AV computer. Hoechst, FITC, and propidium iodide images are shown in blue, green, and red, respectively. The images were then printed (Pictrography, Fuji film Co.).

RESULTS

Purification of DNA-dependent ATPase Q1 and determination of the peptide sequences

DNA-dependent ATPase Q1 was purified from HeLa cells to nearly homogeneity as described (9). The purified ATPase Q1 has DNA helicase activity as we reported previously (9). Figure 1 shows the release of ^{32}P-labeled oligodeoxyribonucleotides from single-stranded circular M13mp19 DNA as a function of incubation time. Thus we designated this DNA-dependent ATPase DNA helicase Q1. The purified helicase Q1 analyzed by SDS–PAGE revealed a single band at 73 kDa. To determine the partial amino acid sequence, the purified DNA helicase Q1 was resolved by preparative SDS–PAGE, and the resulting protein bands in the gel slices were digested with lysylendopeptidase. The peptides in the digest were separated by YMC reverse phase HPLC, and the peptides were sequenced. We determined six partial amino acid sequences.

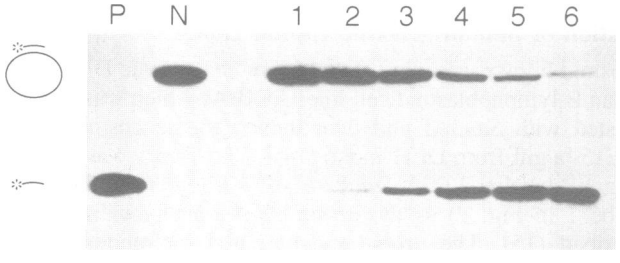

Figure 1. Time course of DNA helicase activity of DNA-dependent ATPase Q1. Nine units of DNA-dependent ATPase Q1 was incubated at 37°C for 0 (lane 1), 5 (lane 2), 10 (lane 3), 30 (lane 4), 60 (lane 5), and 120 (lane 6) min. **p** (positive control), heated in boiling water for 2 min without enzyme;**n** (negative control), incubated at 37°C without enzyme.

Cloning of cDNA of human DNA helicase Q1

One of the determined amino acid sequence, Gly-Val-Val-Ala-Pro-Thr-Leu-Pro-Arg-Glu-Asp-Leu-Glu-Lys, was used to synthesize an oligonucleotide probe. A mixture of 41-mer oligonucleotides, 5'-GGI GTI GTI GCI CCI ACI CTI CCI A/CGI GAI GAT CTI GAI AA-3', was synthesized according to the amino acid sequence. These were used to screen a human KB cell cDNA library integrated into λgt10. The screening of 7.2×10^5 plaques yielded 10 positive plaques, which were classified as two independent clones (φA and φB in Fig. 2). The cDNA inserts from these clones were subcloned into the pUC19 plasmid. Figure 2 shows a schematic representation of the isolated cDNAs, the restriction endonuclease map, and the strategy used to determine the nucleotide sequence.

The nucleotide sequence determined contained an open reading frame that encodes 649 amino acid residues, and the predicted molecular weight was 73329. A consensus AATAAA polyadenylation signal was not present at the 3' non-translated region. However, the closely related sequences, ATTAAA and

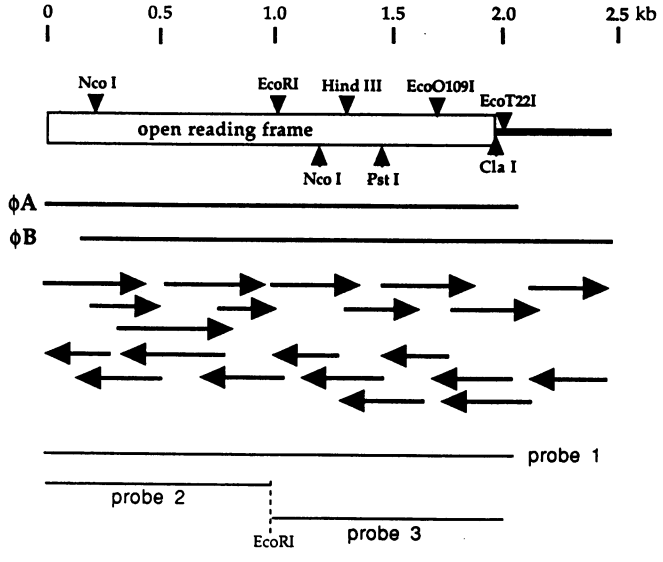

Figure 2. Restriction endonuclease map and sequencing strategy for cDNA of DNA helicase Q1. The scale at the top designates the nucleotide positions (in bases). The unfilled box represents the 649 amino acid coding region, and the 3'-nontranslated region is represented by a thick line. Under the restriction map, two overlapping cDNA clones, φA and φB are shown. The arrows indicate the direction and extent of sequenced fragments. Both strands of the cDNA were sequenced by dideoxy chain termination as described under 'Materials and Methods'. Probes for Southern (Fig. 5A) and Northern (Fig. 5B.) blots are shown at the bottom.

