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Localization of the Fanconi Anemia Complementation Group D Gene to a 200-kb Region on Chromosome 3p25.3

James A. Hejna,[1,*] Cynthia D. Timmers,[1,*] Carol Reifsteck,[1] Donald A. Bruun,[1] Lora W. Lucas,[1] Petra M. Jakobs,[1] SuEllen Toth-Fejel,[1] Nancy Unsworth,[1] Susan L. Clemens,[1] Dawn K. Garcia,[3] Susan L. Naylor,[3] Mathew J. Thayer,[2] Susan B. Olson,[1] Markus Grompe,[1] and Robb E. Moses[1]

[1]Department of Molecular and Medical Genetics and [2]Vollum Institute, Oregon Health Sciences University, Portland; and [3]Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio

Fanconi anemia (FA) is a rare autosomal recessive disease manifested by bone-marrow failure and an elevated incidence of cancer. Cells taken from patients exhibit spontaneous chromosomal breaks and rearrangements. These breaks and rearrangements are greatly elevated by treatment of FA cells with the use of DNA cross-linking agents. The FA complementation group D gene (*FANCD*) has previously been localized to chromosome 3p22-26, by use of microcell-mediated chromosome transfer. Here we describe the use of noncomplemented microcell hybrids to identify small overlapping deletions that narrow the *FANCD* critical region. A 1.2-Mb bacterial-artificial-chromosome (BAC)/P1 contig was constructed, bounded by the marker D3S3691 distally and by the gene *ATP2B2* proximally. The contig contains at least 36 genes, including the oxytocin receptor (*OXTR*), *hOGG1*, the von Hippel-Lindau tumor-suppressor gene (*VHL*), and *IRAK-2*. Both *hOGG1* and *IRAK-2* were excluded as candidates for *FANCD*. BACs were then used as probes for FISH analyses, to map the extent of the deletions in four of the noncomplemented microcell hybrid cell lines. A narrow region of common overlapping deletions limits the *FANCD* critical region to ~200 kb. The three candidate genes in this region are TIGR-A004X28, SGC34603, and AA609512.

Introduction

Fanconi anemia (FA) is a rare autosomal recessive disease characterized by pancytopenia and a specific sensitivity, at the cellular level, to DNA cross-linking agents. FA cells generally show normal resistance to other DNA-damaging agents, such as ultraviolet light, ionizing radiation, or monofunctional DNA-alkylating agents (Weksberg et al. 1979; Klocker et al. 1985; Moustacchi and Diatloff-Zito 1985); however, the results of a recent analysis of FA lymphoblasts from various complementation groups have suggested that some FA cells may be sensitive to bleomycin (Carreau et al. 1999). The primary defect might involve a specific pathway associated with the repair of DNA cross-links, although this hypothesis remains to be proved. The genes for FA complementation groups A, C, and G have been cloned (Strathdee et al. 1992; Fanconi Anaemia/Breast Cancer Consortium 1996; Lo Ten Foe et al. 1996; de Winter et al. 1998), and the FA complementation group E gene (*FANCE*) has been mapped (Waisfisz et al. 1999). There are at least eight FA complementation groups (Joenje et al. 1997). The functions of the genes remain elusive.

The *FANCD* gene has previously been mapped to chromosome 3p22-26 (Whitney et al. 1995). The PD20 cell line that was used to map this gene has been assigned to complementation group D (Jakobs et al. 1996). To map the gene that is defective in the PD20 cells, we used microcell-mediated chromosome transfer (MMCT) followed by a cytogenetic assay for functional complementation. MMCT was used to move individual neomarked human chromosomes into the PD20 immortalized fibroblast cell line. During the fusion process, a donor chromosome can become partially deleted or rearranged. In the initial set of 25 microcell hybrids, one hybrid (PD20-3-8) appeared to contain an intact neomarked p arm of chromosome 3, but it continued to show high levels of chromosomal breaks and radials when exposed to mitomycin C (MMC) and diepoxybutane (DEB). We hypothesized that this was the result of the presence of a small deletion containing the FA complementation group D gene (*FANCD;* MIM 227646). This hypothesis was the basis for a strategy to narrow the *FANCD* region of interest: a large number of microcell hybrids were generated to screen for additional

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Address for correspondence and reprints: Dr. Robb E. Moses, Department of Molecular and Medical Genetics, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Mail Code: L103, Portland, OR 97201. E-mail: mosesr@ohsu.edu

[*] These two authors contributed equally to the work presented in this article.

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noncomplemented clones. If these microcell hybrids carried independent, overlapping deletions, then the region of common overlap would delimit the *FANCD* critical region.

In the course of mapping the small deletions in these noncomplemented microcell hybrids, a bacterial-artificial-chromosome (BAC)/P1 contig was assembled. The contig was useful not only for the fine mapping of markers and expressed-sequence tags (ESTs) in the critical region but, also, for FISH analyses with the use of BACs as probes. Use of BAC probes for FISH analysis of the noncomplemented microcell hybrids determined the extent of deletions on the respective neomarked donor chromosomes. Overlapping deletions in the five noncomplemented microcell hybrids restricted the *FANCD* critical region to ~200 kb.

Material and Methods

Cell Lines

PD20 is an immortalized *FANCD* fibroblast cell line generated by the Oregon Health Sciences University Fanconi Anemia Cell Repository (Jakobs et al. 1996). The mouse cell line A9 as well as the human/mouse hybrid donor cell line GM11713 (A9+3) were obtained from the National Institute of General Medical Sciences Human Genetics Mutant Cell Repository. Murine cell lines, including the human/mouse hybrids, were cultured in Dulbecco modified essential medium (DMEM) with 15% bovine calf serum (Hyclone). Human fibroblasts and microcell hybrids were cultured in α-MEM (minimal essential medium) and 20% FCS (Summit). To all media, we added 2 mM glutamine and 500 μg penicillin-streptomycin/ml.

MMCT

MMCT was performed as described elsewhere (Fournier et al. 1981). In brief, donor cells were split onto 150-mm dishes at ~40% confluence and were allowed to recover for 24 h. Colcemid (0.06 μg/ml for mouse cell lines and 1.0 μg/ml for human cell lines; Sigma) was added, and the cells were incubated for 24–48 h. Micronucleated cells were then trypsinized and were allowed to settle down on "bullets" coated with cross-linked concanavalin A (Sigma). The bullets were then placed into 50-ml centrifuge tubes containing DMEM and 10 μg cytochalasin B (Sigma)/ml and were centrifuged at 14,000 rpm (*g* force 27,000; Beckman JA-17 rotor) for 30 min at 37°C. The resulting pellets were resuspended in DMEM without serum and were filtered through a 5-μm syringe filter (Millex-SV filter unit; Millipore). The microcells were then mixed with phytohemagglutinin solution (final concentration in media 100 μg phytohemagglutinin P [Difco]/ml) and were added to a monolayer of recipient cells. After 15 min, the cells and microcells were fused with 50% polyethylene glycol for 1 min (Mercer and Schlegel 1979), were washed with a serum-free medium, and were allowed to recover overnight without selection. The next day, the cells were split 1:10 into selective media containing 400 μg G418 (GibcoBRL)/ml. After the selection was complete, clones were picked, expanded, and analyzed independently.

Chromosome-Breakage Analysis

Chromosome-breakage analysis was performed by the Cytogenetics Core Lab at Oregon Health Sciences University, Portland, Oregon. For the analysis (Cohen et al. 1982), cells were plated into T_{25} flasks and were allowed to recover overnight. Fibroblast cell lines were then treated with either 40 ng MMC/ml or 200 ng DEB/ml for 2 d. After treatment, the cells were exposed to colcemid for 3 h and were harvested with the use of 0.075 M KCl and 3:1 methanol:acetic acid. Slides were stained with Wright stain, and chromosomes were scored for breaks and radials per cell.

