

## Nucleotide excision repair endonuclease genes in *Drosophila melanogaster*

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Received 1 November 1999; received in revised form 10 December 1999; accepted 10 December 1999

### Abstract

Nucleotide excision repair (NER) is the primary pathway for the removal of ultraviolet light-induced damage and bulky adducts from DNA in eukaryotes. During NER, the helix is unwound around the damaged site, and incisions are made on the 5' and 3' sides, to release an oligonucleotide carrying the lesion. Repair synthesis can then proceed, using the intact strand as a template. The incisions flanking the lesion are catalyzed by different structure-specific endonucleases. The 5' incision is made by a heterodimer of XPF and ERCC1 (Rad1p–Rad10p in *Saccharomyces cerevisiae*), and the 3' incision is made by XPG (Rad2p in *S. cerevisiae*). We previously showed that the *Drosophila* XPF homologue is encoded by the meiotic recombination gene *mei-9*. We report here the identification of the genes encoding the XPG and ERCC1 homologues (XPG<sup>Dm</sup> and ERCC1<sup>Dm</sup>). XPG<sup>Dm</sup> is encoded by the *mus201* gene; we found frameshift mutations predicted to produce truncated XPG<sup>Dm</sup> proteins in each of two *mus201* alleles. These mutations cause defects in nucleotide excision repair and hypersensitivity to alkylating agents and ultraviolet light, but do not cause hypersensitivity to ionizing radiation and do not impair viability or fertility. ERCC1<sup>Dm</sup> interacts strongly in a yeast two-hybrid assay with MEI-9, indicative of the presumed requirement for these polypeptides to dimerize to form the functional endonuclease. The *Drosophila Ercc1* gene maps to polytene region 51D1-2. The nucleotide excision repair gene *mus210* maps nearby (51E-F) but is distinct from *Ercc1*. © 2000 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Flap endonuclease; Nucleotide excision repair; XPG; XPF; ERCC1

### 1. Introduction

The processes of DNA replication, repair, and recombination often involve the formation of distinct

DNA structures that can be resolved by structure-specific nucleases. In nucleotide excision repair (NER), a pathway for the removal of many types of intra-strand DNA lesions, several proteins interact to form an open complex surrounding a lesion (reviewed in Refs. [1–3]). This step is followed by incision of the damaged strand on the 5' side by one structure-specific endonuclease, and on the 3' side by another. The 5' endonuclease is a heterodimer com-

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posed of XPF and ERCC1 (Rad1p and Rad10p in *Saccharomyces cerevisiae*); the 3' endonuclease is XPG (Rad2p in *S. cerevisiae*). These proteins display in vitro activities consistent with these in vivo roles, with both cutting structures that have double-stranded to single-stranded transitions: XPF–ERCC1 cuts strands that transit from 5' double-stranded to 3' single-stranded, whereas XPG cuts strands that transit from 5' single-stranded to 3' double-stranded [4–7].

Double-strand to single-strand transitions are formed in other DNA metabolic pathways, and the XPF–ERCC1 nuclease and its homologues have functions in some of these processes. XPF and ERCC1 function in interstrand crosslink repair [8], and ERCC1 is an essential gene in mice [9,10]. *S. cerevisiae rad1* and *rad10* mutants have defects in mitotic recombination and in the repair of insertional mutations during meiotic recombination [11–14]. *Schizosaccharomyces pombe Swi9<sup>+</sup>* and *Swi10<sup>+</sup>* are required for mating-type switching [15]. In *Drosophila melanogaster*, *mei-9* is required for meiotic recombination, and *mei-9* mutants have increased levels of mitotic recombination [16,17].

In contrast, the enzymatic function of XPG may be limited to its role in NER. Although XPG is involved in transcription-coupled repair of oxidative damage, it is believed to play only a structural role in this pathway, perhaps related to the ability of XPG to interact with PCNA [18,19]. One explanation for the more limited function of XPG enzymatic activity is the existence of proteins with related sequence elements and overlapping biochemical activities, collectively referred to as the FEN-1 family (reviewed in Ref. [20]). Three eukaryotic branches of this family have been described. In addition to the XPG/Rad2p branch, there is a branch containing vertebrate flap endonuclease 1 (FEN-1) and its *S. cerevisiae* orthologue Rad27p, and a branch including *S. pombe* Exo I and its orthologues [21,22].

Given the complex functions of the structure-specific endonucleases, it would be useful to study their roles in a model metazoan. The *Drosophila* homologue of XPF has been described previously as the product of the *mei-9* meiotic recombination gene [23]. The presumptive Exo I orthologue in *Drosophila* has also been identified, although no mutations are known in *Tosca*, the gene encoding it

[24]. We report here the identification of the *D. melanogaster* genes encoding the orthologues of FEN-1 / Rad27p, XPG / Rad2p, and ERCC1/Rad10p. We show that the *Drosophila* XPG orthologue is encoded by the *mus201* gene; the ERCC1 homologue maps to 51D1-2, but no extant mutations exist.

## 2. Materials and methods

### 2.1. GenBank accession numbers

The *mus201* cDNA and predicted protein sequences, along with mutant sequences, are deposited under accession number AF144092. The *Ercc1* genomic and predicted protein sequences are deposited under accession number AF146797.