CATAAA, were located at positions 1973−1979 and 2412−2417 of the cDNA insert, respectively. Thus, differential polyadenylation occurs at the positions which correspond to termini of φA and φB inserts. The amino acid sequences determined by direct protein sequencing were contained in the open reading frame.

Homology between DNA helicase Q1 and *E.coli* RecQ

A search of the Swiss-Prot database indicated that the amino acid sequence of helicase Q1 has high similarity to the *E.coli* DNA recombination protein, RecQ (22). The deduced amino acid sequence of helicase Q1 was compared with *E.coli* RecQ protein. Alignment of the helicase Q1 and *E.coli* Rec Q amino acid sequences revealed that the two proteins contain seven consecutive motifs, I, Ia, II, III, IV, V, and VI (23), which are conserved in two superfamilies of DNA and RNA helicases (Fig. 3). The amino acid sequences of the two proteins are 42% identical (47% homology) in the region that contains 329 amino acids (amino acid 79−408 of helicase Q1) including seven conserved helicase motifs. Regions I, Ia, II, III, IV, V, and VI of the two proteins are 80, 57, 46, 42, 50, 68, and 69% identical, respectively. Figure 4 shows homology in seven conserved regions of helicase Q1 and other DNA and RNA helicases (23,24).

Genomic Southern and Northern blots

The total genomic DNA of HeLa and mouse FM3A cells were digested with restriction endonuclease and the resulting DNA fragments were hybridized with probe 1 (Fig. 2) under conditions of low stringency . As shown in Figure 5A, HeLa and mouse genomic DNA showed several and one hybridization band, respectively, indicating the presence of a homologous gene in the mouse cells. Hybridization under conditions of high stringency revealed the same bands as shown in Figure 5A (data not shown). The size and abundance of helicase Q1 transcripts in HeLa and mouse FM3A cells were examined by Northern blotting using probe 1. Three bands of 4.0, 3.3, and 2.2 kb were detected in HeLa cells, and the 3.3 kb band was the most abundant (Fig. 5B , lane 1 and 3). We could not detect helicase Q1 transcript in mouse FM3A cells under conditions either high (Fig. 5B, lane 2) or low (data not shown) stringency, although the genomic Southern analysis gave positive signal as described above. In addition, we could not detect DNA-dependent ATPase activity of DNA helicase Q1 in FM3A cell extracts (data not shown).

Localization of the DNA helicase Q1 gene

PCR was performed to assign the chromosomal location for the human helicase Q1 gene using a DNA panel of human/rodent somatic cell hybrid cells. The sequences of used primers corresponded to the nucleotide numbers 2196−2219 and 2343−2366 of the cDNA. Thus the size of DNA that will be amplified is 171 base pairs. As shown in Figure 6, only the DNA from hybrid cell line containing the human chromosome 12 exhibited the 171 bp band, indicating that the helicase Q1 gene is localized in chromosome 12.

We next attempted to confirm the above chromosomal assignment and to determine the physical location of the helicase Q1 gene on the human chromosomes by FISH. The genomic DNA (cosmid cHQ1-# 16) and cDNA were biotinylated by nick translation and hybridized with human metaphase chromosome spreads. A set of symmetric double spots appeared in the short arms of chromosome 12 with the genomic DNA (Fig. 7A) and cDNA (Fig. 7B) probes. The G and R band patterns of the