FISH Analysis

Localization of BACs to chromosome 3p25, by means of metaphase FISH, was done by nick translation of BAC DNA with the use of digoxygenin-11-dUTP (Boehringer Mannheim) or biotin-11-dUTP (Enzo Diagnostics), and detection was done with the use of anti-digoxygenin-rhodamine or avidin-fluorescein isothiocyanate (FITC) (Oncor). To order BACs within the contig by means of interphase FISH, we used a rhodamine-tagged probe (Oncor) to chromosome 3p subtelomeric sequences, in addition to pairs of labeled BAC probes, one of which was detected with the use of FITC and the other of which was detected with the use of rhodamine. In a second analysis, we labeled a chromosome 3p subtelomeric probe (a gift from D. H. Ledbetter) with biotin. By comparison of the linear order of colored probes (e.g., red-green-red or green-green-red), the order of a given pair of BACs, with respect to the telomere, was established. A minimum of 50 nuclei were examined for each FISH analysis.

To identify the neomycin-marked chromosome 3 in the microcell hybrids, the eukaryotic expression vector pXT1 (Stratagene), which contains the neomycin-resistance gene, was used as a FISH probe. After Qiagen purification, the vector was nick-translated with digoxygenin-11-dUTP (Boehringer Mannheim) and was detected with anti-digoxigenin FITC (Oncor). The cells were counterstained with propidium iodide, and a minimum of 20 metaphases were examined.

Dinucleotide-Repeat Analysis

Primers for the microsatellite markers were either obtained from Research Genetics or custom synthesized. PCR was performed on 50 ng genomic DNA, with the use of 0.2 μM each primer, 0.25 mM each dNTP, 1.5–2.0 mM $MgCl_2$, 1 × PCR buffer (PE Biosystems), and 0.25 U *Taq* DNA polymerase (PE Biosystems) for 35 cycles. PCR products were resolved on an 8% denaturing polyacrylamide gel, were transferred onto Hybond N membrane (Amersham), and were probed with a $\gamma[^{32}P]$-ATP–end-labeled, 70-base CA-repeat oligonucleotide. Genomic DNA was prepared as described elsewhere (Miller et al. 1988). PCR products were run on 2.5% agarose gels and were stained with ethidium bromide.

BACs/P1s

BACs were obtained from Research Genetics after screening of the California Institute of Technology (CIT)–arrayed human BAC library (Shizuya et al. 1992; Kim et al. 1996), by means of PCR done on hierarchically pooled BACs. P1s were obtained from the library of N. Sternberg (Shepherd et al. 1994). BAC DNA was purified by use of a modified protocol from Qiagen. BAC clones were grown overnight in 500 ml Luria-Bertani broth supplemented with 10 μg thymine/ml and 12.5 μg chloramphenicol/ml. The Maxi-prep protocol (Qiagen) for single-copy plasmids was followed, with the following changes: (1) volumes of buffers P1, P2, and P3 were each 40 ml; (2) after isopropanol precipitation of DNA from the supernatant obtained from the alkaline lysis and after resuspension in 12 ml QBT buffer (Qiagen), additional ribonuclease A was added to a final concentration of 50 μg/ml prior to loading of the DNA onto the Qiagen Tip-500 column; and (3) the final DNA precipitate was dissolved in 300 μl 10-mM Tris, pH 8.0, and 0.1-mM EDTA. M13 universal forward and reverse primers were used for end sequencing of the BACs. BAC end sequences were analyzed by use of the BLAST algorithm (Altshul et al. 1990) located on the Baylor College of Medicine (BCM) Search Launcher site (Smith et al. 1996). Repetitive sequences were identified by use of RepeatMasker. BAC walking was done by designing new primers from nonrepetitive BAC end sequences and by screening the CIT-arrayed BAC library. P1 clones were purified and were analyzed similarly.

cDNA Clones

I.M.A.G.E. Consortium (Lawrence Livermore National Laboratory [LLNL]) cDNA clones corresponding to ESTs in GenBank (Lennon et al. 1996) were purchased from Genome Systems. These cDNA clones—denoted by I.M.A.G.E. Consortium clone accession numbers 180467, 1018392, 178261, 198202, 221127, 41789, 31702, 221330, 113501, 82388, and 137340—corresponded to ESTs NIB327, SGC30425, WI-15198, SGC34603, SGC31307, WI-13447, WI-14334, SGC31297, SGC33503, SGC33325, and WI-8719, respectively. The cDNA inserts from these clones were digested with the appropriate restriction enzymes and were used as probes for Southern and northern blot hybridizations. Additional cDNA clones were obtained by means of a PCR-based screen of a human lymphoblast cDNA library (provided by M. Buchwald; Strathdee et al. 1992) or were amplified either by PCR from Marathon libraries (Clontech) or by reverse transcriptase (RT)-PCR.

Southern Blot Hybridizations

Southern blot hybridization was performed by means of standard procedures (Southern 1975). BACs were digested with *Hin*dIII, were resolved on a 0.8% agarose gel, and were transferred onto Hybond N^+ membranes (Amersham). cDNA probes were labeled with random hexamers and $\alpha[^{32}P]$-dCTP. Hybridizations were performed at 65°C in 6 × SSPE, 0.5% SDS, 5 × Denhardt reagent, and 100 μg salmon sperm DNA/ml. The membranes were washed for 15 min in 2 × SSC and 0.1% SDS at room temperature, then for 15 min in 1 × SSC and 0.1% SDS at 65°C, and then for 5 min in 0.1 × SSC and 0.1% SDS at 65°C for higher stringency. Inter-*Alu* PCR products were labeled in the same manner as were the cDNA probes; they were then prehybridized with 500 μg human placental DNA. Blots were also prehybridized with human placental DNA.

Northern Hybridizations

Human adult and fetal multitissue mRNA blots were purchased from Clontech. Blots were probed with cDNA probes labeled as described above, were hybridized with the use of the ExpressHyb hybridization solution (Clontech) at 65°C, and were washed at high stringency, also as described above.

Inter-Alu PCR

Three separate PCR reactions were performed on 10 ng purified BAC DNA, by use of 3′ (5′-ACAGAGCGAGACTCCGTCTC-3′) and 5′ (5′-GTGAGCCACCGCGCCCGGCC-3′) *Alu* primers. One reaction included both primers, and the other two reactions included each primer by itself. Products from all three reactions were pooled and were used as probes for Southern-blot-hybridization analyses. PCR was done with 0.2 μM primer, 0.25 mM each dNTP, 2.0 mM $MgCl_2$, 1 × PCR buffer, and 0.5 U *Taq* DNA polymerase (PE Biosystems). The PCR conditions were as follows: 30 cycles for 1 min,

15 s at 94°C; 3 min at 72°C; and extension for 10 min at 72°C at the end of the PCR reaction. The PCR products were combined and were purified with the use of a Qiaquick PCR purification column (Qiagen).