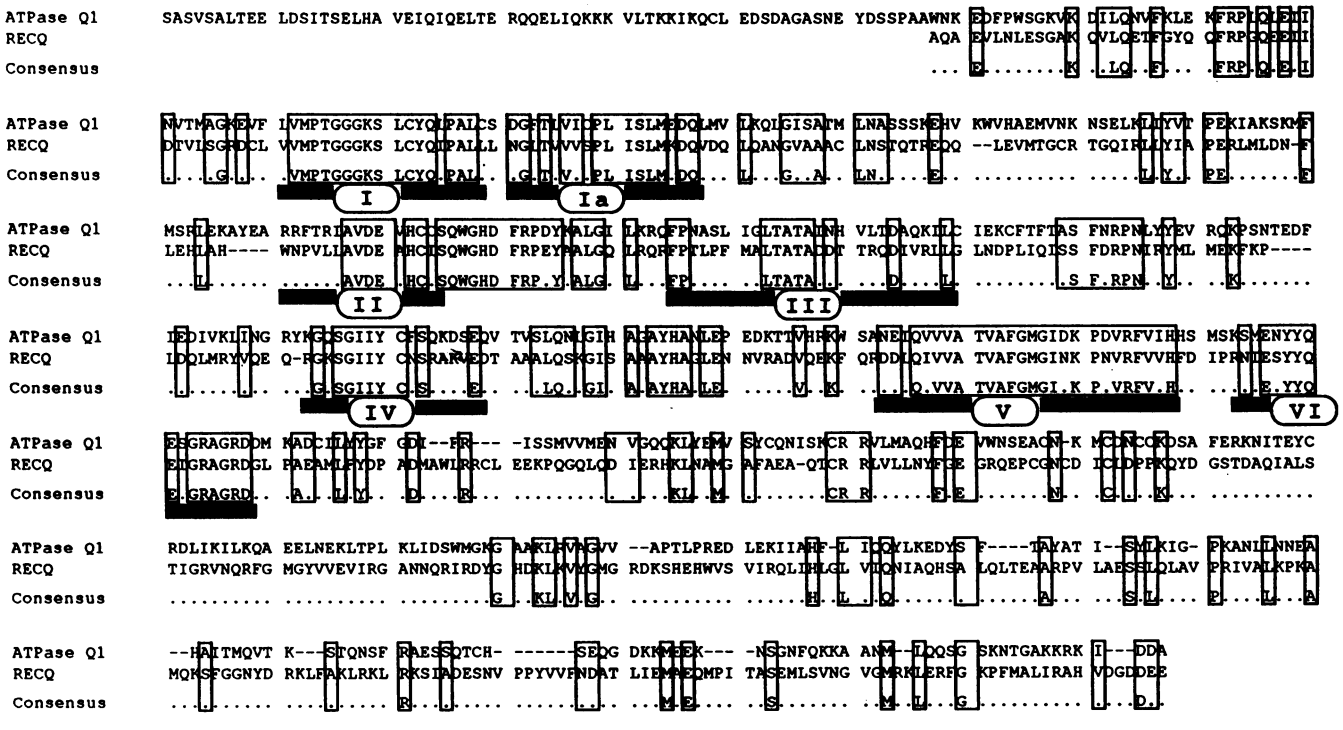

Figure 3. The similarity of human DNA helicase Q1 and *E.coli* RecQ protein. Identical and conserved substituted amino acids (A=S, D=N=E, S=N, V=I) are boxed. Conserved helicase domains (identified by roman numerals) are thickly underlined according to Gorbalenya *et al.* (23).

chromosomes indicated that the physical location of the helicase Q1 gene is 12p12. Consistent fluorescence signals were not evident on other chromosomes using these probes.

DISCUSSION

In this study we cloned a cDNA encoding DNA helicase Q1 prepared from a human KB cell cDNA library, and several issues were revealed as follows.

Firstly, the helicase Q1 cDNA contains a 1947 bp open reading frame encoding a 649 amino acid protein with a molecular weight of 73 329. Although the N terminal region of the cDNA does not contain the initiation codon, the predicted molecular weight is very close to the molecular mass of the purified helicase Q1 estimated by SDS−PAGE at 73 kDa. In addition, sequencing of a cDNA clone from another human cDNA library indicated that the first three nucleotides are ATG and not TCG. Thus this cDNA contains almost the entire coding sequence for helicase Q1.

Secondly, the predicted amino acid sequence of helicase Q1 has homology with the *E.coli* recombination protein, RecQ (Fig. 3). *E.coli* RecQ protein has both DNA-dependent ATPase and DNA helicase activities, translocating in the 3′ to 5′ direction on the DNA to which it binds (22,25). DNA helicase Q1 also translocates in the 3′ to 5′ direction (9). Thus both helicase Q1 and RecQ protein have similar biochemical activity as well as similar amino acid sequences.

Thirdly, the seven motifs conserved in DNA and RNA helicase super family (23,24,26) are also conserved in both DNA helicase

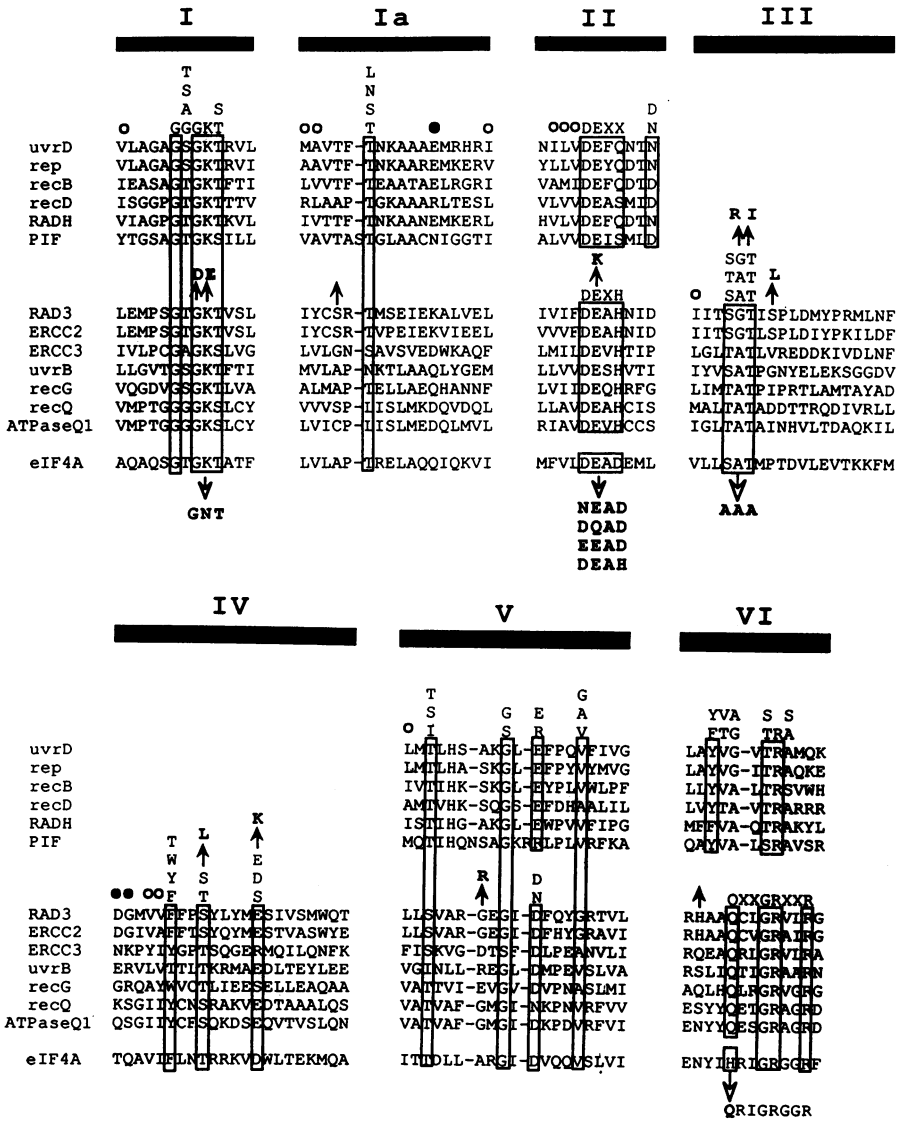

Figure 4. A Comparison of DNA/RNA helicase motifs. UvrD (45), rep (46), recB (47), recD (48), and PIF (49) were aligned by Hodgman (24). UvrB (50), recQ (22), ERCC2 , ERCC3 , RAD3 (51) and eIF4A (52) were aligned by Gorbalenya *et al.*, (23), Weber, *et al.* (2), and Weeda *et al.* (3). RecG (53), RADH (54), and helicase Q1 were aligned according to the method of Hodgman (24) and Gorbalenya *et al.* (23). Identical amino acids or conserved substitutions are boxed. Hydrophobic or hydrophilic amino acids conserved in all helicases are represented as ○ or ●, respectively. The amino acid positions of characterized missense mutations in rad3 alleles are indicated (↑) and the replaced amino acids are printed in bold characters. The amino acid positions of mutagenized eIF4A are indicated (↓), and the replaced amino acids are represented by bold characters.

Q1 and RecQ. The importance of those motifs have been studied in detail with the mammalian translation initiation factor eIF-4A and the yeast repair protein RAD3 by mutational analysis (27–30).

The sequence of GXGKS/T in motif I may be responsible for ATP binding, because replacement of Lys with Asn in XXXXGKT of eIF-4A abolished ATP binding activity (27). Motif II is classified into three families, DEXX (24), DEAD (26), and the DEXH (23,31). Both RecQ and helicase Q1 belong to DEXH family. This motif is a variant of the Walker ATPase B motif (32), and an X-ray crystallographic study has shown that the first aspartate residue is in close proximity to the ATPase A motif (motif I) and that it binds Mg^{2+} ions through a water molecule (33,34).

Motif III, TAT for helicase Q1 and RecQ, SAT for eIF-4A, or SGT for RAD3, may be essential for helicase activity because the eIF-4A replaced SAT with AAA has both ATP binding and ATPase activities but no RNA helicase activity (27). Replacement of SGT with SGI or SRI in RAD3 protein resulted in a deficiency of the DNA repair function *in vivo* (29).