Results

Analysis of Microcell Hybrid Clone PD20-3-8

In the assignment of the *FANCD* region to chromosome 3p22-26, a microcell hybrid cell line, PD20-3-8, was generated (Whitney et al. 1995). This cell line showed no increased resistance to MMC and DEB, yet it maintained a neomycin-marked chromosome 3 from the GM11713 donor cell line. Marker analysis of the hybrid was attempted, but there were few informative markers in the chromosome 3p22-26 region. Therefore, the neomycin-marked chromosome 3 was isolated in a mouse background, by use of the PD20-3-8 cell line as an MMCT donor and by use of the $HPRT^-$ immortalized mouse fibroblast cell line A9 as a recipient. After fusion of PD20-3-8 micronuclei with A9 cells, the neomycin-marked chromosome 3 was selected with the use of G418, and carryover human cells were counterselected with the use of 6-thioguanine. Three independent PD20-3-8/A9 microcell hybrids, C6–C8, were shown, by means of Southern blot analysis, to carry the neomycin-marked human chromosome 3. With only the single neomycin-marked chromosome 3 present in the A9 mouse background, every mapped EST or sequence-tagged site (STS) marker in the chromosome 3p22-26 region could potentially be used to determine the extent of the deletion. One marker that was absent in microcell hybrids C6–C8 was D3S1597. An additional 15 markers—AFMA216ZG1, SGC33325, SGC31297, SGC33503, SGC30425, SGC30473, SGC34603, WI-14334, SGC31307, CHLC.GGAT2A11, NIB327, D3S3691, NIB673, WI-6956, and WI-8719—12 of which were ESTs, were also found to be deleted in these clones. The breakpoints of the deletion in the PD20-3-8 hybrid were delimited by markers D3S3591 and D3S3589. On the basis of the CEPH genetic map of this region, this corresponded to a distance of ~8 cM. Using the markers neighboring D3S1597 as a framework, we then constructed a BAC/P1 contig.

Assembly of the Contig

The final BAC/P1 contig covering ~1.2 Mb between D3S3691 and the *ATP2B2* gene is shown in figure 1. The size of the contig was estimated from the size of the genomic inserts on pulse-field gel electrophoresis and an average overlap of ~30 kb through the minimal tiling of BACs in the contig. This is consistent with an estimated physical distance for the region (Szymanski et al. 1993). The assembly was begun by screening the CIT human BAC library (Kim et al. 1996) with the use of markers previously mapped between D3S3591 and D3S1293 (e.g., markers SGC33325, SGC31297, SGC33503, SGC30425, SGC30473, SGC34603, WI-14334, SGC31307, CHLC.GGAT2A11, NIB327, D3S3691, NIB673, WI-6956, and WI-8719) by the Whitehead Institute for Biomedical Research/MIT Center for Genome Research, the Stanford Human Genome Center, Généthon, and the Cooperative Human Linkage Center. This established a framework of BACs from which multiple walks could be initiated; at that time, ~20 STS and EST markers had been assigned to the chromosome 3p25 region. BAC DNA was purified, and the localization to chromosome 3p25 was verified, by use of FISH, to metaphase spreads of normal human cells. The ends of the human genomic inserts were sequenced with the use of the M13 universal forward and reverse sequencing primers flanking the p-BeloBAC-11 vector multicloning site. Use of the RepeatMasker algorithm available from the BCM Search Launcher identified repeat-free sequences from which the PCR primer pairs were designed. Usable sequence was obtained ~70% of the time. These PCR primers were then used to search the arrayed BAC library for overlapping BACs. In addition, the new primers were tested against the initial set of framework BACs, with the objective of linking small clusters of BACs together. As the size of these clusters increased, testing of newly designed primers on the assembly linked more clusters together. A list of PCR primers developed from the BAC end sequence is given intable 1.

Southern blot analysis and FISH were used to verify the location of BACs within the contig. For Southern blot analyses, BACs were digested with *Hin*dIII and were probed with cDNAs obtained either from the I.M.A.G.E. Consortium (LLNL; Lennon et al. 1996) or from RT-PCR. Inter-*Alu* PCR products of BAC DNA were also used as probes. For this type of Southern blot test, probes and membranes were prehybridized with human placental DNA to block repetitive DNA sequences. For interphase FISH, entire BACs were used as probes in a pairwise ordering, with respect to the p-terminus of chromosome 3. One BAC probe was detected with the use of an FITC (green) secondary label, whereas the second BAC probe was detected with the use of a rhodamine (red) secondary label. Two chromosomal spreads, one with the p-terminus labeled with FITC and the other with the p-terminus labeled with rhodamine, were then compared. For example, if the linear order on one spread was green-green-red and that on the second spread was red-green-red, then the BAC labeled with FITC would be closer to the p-terminus than would the

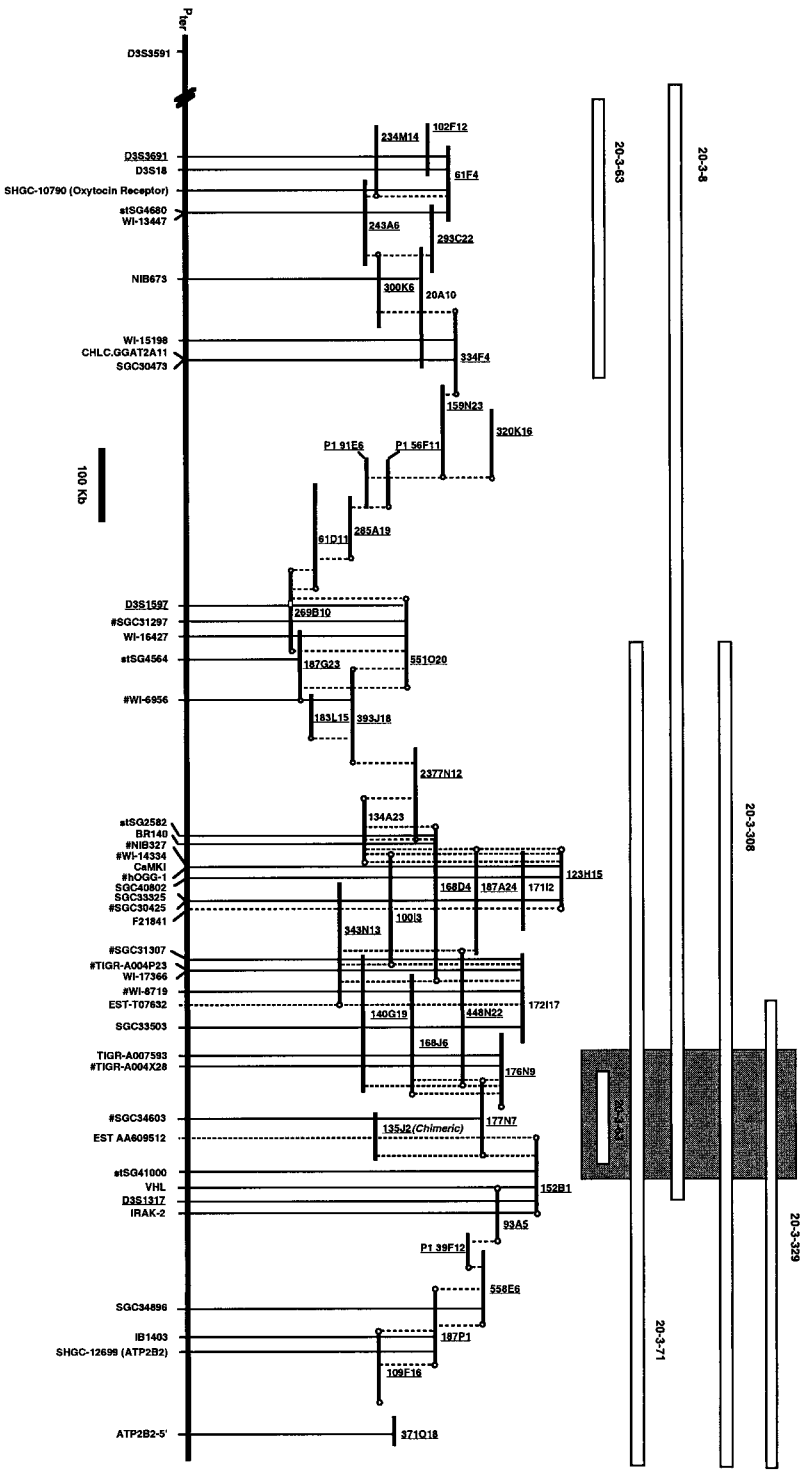

Figure 1 BAC contig in chromosome 3p25-26. A total of 38 BACs and three P1 clones covering ~1.2 Mb in chromosome 3p25-26 are represented. Marker D3S3591 is telomeric to the contig. BACs corresponding to this marker were identified but did not overlap the contig. The distance between D3S3591 and D3S3691 is estimated to be 4.2 cR (GeneMap'99). Markers D3S3589 and D3S1293 are centromeric to the contig, with D3S1293 located ~12.8 cM from D3S1597 (GeneMap'99). Underlined BACs were confirmed by FISH, with use of the two-color strategy presented in figure 2. Markers confirmed by Southern blot tests are denoted by the number symbol (#). The small open rectangle on BAC210B10 denotes the absence of marker D3S1597 on this BAC, possibly as a result of a small deletion or polymorphism. Ends of BACs tagged with open circles represent sequences from which new PCR primers were derived, and dashed lines indicate the correspondence of these PCR primers on overlapping BACs. Markers grouped together, such as TIGR-A004P23 and WI-17366, are not yet ordered with respect to one another. Deletions on the donor chromosome of noncomplemented microcell hybrids are shown beside the contig, with the common overlapping deleted region appearing in a box.

Table 1

List of New STS Markers Derived from the BAC End Sequence

Primer	BAC	Primer Sequence	Product Size (bp)
MG316	300K6	GACATTCTTCCAACCACTTTCC	303
MG317	300K6	GGTGGATCAGGCTAGAAAAGG	
234F-1	234M14	CAGACATCACCCCATCACTG	165
234F-2	234M14	GAGCCTTCATCTTTGGGTAGG	
187R-1	187A24	CAAGTGAATCTTGAGCAACTGC	124
187R-2	187A24	TCTGTTCACTGACCACAGCTG	
187F-1	187A24	TCCAGTGAACAAACATAGCACC	114
187F-2	187A24	TTTTAGCACTGGTGGGCAG	
183R-1	183K15	AACCTTTATGGGAACCAATGC	139
183R-2	183L15	TTACAAAAGTGTGGGAGCCC	
293C22F-1	293C22	TTACCTTCTGCCAAAATGGC	153
293C22F-2	293C22	TGCTCCTTGACTTATGATGGG	
20A10R-1	20A10	ACTTAGGCAGTGTTTCCCTCC	218
20A10R-2	20A10	CCAAAAGTGTGAGGGAGGAC	
234R-3	234M14	GAGGAAATGCATCCTTCTGC	101
234R-4	234M14	GCTGAAGGGGTTAGTTCGC	
123F-1	123H15	TGCAAAATGAGGACAACAAG	177
123F-2	123H15	CTCAAGGCTCCGTCAAACTC	
343R-1	343N13	CCTACTCCCAACCCTGGAG	153
343R-2	343N13	TTGAGTCCCATCCTCCAAAC	
102R-1	102F6	GCACATTAAAACTCACTCCTGC	183
102R-2	102F6	GAGATGGGGCACCAGAAAC	
168JF-1	168J6	ACTGTGCTCTCCCTCCCC	178
168JF-2	168J6	GAAGAATTTGTTAGTGGCCTGG	
334F-1	334F6	CCTCTGGTAGTCTGCTTGGC	147
334F-2	334F6	AGATGTGGTCAGGCTCGG	
334R-1	334F6	TTTGTGCATTTGAGTATTTGGC	392
334R-2	334F6	AAGTGGGATGCAGACTGACC	
269R-1	269B10	GTCACTTTGCATTCCCTACTCC	174
269R-2	269B10	GGTAAGGCAGAAAGGAGCG	
269F-1	269B10	CCATATTTTTTGTGTTCACAAGG	165
269F-2	269B10	AGAACCCCTCTTACAAGTTCCC	
177F-1	177N7	CAGGGTCATGATATTCAACTCC	104
177F-2	177N7	CAAGACTTTTCCAGGCAGTG	
177R-1	177N7	TCATTTCAGACAAATCCATTGC	196
177R-2	177N7	GCTGGGATTATAGGCGTGAC	
159F-1	159N23	GAGCCGAAAGTAAGGGCTG	261
159F-2	159N23	TTGAGGAAGGAGAGCTAAGTGG	
93-R1	93A5	ACCATTGGATTGCACCTACCAC	220
93-R2	93A5	TGAGATGGATTTCCCAGCAGTC	
P39T-1	P1 39F12	TTCATATTCTTCCCCCTCCC	136
P39T-2	P1 39F12	CTAACCACAAAAACCTTCCAGG	
P91S-1	P1 91E6	GCATATGCTTCAGAATCCCTG	127
P91S-2	P1 91E6	AAAGTCCCATGGCTGTGAAG	
P56T-1	P1 56F11	TGCACAGGAATGCTTAGCAC	259
P56T-2	P1 56F11	GTTGAATAGTCTTCCTCCCACG	
P56S-1	P1 56F11	CTGAAGAAGATTGAAACCCACC	150
P56S-2	P1 56F11	CATGAGCCGTGATTGAACC	
61D11R-1	61D11	GGTCACAGAAAGTTCTAAGTGG	95
61D11R-2	61D11	GCCCTACGTCCTTTATGG	
61D11T-1	61D11	TGTTCCAGATGAATCCAAAGCC	130
61D11T-2	61D11	GGTAGCAGGAGGTTAAATGCACC	

Table 1 Continued

Primer	BAC	Primer Sequence	Product Size (bp)
183F-3	183L15	CACTTTACACTTTCCTGGACTCCAC	130
183F-4	183L15	TTGGGTCTGCCACTTAAAAGAGAG	
183NS3	183L15	CGGGTGTTCAAAAAATACCAGG	95
183NS4	183L15	ACAGGTGAGAAGTTTCTCCCCC	
39T-3	P1 39F12	TGAAACAGAGCAATGCCTGTCC	300
39T-4	P1 39F12	TGCAAGGCACTGAGGAGATGAG	
393F-1	393J18	ACGGGCAACAGATAGAAATTC	118
393F-2	393J18	TGACACATAGATAAAAGTGCTTGAG	
393R-1	393J18	GCCTCTAAGTGTTCAAGTGAAAGG	105
393R-2	393J18	GTGCTGGGATAACAAGCGTG	
558F-1	558E6	TTATCACCCCACTTTATAGAGCAG	142
558F-2	558E6	TTTCACCAGGCGGATAGTTC	
187PF-1	187P1	CCCAGGTTATGGAACTCAGC	92
187PF-2	187P1	TATGTGCTGCTCCATCCTTG	
187PR-1	187P1	CAACTTGTGTCTCAGTGCAGTTC	112
187PR-2	187P1	GATTTCCAATACAGGCAGTGG	
109F-1	109F16	CATGGCAGTCATTACTCTGTTTC	108
109F-2	109F16	ATCTGAAGGGGATGTTGAGG	
109R-1	109F16	CCATATTGGGCACTGACTTATC	146
109R-2	109F16	GACTAAAGCAGTTTTCAGGGC	
168DF-1	168D4	CTCCACTGGATGTAACAATGTTC	113
168DF-2	168D4	CCTTCCCATCACTTTGGTGG	
168DR-1	168D4	CCAACCCACATCTCACAGGAG	151
168DR-2	168D4	CCCTTATCCTGCCCTCAATG	
100F-1	100I3	TTCTGGAAGTCAGGCTGGC	99
100F-2	100I3	TCATTTATTTCCAGCCCCC	
100R-1	100I3	CCAAGGCTATCCAGTGAACAAAC	181
100R-2	100I3	AAAGAATCGGGGAAGAAGGAAG	
140F-1	140G19	TCAGTGATCTCTGTCAGGGAGG	140
140F-2	140G19	CGAGGCTGAGGAATTGTTTTAC	
172F-1	172I17	CCACAGCTTCATCAAGGGTG	110
172F-2	172I17	TGCAGTTATGGATGGAGGATG	
448F-1	448N22	ACAGGCTAGATTTGCATGTTTC	100
448F-2	448N22	CGGTATAAAGGGAGAATGGC	
448R-1	448N22	GACAACACTGAAAAGGGAACG	120
448R-2	448N22	CCACCATTTTGCATACGATG	
134F-1	134A23	AGACCTCAGGACATCAGAGGC	105
134F-2	134A23	GCATGAGCAGATGGAGTATGG	
134R-1	134A23	GTTGACCCCAAGGTGAAATG	104
134R-2	134A23	GACAATGCTAACGCCTTTGC	
320F-1	320K16	GGTCTTTTTCAACTCTGTGGC	156
320F-2	320K16	AGCAATTTCAGCAGGTCAGC	
558R-1	558E6	CCCAAGTTCCATGAAGTGTATG	441
558R-2	558E6	TGATGAGTACACAGGGTTCAGAG	
551R-1	551O20	GCAAAGGAGTTTTCTGGGAGGC	118
551R-2	551O20	AAGTGTCAAGCAGGGCTTCTGGAC	
551F-1	551O20	GTATGTCCATGATGAAAGCTGCTG	116
551F-2	551O20	AAATATGCCCCTGAGGTTTTCC	
152R-1	152B1	GTTGCATACAGCAAGAGGATAGAGC	246
152R-2	152B1	CAACAAAGGACTGGTCAGGAAGC	
152F-1	152B1	CCTTTCTCCTCTCCACCCTTAAAC	187
152F-2	152B1	CTTTTTCCAGCCTCCTGAAGAAC	