The function of motif IV is unknown. However, replacing Ser with Leu in RAD3 protein abolishes DNA repair activity *in vivo* (29). Serine or threonine is conserved in most helicases (Fig. 4).

Motif V of RAD3 protein is essential for both DNA repair and the viability of cells *in vivo* (29), and S/T and G/S are conserved in all helicases, indicating that these amino acids are essential for enzyme function. The replacement of Gly with Arg

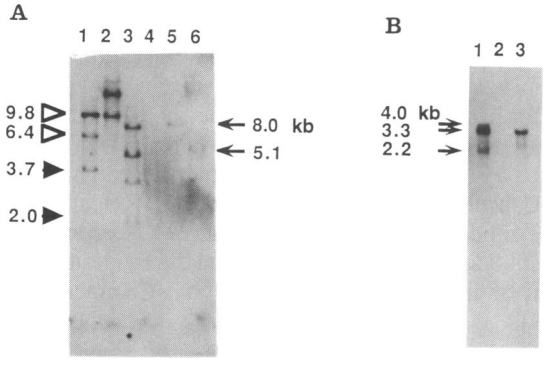

Figure 5. Genomic Southern and Northern blots. **A**: Human (HeLa cells) or mouse (FM3A cells) genomic DNA (10 μg) was digested with *Eco*RI, *Bam*HI, or *Hind*III, and the digests were size fractionated on a 1 % agarose gel. Southern transfer and hybridization with probe 1 (Fig. 2) proceeded according to 'Materials and Methods'. Lane 1, *EcoR*I digests of HeLa genomic DNA; lane 2, HeLa digested with *Bam*HI; lane 3, HeLa digested with *Hind*III; lane 4, FM3A digested with *Eco*RI; lane 5, FM3A digested with *Bam*HI; and lane 6, FM3A digested with *Hind*III. The bands of lane 1 hybridized with probe 2 or 3 are indicated as or, respectively. **B**: RNA was size fractionated on a 1.2 % agarose gel containing formaldehyde. Hybridization with probe 1 (Fig. 2) proceeded under conditions of high stringency according to 'Materials and Methods'. Lane 1, 2 μg of HeLa poly(A)$^+$RNA; lane 2, 2 μg of mouse FM3A poly(A)$^+$RNA and lane 3, 10 μg of HeLa total RNA.

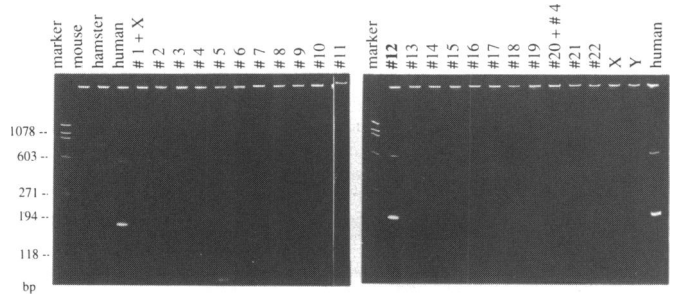

Figure 6. Chromosome assignment of the human DNA helicase Q1 gene by PCR. PCR proceeded under the conditions described under 'Materials and Methods' to assign the human chromosome bearing the helicase Q1 gene. The DNAs from the human/rodent somatic cell hybrid lines (NIGMS Somatic Cell Hybrid Mapping Panel # 2) were used as a panel. Each reaction contained DNA from mouse 3T6 cells ('mouse' on top of the lane as a negative control), Chinese hamster RJK88 cells (hamster as a negative control), human IMR91 cells (human as a positive control), and the number of human chromosome contained in the human/rodent hybrid cell lines is indicated on top of the lane. The product (171 bp) was only amplified in the reactions containing genomic DNA from human chromosome 12. *Hae*III-digested φX174 DNA was used as a size marker.