BAC labeled with rhodamine. This allowed for the pairwise ordering of BACs at a resolution of 50–100 kb (Trask et al. 1991). An example is presented in figure 2*A* and *B*.

On one occasion, gaps were filled with P1 clones. Another gap between BACs 393J18 and 134A23 (fig. 1) was filled by BAC 2377N12. The end sequence of 2377N12 (GenBank accession number AQ112748; Institute for Genome Research BAC End Sequencing Project) shared homology with the 5′ end sequence of I.M.A.G.E. Consortium cDNA clone 43420 (GenBank accession number H12922) in the UniGene human-sequence cluster Hs. 157071 (the 3′ end of the same clone has been placed in cluster Hs. 27556, along with EST stSG2582). FISH analysis indicates that BAC 2377N12 maps to chromosome 3p25-26 and that it contains the PCR markers derived from both of the BACs flanking the gap. Coverage in the middle of the contig is rather poor and may reflect either an unstable region or sequences that are difficult to clone. The entire contig has been submitted to the Baylor College of Medicine Human Genome Sequencing Center, as a contribution to the human-genome-sequencing effort.

Locating cDNAs on the Contig

During the construction of the contig, new ESTs were continually binned to the chromosome 3p25 region by the human genome centers (Deloukas et al. 1998), and these ESTs were mapped directly, by use of PCR, to

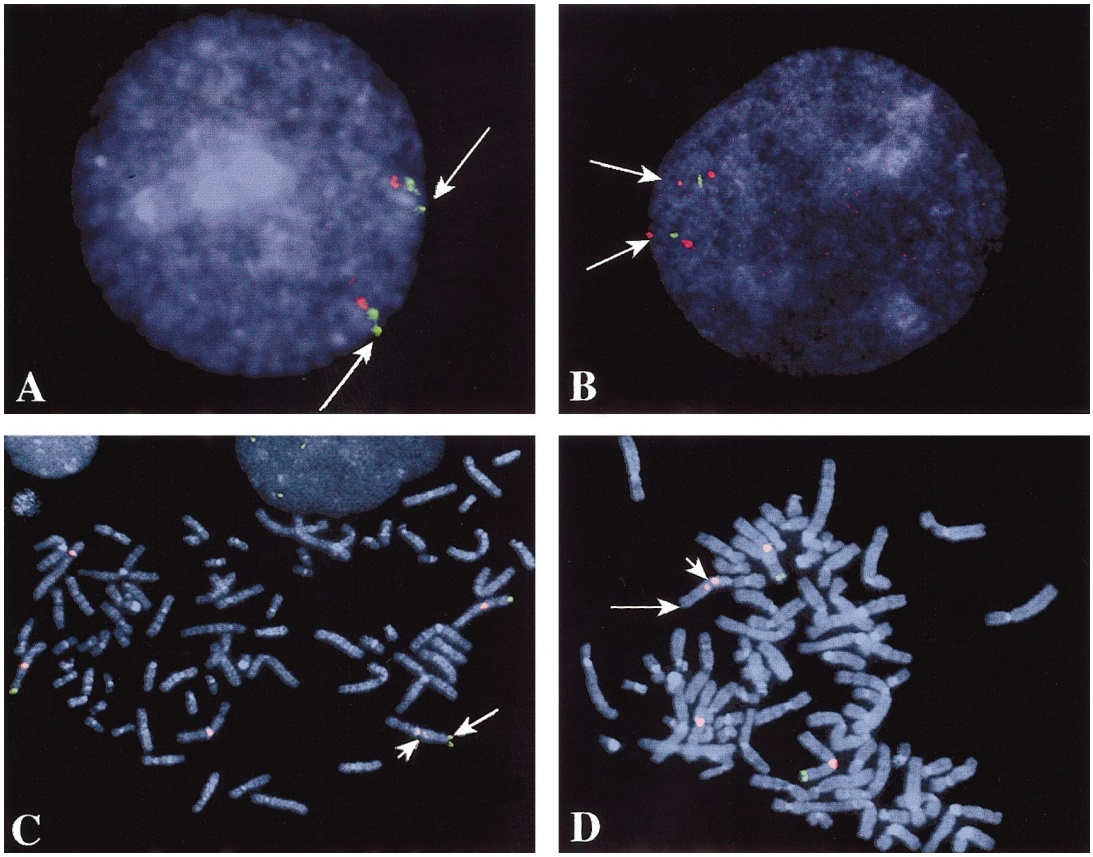

Figure 2 Interphase FISH mapping of two BACs (*A* and *B*) from the contig on a normal human interphase and deletion mapping on noncomplemented microcell hybrids (*C* and *D*). To determine the order of the BACs, two analyses were performed with each BAC pair. The BACs were labeled in the same color, respectively, in both analyses, with the chromosome 3p subtelomere labeled in green in the first analysis and labeled in red in the second analysis. *Panel A* shows green (3p subtelomere [*arrow*])–green (BAC 551O20)–red (BAC168D4). This order is confirmed by the results of the second analysis (*B*), which show red (3p subtelomere [*arrows*])–green (BAC 551O20)–red (BAC 168D4). *C* and *D*, Noncomplemented microcell hybrid cells were probed with BACs from the contig, and the presence or absence of a signal on the donor chromosome 3 was determined. The donor chromosome in this example was identified by a chromosome 3–specific α-satellite probe (*red*) that was split on the donor chromosome (*short arrow*). *Panel C* shows PD20-3-71 cells probed with BAC 551O20 (*green*) and the a-satellite probe. In this example, the BAC hybridized to the donor chromosome (*long arrow*). *Panel D* shows PD20-3-71 cells probed with BAC 134A23 (*green*) and the α-satellite probe. In this example, the BAC failed to hybridize to the donor chromosome (*long arrow*), although it hybridized to the PD20 resident chromosomes. The deletion on the donor chromosome thus included BAC 134A23.

specific BACs in the contig. Under the assumption that there is one gene per EST, the total number of genes in this region is at least 36, with a gene density of approximately one gene per 40 kb. BLAST homology searches with BAC end sequences revealed the presence of the von Hippel-Lindau tumor-suppressor gene (*VHL*) near the centromeric end of the contig. A 400-kb cosmid contig covering *VHL* and *ATP2B2* has been reported (Kuzmin et al. 1994); it partially overlaps the BAC contig described here. The order of markers in this overlapping region is in agreement with that of Kuzmin et al. (1994)

Additional ESTs that were identified, on the basis of BLAST homology searches, from end sequences of BACs were T07632, AA609512, F21841, and AA715826 (GenBank accession numbers), located on BAC 343N13, BAC 152B1, BAC 123H15, and BAC 293C22, respectively. On the basis of both the results of multitissue northern blot tests and the fact that the only library from which the cDNA could be amplified was derived from testis, the EST AA609512 appears to be testis specific.

To assess the pattern of expression of several of the cDNAs in the region, tissue-specific northern blot tests were hybridized with cDNA probes. A summary of these results is given in table 2. Two genes—NIB673 and WI-6956—appear to be highly expressed in the brain. These are of potential interest with regard to two rare syndromes, 3p− syndrome and duplication 3p syndrome (or trisomy 3p syndrome; Martin and Steinberg 1983; Phipps et al. 1994; Drumheller et al. 1996), which both involve mental retardation as one of several clinical manifestations.

Other well-characterized genes that have been placed on the contig are the oxytocin receptor (*OXTR*) on BACs 234M14, 61F4, and 243A6; calmodulin kinase-1 (Haribabu et al. 1995), which was shown to partially overlap the 3′ end of *hOGG1* (Aburatani et al. 1997); and *ATP2B2* (Brandt et al. 1992), which was mapped to the YAC/cosmid contig containing *VHL* (Kuzmin et al. 1994). Interestingly, *ATP2B2* has recently been associated with mutations that cause deafness and imbalance (Kozel et al. 1998; Street et al. 1998). End sequence from BAC 152B1 identified the gene *IRAK-2* by means of BLAST search homology.

Table 2

Northern Blot Analysis of Mapped ESTs

EST	Size (kb)	Expression Pattern
NIB673	7.5 and 9.5	Highly expressed in brain; low expression in heart, placenta, lung, skeletal muscle, and pancreas
SGC31307	3.0	Ubiquitous expression, with the highest levels in the liver, heart, skeletal muscle, and pancreas; low expression in brain
SGC30425	6.0	Highly expressed in liver
SGC34603	5.0	Ubiquitous, low-level expression, with highest expression in placenta and lowest expression in skeletal muscle
WI-6956	4.4	Highly expressed in brain; lower expression in kidney and pancreas
SGC33503	1.0,[a] 1.4, 2.6,[a] and 3.0	Ubiquitous expression, with highest levels in heart and skeletal muscle
EST-AA609512	1.1	Testis specific

[a] These transcripts appear to be present only in heart and skeletal muscles.

Testing hOGG1 *and* IRAK-2 *for Functional Complementation*

The human homologue of the yeast gene *ogg* was considered to be a candidate for *FANCD*. Primary FA cell lines have been reported to be oxygen sensitive (Saito et al. 1993), and the human gene encodes an 8-hydroxyguanine glycosylase, an enzyme responsible for the repair of one of the prevalent lesions produced by reactive oxygen species (Aburatani et al. 1997; Lu et al. 1997; Radicella et al. 1997; Roldan-Arjona et al. 1997). The full-length normal *hOGG1* cDNA was obtained, by means of RT-PCR, from control cell line PD138. Two different normal splice variants, one of which is 17 bp longer than the other, were separately subcloned into the pREP4 expression vector. In addition, the analogous two cDNA splice variants were amplified from PD20-immortalized *FANCD* fibroblasts and were subcloned into pREP4. PD20 *FANCD* cells were electroporated with each of the expression constructs, respectively, and, after selection with hygromycin B, individual clones were cytogenetically tested for genomic stability after treatment with MMC and DEB. None of the transformed PD20 fibroblasts showed improved genomic stability after treatment with these agents. Expression of the normal *hOGG1* cDNA from the Rous-sarcoma-virus promoter of the pREP4 vector was verified by use of the northern blot test. In addition, RT-PCR products from normal cells and PD20 cells were sequenced, and no sequence alterations were detected. On the basis of these results, the *hOGG1* gene was excluded as a candidate for the *FANCD* gene.

IRAK-2 (Muzio et al. 1997) was tested because FA cells have been shown to be hypersensitive to interferon-

γ (Whitney et al. 1996). Conceivably, an interleukin-1 receptor–associated kinase might also interact with several different signal transduction pathways. A full-length cDNA clone was obtained by screening of the Strathdee library and was tested for functional complementation of PD20 cells. There was no indication that *IRAK-2* cDNA corrected the genomic instability in PD20 cells after treatment with MMC and DEB. Mutation detection was not attempted. As described below, narrowing of the *FANCD* critical region ultimately excluded both *hOGG-1* and *IRAK-2* as candidate genes.

Production and Analysis of Additional Noncomplemented Hybrids

To refine the *FANCD* critical region, additional microcell hybrids were generated by use of the GM11713 donor and PD20 recipient cells, as previously described. Two hundred fifty independent neomycin-resistant hybrid cell lines were tested for complementation with the chromosomal-breakage assay. Of the 250 clones, 7 retained high levels of chromosomal breakage after treatment with MMC and DEB. The seven noncomplemented clones were analyzed for deletions by use of the informative markers D3S3591, D3S1597, D3S1317, and D3S1263. Three of these clones lacked all four markers. This indicated that the donor chromosome had a deletion larger than the current region of interest; consequently, these three clones were not studied further. The four remaining noncomplemented clones—PD20-3-63, PD20-3-71, PD20-3-308, and PD20-3-329—retained the D3S1597 marker in common but lacked at least one of the other informative markers, suggesting the presence of deletions that were partially overlapping or that were smaller than the original PD20-3-8 deletion.

The BAC/P1 contig was exploited in mapping of the deletions in the four new noncomplemented hybrid clones. A FISH probe was developed to identify the neomycin marker on the donor chromosome 3, and BAC FISH probes were then used to determine the presence or absence of the corresponding region on the donor chromosome. The recipient chromosome 3 signals served as internal controls, and no large deletions were ever observed on the recipient (*FANCD*) chromosomes in the chromosome 3p25-26 region, implying that the genetic defect in the PD20 cell line is not a large deletion. Figure 1 summarizes the contig and the breakpoints of the deletions in the noncomplemented hybrid cell lines. Each of the 41 BACs or P1s depicted in figure 1 was independently used as a FISH probe in mapping of the deletions. Hybrids PD20-3-71, PD20-3-308, and PD20-3-329 all had deletions extending beyond the proximal end of the contig. The distal breakpoint in both PD20-3-71 and PD20-3-308 lay within the region represented by BAC 187G23. In this region of the contig, the depth of coverage in the CIT BAC library was less than the sixfold average, suggesting that this might be a hot spot for chromosomal breaks. The distal breakpoint for the deletion in PD20-3-329 was within BAC 343N13. PD20-3-63 carried two deletions on the donor chromosome. One deletion began within BAC 334F4 and extended beyond the distal end of the contig, whereas a second, very small deletion was detected only by the absence of a hybridization signal from BACs 135J2 and 177N7. The deletions in the donor chromosomes from all five noncomplemented hybrid cell lines overlapped in this one small region. The size of the insert in BAC 177N7 was determined to be 100 kb, by means of pulsefield gel electrophoresis. Assuming that the deletion in PD20-3-63 could extend somewhat beyond the region delimited by BAC177N7 and that it could disrupt the function of the genes flanking the deletion, we estimated that the *FANCD* gene must lie within a region of ~200 kb, centered around the EST SGC34603. The ESTs SGC34603, TIGR-A004X28, and AA609512 are currently being evaluated as candidates for the *FANCD* gene.