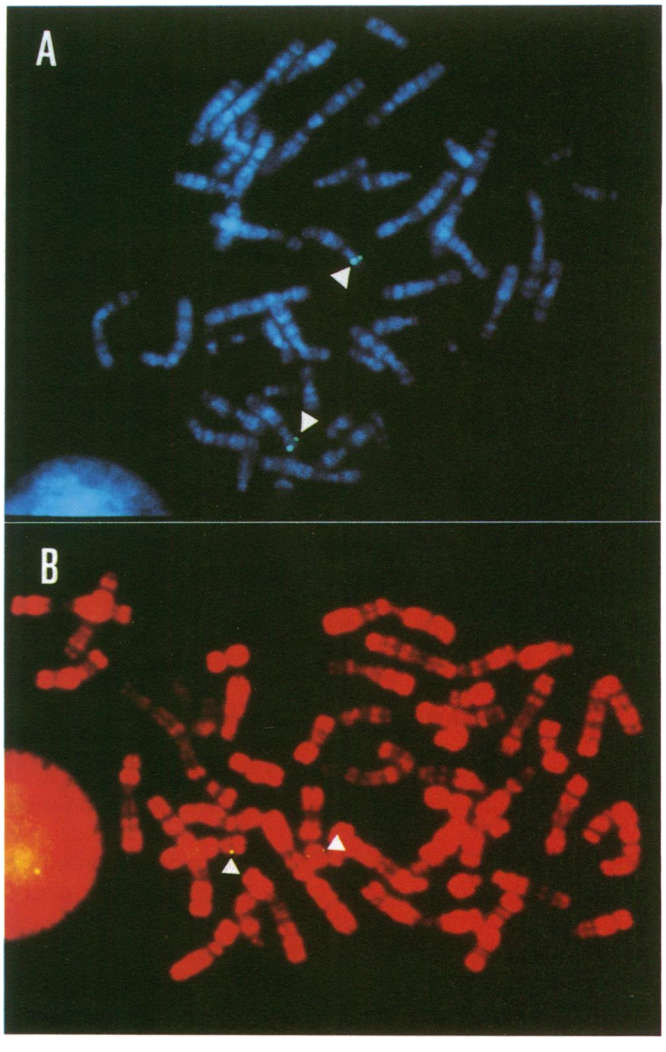

Figure 7. Fluorescence *in situ* hybridization of the DNA helicase Q1 gene to human metaphase chromosomes. Fluorescence *in situ* hybridization was performed using the cosmid cHQ1-# 16 (**A**) and cDNA (**B**) as a probe as described under 'Materials and Methods'. The digital images were pseudocolored to distinguish the different fluorochromes. Photographs were printed by Fuji Pictrography. Both the cosmid (A) and cDNA (B) clones were localized on chromosome band 12p12 by G- and R-banding, respectively. The arrow heads indicate fluorescence signals.

in VARGEGI of RAD3 protein resulted in a deficiency in the DNA repair function *in vivo* (29).

The motif VI of helicase Q1, QXXGRXXR, is highly conserved in the RNA/DNA helicase family. The signature of DEAD family is 'HXXGRXXR' and that of DEXH family is 'QXXGRXXR' (23) as shown in Figure 4, indicating a sort of compensation. Mutational analysis of eIF-4A suggests that the His residue in HRIGRGGR of eIF-4A is important for both ATPase and RNA helicase activity (27).

Fourthly, we confirmed the existence of a mouse homologue of human helicase Q1 gene by genomic Southern blotting (Fig. 5A, lanes 5 and 6). We therefore speculate that there is a RecQ related gene in a wide range of species from prokaryotes to higher eukaryotic cells. Both the mRNA and DNA-dependent ATPase activity of helicase Q1 were detected in human HeLa cells (Fig. 5B, lanes 1 and 3) and DNA-dependent ATPase activity of Q1 was also detected in human fibroblasts and lymphoblasts (8). However, we could not detect DNA-dependent ATPase activity of DNA helicase Q1 in mouse FM3A cells and Swiss 3T3 cells (data not shown). These results indicate that the level of expression of DNA helicase Q1 message is variable in different species.

Fifthly, we mapped the human helicase Q1 gene to chromosome 12q12 by both PCR assay using DNAs from human/rodent hybrid cell panels and FISH (Figs.6 and 7). The result from FISH analysis with a cDNA clone confirmed that helicase Q1 is transcribed from a single gene. Several reports have suggested that some types of human cancer are caused by a dysfunction of the gene products functioning in DNA metabolism, especially in DNA repair (reviewed in 35). Since human helicase Q1 may be involved in DNA repair or recombination, it is possible that a genetic loss or alteration of the helicase Q1 gene causes human hereditary diseases associated with aberrant cell proliferation. Among current reports of clinical disorders, such as chromosome aberrations and uniparental disomy (36), chromosome changes (37) or gene loss (38) in human neoplasia, there are no descriptions of genetic aberrations at the 12q12 region associated with disorders or neoplasia.

The question remains as to the function of DNA helicase Q1. Because of its homology with *E.coli* RecQ protein, studies of the processes involving RecQ protein should shed some light on the functions of helicase Q1. In *E.coli* , there are three distinct homologous recombination pathways, recBCD, recE, and recF (39, 40). The Rec E pathway requires the gene products of RecA, E, F, J, O, and Q whereas that of RecF requires those of RecA, F, J, N, O, Q, and Ruv. Thus RecQ protein is required for both the RecE and RecF pathways. The recF and recJ mutants were reduced or abolished in the activity to repair single-stranded gaps in post replicational repair (41). Furthermore, plasmid recombination is markedly reduced by recF, recJ, or recQ mutations (40). Thus, DNA without double stranded ends or having single stranded gaps may follow the Rec F pathway. It could be considered that helicase Q1 is involved in processes similar to those described above.