Discussion

Mapping of FANCD

The International Radiation Hybrid Mapping Consortium, with its wealth of STS and EST markers in easily searchable databases, in conjunction with the development of arrayed BAC libraries, allows for the assembly of BAC contigs covering several megabases of sequence. These contigs will accelerate the development of human sequence data as well as the fine mapping of critical regions in the search for genes implicated in genetic diseases. In the case of *FANCD,* BACs from the chromosome 3p25-26 contig were used as FISH probes, to map the deletions in the donor chromosome in five noncomplemented microcell hybrid cell lines. Although it is possible that a noncomplemented clone could arise from the spontaneous deletion of another FA gene in the recipient cell, this event would be extremely unlikely, since all known FA mutations are recessive—that is, such an event would have to entail the loss of both alleles. The *FANCD* critical region is contained within ~200 kb centered around the EST SGC34603. The corresponding cDNA as well as cDNAs corresponding to the ESTs TIGR-A004X28 and AA609512 are thus the leading candidates for the *FANCD* gene.

BACs have been demonstrated to directly complement mutant cell lines (Hejna et al. 1998), and several experiments have been performed with BACs from the chromosome 3p25 contig, to test for functional complementation after electroporation of BACs into PD20 fibroblasts. Direct functional complementation of PD20

cells with BACs has thus far been unsuccessful. The BAC vector has no selectable marker; consequently, complementation was tested by means of direct selection with DEB after electroporation. In control experiments, PD20 fibroblasts showed an appreciable background of DEB-resistant colonies under continuous DEB selection, presumably as a result of reversion of the *FANCD* gene. This background could be eliminated by retrofitting the BACs with a selectable marker, such as hygromycin resistance. Now that the *FANCD* region of interest is covered by only three or four BACs, such a project is feasible.

An advantage of using BACs for contig building is that they are only rarely chimeric. In the present study, we found only one BAC that appeared to be chimeric. It was noted, in the earlier YAC/cosmid contig covering the *VHL* region, that many of the YACs were chimeric toward the telomeric end of the contig (Kuzmin et al. 1994). One basis for this observation might be that there is an inherently unstable site in that vicinity. We have observed amplification of this entire region in PD20 fibroblasts, under DEB selection (D. A. Bruun, unpublished data); the breakage-fusion-bridge model of amplification would predict a fragile site in the vicinity (Coquelle et al. 1998). In addition, the representation of BACs in the CIT BAC library decreased from approximately sixfold in the region adjacent to *VHL* to onefold through the middle of the contig, suggesting that the DNA in this region is difficult to clone or to maintain in other vectors as well. In addition to deletion breakpoints associated with von Hippel-Lindau disease or tumors, the region has been associated with translocation breakpoints and fragile sites (Porfirio et al. 1991; Sbrana and Musio 1995; Schmidt et al. 1998).

The region has recently been associated with loss of heterozygosity in ovarian cancer (Fullwood et al. 1999). BAC FISH probes from the contig may narrow the region of interest for a putative tumor-suppressor gene in the vicinity. The cDNA Br140, which maps to BACs 134A23, 100I3, and 168D4, has been completely sequenced (Thompson et al. 1994) and belongs to a family of zinc-finger, leucine-zipper motif genes that have been disrupted in translocations associated with acute myeloid leukemia (Gregorini et al. 1996). The above-mentioned BACs would serve as excellent probes with which to test whether a similar translocation breakpoint might disrupt the Br140 gene in related leukemias.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

BCM Search Launcher, http://www.hgsc.bcm.tmc.edu/SearchLauncher/ (for the BLAST and RepeatMasker algorithms)

CEPH, http://www.cephb.fr/ (for genetic map of chromosome 3p)

Cooperative Human Linkage Center, The, http://lpg.nci.nih.gov/CHLC/ (for markers mapped between D3S3591 and D3S1293)

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/index.html (for nucleotide sequences [accession numbers AQ112748, H12922, T07632, AA609512, F21841, and AA715826])

GeneMap'99, http://www.ncbi.nlm.nih.gov/genemap/ (for markers mapped between D3S3591 and D3S1293)

Généthon, http://www.genethon.fr/ (for markers mapped between D3S3591 and D3S1293)

I.M.A.G.E. Consortium, The, http://image.llnl.gov/ (for cDNA clones [accession numbers 180467, 1018392, 178261, 198202, 221127, 41789, 31702, 221330, 113501, 82388, and 137340])

Institute for Genome Research BAC End Sequencing Project, The, http://www.tigr.org/tdb/humgen/bac_end_search/bac_end_intro.html (for BAC 2377N12 end sequence)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for FANCD [MIM 227646])

RepeatMasker, http://ftp.genome.washington.edu/RM/RepeatMasker.html (to check BAC end sequence for repetitive elements)

Stanford Human Genome Center, http://www-shgc.stanford.edu/ (for markers mapped between D3S3591 and D3S1293)

UniGene Human Sequences Collection, National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/UniGene/Hs.Home.html (for human-sequence-cluster accession numbers 157071 and 27556)

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, http://www.genome.wi.mit.edu/ (for markers mapped between D3S3591 and D3S1293)

References

Aburatani H, Hippo Y, Ishida T, Takashima R, Matsuba C, Kodama T, Takao M, et al (1997) Cloning and characterization of mammalian 8-hydroxyguanine–specific DNA glycosylase/apurinic, apyrimidinic lyase, a functional mutM homologue. Cancer Res 57:2151–2156

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Brandt P, Ibrahim E, Bruns GA, Neve RL (1992) Determination of the nucleotide sequence and chromosomal localization of the *ATP2B2* gene encoding human Ca^{2+}-pumping ATPase isoform PMCA2. Genomics 14:484–487

Carreau M, Alon N, Bosnoyan-Collins L, Joenje H, Buchwald M (1999) Drug sensitivity spectra in Fanconi anemia lymphoblastoid cell lines of defined complementation groups. Mutat Res 435:103–109

Cohen MM, Simpson SJ, Honig GR, Maurer HS, Nicklas

JW, Martin AO (1982) The identification of Fanconi anemia genotypes by clastogenic stress. Am J Hum Genet 34: 794–810

Coquelle A, Toledo F, Stern S, Bieth A, Debatisse M (1998) A new role for hypoxia in tumor progression: induction of fragile site triggering genomic rearrangements and formation of complex DMs and HSRs. Mol Cell 2:259–265

Deloukas P, Schuler GD, Gyapay G, Beasley EM, Soderlund C, Rodriguez-Tome P, Hui L, et al (1998) A physical map of 30,000 human genes. Science 282:744–746

de Winter JP, Waisfisz Q, Rooimans MA, van Berkel CG, Bosnoyan-Collins L, Alon N, Carreau M, et al (1998) The Fanconi anaemia group G gene *FANCG* is identical with *XRCC9*. Nat Genet 20:281–283

Drumheller T, McGillivray BC, Behrner D, MacLeod P, McFadden DE, Roberson J, Venditti C, et al (1996) Precise localisation of 3p25 breakpoints in four patients with the 3p− syndrome. J Med Genet 33:842–847

Fanconi Anaemia/Breast Cancer Consortium, The (1996) Positional cloning of the Fanconi anaemia group A gene. Nat Genet 14:324–328

Fournier RE (1981) A general high-efficiency procedure for production of microcell hybrids. Proc Natl Acad Sci USA 78:6349–6353

Fullwood P, Marchini S, Rader JS, Martinez A, Macartney D, Broggini M, Morelli C, et al (1999) Detailed genetic and physical mapping of tumor suppressor loci on chromosome 3p in ovarian cancer. Cancer Res 59:4662–4667

Gregorini A, Sahin FI, Lillington DM, Meerabux J, Saha V, McCullagh P, Bocci M, et al (1996) Gene BR140, which is related to AF10 and AF17, maps to chromosome band 3p25. Genes Chromosomes Cancer 17:269–272

Haribabu B, Hook SS, Selbert MA, Goldstein EG, Tomhave ED, Edelman AM, Snyderman R, et al (1995) Human calcium-calmodulin dependent protein kinase. I. cDNA cloning, domain structure and activation by phosphorylation at threonine-177 by calcium-calmodulin dependent protein kinase I kinase. EMBO J 14:3679–3686

Hejna JA, Johnstone PL, Kohler SL, Bruun DA, Reifsteck CA, Olson SB, Moses RE (1998) Functional complementation by electroporation of human BACs into mammalian fibroblast cells. Nucleic Acids Res 26:1124–1125

Jakobs PM, Sahaayaruban P, Saito H, Reifsteck C, Olson S, Joenje H, Moses RE, et al (1996) Immortalization of four new Fanconi anemia fibroblast cell lines by an improved procedure. Somat Cell Mol Genet 22:151–157

Joenje H, Oostra AB, Wijker M, di Summa FM, van Berkel CG, Rooimans MA, Ebell W, et al (1997) Evidence for at least eight Fanconi anemia genes. Am J Hum Genet 61: 940–944

Kim UJ, Birren BW, Slepak T, Mancino V, Boysen C, Kang HL, Simon MI, et al (1996) Construction and characterization of a human bacterial artificial chromosome library. Genomics 34:213–218

Klocker H, Burtscher HJ, Auer B, Hirsch-Kauffmann M, Schweiger M (1985) Fibroblasts from patients with Fanconi's anemia are not deficient in excision of thymine dimer. Eur J Cell Biol 37:240–242

Kozel PJ, Friedman RA, Erway LC, Yamoah EN, Liu LH, Riddle T, Duffy JJ, et al (1998) Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2. J Biol Chem 273:18693–18696

Kuzmin I, Stackhouse T, Latif F, Duh FM, Geil L, Gnarra J, Yao M, et al (1994) One-megabase yeast artificial chromosome and 400-kilobase cosmid-phage contigs containing the von Hippel-Lindau tumor suppressor and Ca^{2+}-transporting adenosine triphosphatase isoform 2 genes. Cancer Res 54:2486–2491

Lennon G, Auffray C, Polymeropoulos M, Soares MB (1996) The I.M.A.G.E. Consortium: an integrated molecular analysis of genomes and their expression. Genomics 33:151–152

Lo Ten Foe JR, Rooimans MA, Bosnoyan-Collins L, Alon N, Wijker M, Parker L, Lightfoot J, et al (1996) Expression cloning of a cDNA for the major Fanconi anaemia gene, *FAA*. Nat Genet 14:320–323

Lu R, Nash HM, Verdine GL (1997) A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer. Curr Biol 7: 397–407

Martin NJ, Steinberg BG (1983) The dup(3) (p25 leads to pter) syndrome: a case with holoprosencephaly. Am J Med Genet 14:767–772

Mercer WE, Schlegel RA (1979) Phytohemagglutinin enhancement of cell fusion reduces polyethylene glycol cytotoxicity. Exp Cell Res 120:417–421

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Moustacchi E, Diatloff-Zito C (1985) DNA semi-conservative synthesis in normal and Fanconi anemia fibroblasts following treatment with 8-methoxypsoralen and near ultraviolet light or with X-rays. Hum Genet 70:236–242

Muzio M, Ni J, Feng P, Dixit VM (1997) IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. Science 278:1612–1615

Phipps ME, Latif F, Prowse A, Payne SJ, Dietz-Band J, Leversha M, Affara NA, et al (1994) Molecular genetic analysis of the 3p− syndrome. Hum Mol Genet 3:903–908

Porfirio B, Smeets D, Beckers L, Caporossi D, Tedeschi B, Vernole P, Joenje H, et al (1991) Fragile sites and chromosome instability: the distribution of breaks induced by *cis*-diamine-dichloro-platinum (II) in Fanconi anemia lymphocyte cultures. Hum Genet 86:256–260

Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S (1997) Cloning and characterization of *hOGG1*, a human homolog of the *OGG1* gene of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 94:8010–8015

Roldan-Arjona T, Wei YF, Carter KC, Klungland A, Anselmino C, Wang RP, Augustus M, et al (1997) Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine–DNA glycosylase. Proc Natl Acad Sci USA 94:8016–8020

Saito H, Hammond AT, Moses RE (1993) Hypersensitivity to oxygen is a uniform and secondary defect in Fanconi anemia cells. Mutat Res 294:255–262

Sbrana I, Musio A (1995) Enhanced expression of common fragile site with occupational exposure to pesticides. Cancer Genet Cytogenet 82:123–127

Schmidt HH, Pirc-Danoewinata H, Panzer-Grumayer ER,

Sill H, Sedlmayr P, Neumeister P, Linkesch W, et al (1998) Translocation (3;5) (p26;q13) in a patient with chronic T-cell lymphoproliferative disorder. Cancer Genet Cytogenet 104:82–85

Shepherd NS, Pfrogner BD, Coulby JN, Ackerman SL, Vaidyanathan G, Sauer RH, Balkenhol TC, et al (1994) Preparation and screening of an arrayed human genomic library generated with the P1 cloning system. Proc Natl Acad Sci USA 91:2629–2633

Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor–based vector. Proc Natl Acad Sci USA 89: 8794–8797

Smith RF, Wiese BA, Wojzynski MK, Davison DB, Worley KC (1996) BCM Search Launcher—an integrated interface to molecular biology data base search and analysis services available on the World Wide Web. Genome Res 6:454–462

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Strathdee CA, Gavish H, Shannon WR, Buchwald M (1992) Cloning of cDNAs for Fanconi's anaemia by functional complementation. Nature 356:763–767

Street VA, McKee-Johnson JW, Fonseca RC, Tempel BL, Noben-Trauth K (1998) Mutations in a plasma membrane Ca^{2+}-ATPase gene cause deafness in deafwaddler mice. Nat Genet 19:390–394

Szymanski SC, Hummerich H, Latif F, Lerman MI, Rohrborn G, Schroder E (1993) Long range restriction map of the von Hippel-Lindau gene region on human chromosome 3p. Hum Genet 92:282–288

Thompson KA, Wang B, Argraves WS, Giancotti FG, Schranck DP, Ruoslahti E (1994) BR140, a novel zinc-finger protein with homology to the TAF250 subunit of TFIID. Biochem Biophys Res Commun 198:1143–1152

Trask BJ, Massa H, Kenwrick S, Gitschier J (1991) Mapping of human chromosome Xq28 by two-color fluorescence in situ hybridization of DNA sequences to interphase nuclei. Am J Hum Genet 48:1–15

Waisfisz Q, Saar K, Morgan NV, Altay C, Leegwater PA, de Winter JP, Komatsu K, et al (1999) The Fanconi anemia group E gene, *FANCE*, maps to chromosome 6p. Am J Hum Genet 64:1400–1405

Weksberg R, Buchwald M, Sargent P, Thompson MW, Siminovitch L (1979) Specific cellular defects in patients with Fanconi anemia. J Cell Physiol 101:311–323

Whitney M, Thayer M, Reifsteck C, Olson S, Smith L, Jakobs PM, Leach R, et al (1995) Microcell mediated chromosome transfer maps the Fanconi anaemia group D gene to chromosome 3p. Nat Genet 11:341–343

Whitney MA, Royle G, Low MJ, Kelly MA, Axthelm MK, Reifsteck C, Olson S, et al (1996) Germ cell defects and hematopoietic hypersensitivity to γ-interferon in mice with a targeted disruption of the Fanconi anemia C gene. Blood 88:49–58