We found that the elution profile of DNA-dependent ATPase activity of helicase Q1 on a FPLC Mono Q column is altered in all cell lines belonging to the XP complementation group C tested (8). However, purified helicase Q1 did not complement XP-C cells for DNA repair activity tested by microinjection and cell-free repair assay (unpublished data). In addition, a complex consisting of 125 and 58 kDa proteins which complement XP-C cell extracts has been purified from HeLa cells, and the cDNAs coding these proteins have also been cloned (42). The 125 kDa subunit was identified as a N-terminally extended version of the XPCC gene product reported by Legerski and Peterson (10). These proteins have no homology with helicase Q1. Thus, helicase Q1 is not the corresponding protein responsible for the defect in XP-C cells. We are now attempting to isolate the genes encoding the proteins that interact with helicase Q1, using the two hybrid system. This will allow us to investigate the processes in which helicase Q1 is involved and to clarify why the elution profile of helicase Q1 is altered in XP-C cells.

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REFERENCES

1. Friedberg, E. C. (1985) DNA repair, W. H. Freeman & Co, San Francisco.
2. Weber, C. A., Salazar, E. P., Stewart, S. A. and Thompson, L. H. (1990) *EMBO J.*, **9**, 1437–1447.
3. Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. J. and Hoeijmakers, J. H. J. (1990) *Cell*, **62**, 777–791.
4. Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L. and Prakash, S. (1993) *Nature*, **365**, 852–855.
5. Schaeffer, L., Roy, R., Humbert, S., Moncollin, V., Vermeulen, W., Hoeijmakers, J. H. J., Chambon, P. and Egly, J.-M. (1993) *Science*, **260**, 58–63.
6. Vermeulen, W., Stefanini, M., Giliani, S., Hoeijmakers, J. H. J. and Bootsma, D. (1991) *Mutation Res.*, **255**, 201–208.
7. Flejter, W. L., McDaniel, L. D., Johns, D., Friedberg, E. C. and Schultz, R. A. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 261–265.
8. Yanagisawa, J., Seki, M., Ui, M. and Enomoto, T. (1992) *J. Biol. Chem.*, 267, 3585–3588.
9. Seki, M., Yanagisawa, J., Kohda, T., Sonoyama, T., Ui, M. and Enomoto, T. (1994) *J. Biochem.*, **115**, 523–531.
10. Legerski, R. and Peterson, C. (1992) *Nature*, **359**, 70–73.
11. Hoshino, S., Miyazawa, H., Enomoto, T., Hanaoka, F., Kikuchi, Y., Kikuchi, A. and Ui, M. (1989) *EMBO. J.*, **8**, 3807–3814.
12. Seki, M., Enomoto, T., Watanabe, Y., Tawaragi, Y., Kawasaki. K., Hanaoka, F., and Yamada, M. (1986) *Biochemistry* **25**, 3239–3245.
13. Seki, M., Enomoto, T., Yanagisawa, J., Hanaoka, F. and Ui, M. (1988) *Biochemistry* **27**, 1766–1771.
14. Kawai, J., Ando, A., Sato, T., Nakatsuji, T., Tsuji, K. and Inoko, H. (1989) *J. Immunol.*, **142**, 312–317.
15. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning*; A Laboratory Manual (second edition), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
16. Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem*, **132**, 6–13.
17. Ozawa, K., Dean, F. B., Chen, M., Lee, S.-H., Shiratori, A., Murakami, Y., Sakakura, T., Hurwitz J. and Eki, T. *Cell Struct. Funct.*, **18**, 221–230.
18. Adams, M. D., Kelley, J. M., Gocayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., Merril, C. R., Wu, A., Olde, B., Moreno, R. F., Kerlavage, A. R., McCombie, W. R. and Venter, J. C. (1991) *Science*, **252**, 1651–1656.
19. Takahashi, E., Hori, T., O'Connel, P., Leppert, M. and White, R. (1990) *Hum. Genet.*, **86**, 14–16.

20. Lichter, P., Cremer, T., Borden, J., Manuelidis, L. and Ward, D. C. (1988) *Hum. Genet.*, **88**, 119–121.
21. Takahashi, E., Yamauchi, M., Tsuji, H., Hitomi, A., Meuth, M. and Hori, T. (1991) *Hum. Genet.*, **88**, 119–121.
22. Irino, N., Nakayama, K. and Nakayama, H. (1986) *Mol. Gen. Genet.*, **205**, 298–304.
23. Gorbalenya, A. E., Koonin, E. V., Donchenko, A. P. and Blinov, V. M. (1989) *Nucleic Acid Res.*, **17**, 4713–4730.
24. Hodgman, T. C., (1988) *Nature*, **333**, 22–23.
25. Umezu, K., Nakayama, K. and Nakayama, H. (1990) *Proc. Natl. Acad. Sci. USA* , 87, 5363–5367.
26. Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Nielsen, P. J., Nishi, K., Shnier, J. and Slonimsky, P. P. (1989) *Nature*, **337**, 121–122.
27. Pause, A. and Sonenberg, N. (1992) *EMBO. J.*, **11**, 2643–2654.
28. Naumovski, L., Chu, G., Berg, P. and Friedberg, E. C. (1985) *Mol. Cell. Biol.*, **5**, 17–26.
29. Naumovski, L. and Friedberg, E. C. (1986) *Mol. Cell. Biol.*, **6**, 1218–1227.
30. Sung, P., Higgins, D., Prakash, L. and Prakash, S. (1988) *EMBO J.*, **7**, 3263–3269.
31. Koonin, E. V., Choi, G. H., Nuss, D. L., Shapira, R. and Carrington, J. C. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 10647–10651.
32. Walker, J., E., Sarsate, M., Runswick, M. J. and Gay, N. J. (1982) *EMBO J.*, **1**, 945–951.
33. Pai, E. F., Krengel, U., Petsko, G. A., Gody, R. S., Katsch, W. and Wittinghofer, A. (1990) *EMBO. J.*, **9**, 2351–2359.
34. Story, R. S. and Steitz, T. A. (1992) *Nature*, 349, 494–499.
35. Cleaver, J. C. (1994) *Cell*, **76**, 1–4.
36. Frezal, J. and Schinzel, A. (1991) *Cytogenet. Cell Genet.*, **58**, 986–1052.
37. Mitelman, F., Kaneko, Y. and Trent, J. (1991) *Cytogenet. Cell Genet.*, **58**, 1053–1079.
38. Seizinger, B. R., Klinger, H. P., Junien, C., Nakamura, Y., Beau, M. L.,Cavenee,W., Emanuel, B., Ponder, B., Naylor, S., Mitelman, F., Louis, D., Menon, A., Newsham, I., Decker, J., Kaelbling, M., Henry, I. and Deimling, A. V. (1991) *Cytogenet. Cell Genet.*, **58**, 1080–1096.
39. Smith, G. R. (1988) *Microbiological Review*, **52**, 1–28.
40. Smith, G. R. (1989) *Cell*, **58**, 807–809.
41. Lovvet, S. T. and Clark, A. J. (1983) *J. Bacteriol*, **153**, 1471–1478.
42. Masutani, C., Sugasawa, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takio, K., Tanaka, K., van der Spek, P. J., Boosma, D., Hoeijmakers, J. H. J. and Hanaoka, F. (1994) *EMBO J.*, **13**, 1831–1843.
43. Laemmli, U. K. (1970) *Nature*, **227**, 680–685.
44. Oakley, B. R., Kirsch, D. R. and Morris, N. R. (1980) *Anal. Biochem.*, **105**, 361–363.
45. Gustafson, G. and Armour, S. L. (1986) *Nucleic Acids Res.*, **14**, 3895–3909.
46. Gilchrist, C. A. and Denhardt, D. T. (1987) *Nucleic Acids Res.*, **15**, 465–475.
47. Finch, P. W., Storey, A., Brown, K., Hickson, I. D. and Emmerson, P. T. (1986) *Nucleic Acids Res.*, **14**, 8573–8582.
48. Finch, P. W. and Emmerson, P. T. (1984) *Nucleic Acids Res.*, **12**, 5789–5799.
49. Foury, F. and Lahaye, A. (1987) *EMBO. J.*, **6**, 1441–1449.
50. Backendorf, C., Spaink, H., Barbeiro, A. P. and van de Putte. P., (1986) *Nucleic Acids Res.*, **14**, 2877–2890.
51. Reynolds, P., Higgins, D. R., Prakash, L. and Prakash, S. (1985) *Nucleic Acids Res.*, **13**, 2357–2372.
52. Nielsen, P. J., McMaster, G. K. and Trashsel, H. (1985) *Nucleic Acids Res.*, **13**, 6867–6880.
53. Lloyd, R. G. and Sharples, G. J. (1991) *J. Bacteriol.*, **173**, 6837–6843.
54. Aboussekhra, A., Chanet, R., Zgaga, Z., Cassier-Chauvat, C., Heude, M. and Fabre, F., (1989) *Nucleic Acids Res.*, **17**, 7211–7219.