### 2.2. Sequence analysis

Sequence comparisons were performed with the Wisconsin Package, Version 9.1 (Genetics Computing Group). The alignment of FEN-1 family members was generated with the PILEUP program. Protein sequence distances were determined with the DISTANCES program, using the Kimura scoring method, and a phylogenetic tree was constructed with the GROWTREE program, using the neighbor-joining method.

### 2.3. *Drosophila mus201* cDNAs

We screened a 0–4 h embryonic cDNA library, and recovered and characterized several cDNAs encoding *Drosophila* XPG. The longest of these are 4119 bp, beginning at the last nucleotide of a genomic *PvuII* site. Coordinates are given relative to the cDNA sequence in GenBank AF144092.

### 2.4. Deficiency mapping of *mus201* and *mus210*

Except where indicated, females homozygous for the mutation being mapped were crossed to *Df/CyO* males. Adults were allowed to mate and lay eggs for 2 days before being removed. One day later, 0.25 ml of 0.05% (v/v) methyl methanesulfonate (MMS) in water was added to the medium. Progeny were

counted and scored from the 12th to the 18th day after the beginning of the egg laying period. All crosses were conducted at 25°C.

### 2.5. Sequencing of mutants

The XPG<sup>Dm</sup> coding region was sequenced for the mutations *mus201<sup>D1</sup>* and *mus201<sup>A1</sup>*. Individual flies homozygous for the mutation were homogenized, and PCR was performed using gene-specific primers. Products from several independent amplifications were pooled, isolated on an agarose gel, and sequenced. Mutations were confirmed by sequencing the opposite strand from an independent amplification.

### 2.6. Isolation of additional *mus210* mutants

To isolate additional mutations in *mus210*, we obtained EMS-mutagenized chromosome 2 lines (kindly provided by Pelin Cayirlioglu and Bob Duronio) as *P{neoFRT}40A\*/CyO* stocks. Males from these stocks were crossed to *mus210<sup>G1</sup>/CyO* virgin females. After three days, parents were transferred into new vials; 1 day later 250- $\mu$ l 0.05% MMS was added to the first vial. Vials were subsequently scored for the absence of Cy<sup>+</sup> progeny in the treated vial. These were retested for MMS hypersensitivity by crossing *P{neoFRT}40A\*/CyO* males to *Df(2R)Jp1/CyO* virgin females. Two new alleles, designated *mus210<sup>C1</sup>* and *mus210<sup>C2</sup>*, were recovered from approximately 900 lines tested.

### 2.7. Two-hybrid assay

We used the two-hybrid vectors and strains described in James et al. [25]. For pGBD–MEI-9, a fragment of a *mei-9* cDNA from a *PvuII* site (encoding residues 251–2) to the 3' end was cloned into the *SmaI* to *SalI* sites of pGBD–C1. For pGAD–ERCC1<sup>Dm</sup>, the entire GM10122 cDNA insert (encoding all but the first three residues of ERCC1<sup>Dm</sup>) was cut out using the *SmaI* site at the 5' end and the *XhoI* site at the 3' end, and subcloned into the *SmaI* and *SalI* sites of pGAD–C1. Constructs were sequenced to ensure that they were in the proper reading frame, then transformed separately and together into yeast strain PJ69-4a.

Single transformants were selected on CM plates lacking tryptophan (for pGBD–MEI-9) or leucine (for pGAD–ERCC1<sup>Dm</sup>). The double-transformant was selected for on CM plates lacking both tryptophan and leucine. Single and double transformants were streaked onto identical plates also lacking histidine, to test for activation of the *GALI-HIS3* reporter, or adenine, to test for activation of the *GAL2-ADE2* reporter. Plates were incubated at 30°C for 2 to 5 days.

## 3. Results

### 3.1. Identification of *Drosophila* FEN-1 family genes

The sole *Drosophila* member of the FEN-1 gene family previously reported is *Tosca*, which encodes the putative Exo I orthologue [24]. We found two new *Drosophila* FEN-1 family members through database searches, one on P1 DS02110 in 29C1-5, and another on P1s DS06306 and DS07321 in 53E1-2 (also represented by EST GM10241). A comparison of the predicted protein sequences with other FEN-1 family members is shown in Fig. 1A. As in other organisms, these sequences can be assigned to three branches of a single family, based on sequence similarities and structural features. This comparison indicates that the sequence in 29C encodes the orthologue of XPG and Rad2p (XPG<sup>Dm</sup>), and the sequence in 53E encodes the orthologue of FEN-1 and Rad27p. A detailed analysis of the XPG<sup>Dm</sup> sequence was published recently by Houle and Friedberg [26].

### 3.2. *Drosophila* XPG is encoded by *mus201*

The P1 clone encoding XPG<sup>Dm</sup> maps to polytene bands 29C1-5. The *mus201* gene seemed to be a likely candidate for this gene, since it had been previously mapped to the interval between *dp* (25A3) and *pr* (34D4), and mutations in it cause defects in NER [27]. This gene is represented by two extant alleles, *mus201<sup>A1</sup>* and *mus201<sup>D1</sup>* (as described below, the mutation designated *mus(2)201<sup>G1</sup>* by Luchkina et al. [28] is not allelic to these mutations). We used deficiencies to map these alleles to cytological region 29C1-2 to 29D1-2, between the distal breakpoints of *Df(2L)N22-14* and *Df(2L)N22-5* (Table 1). This is an interval of approximately 40 kb

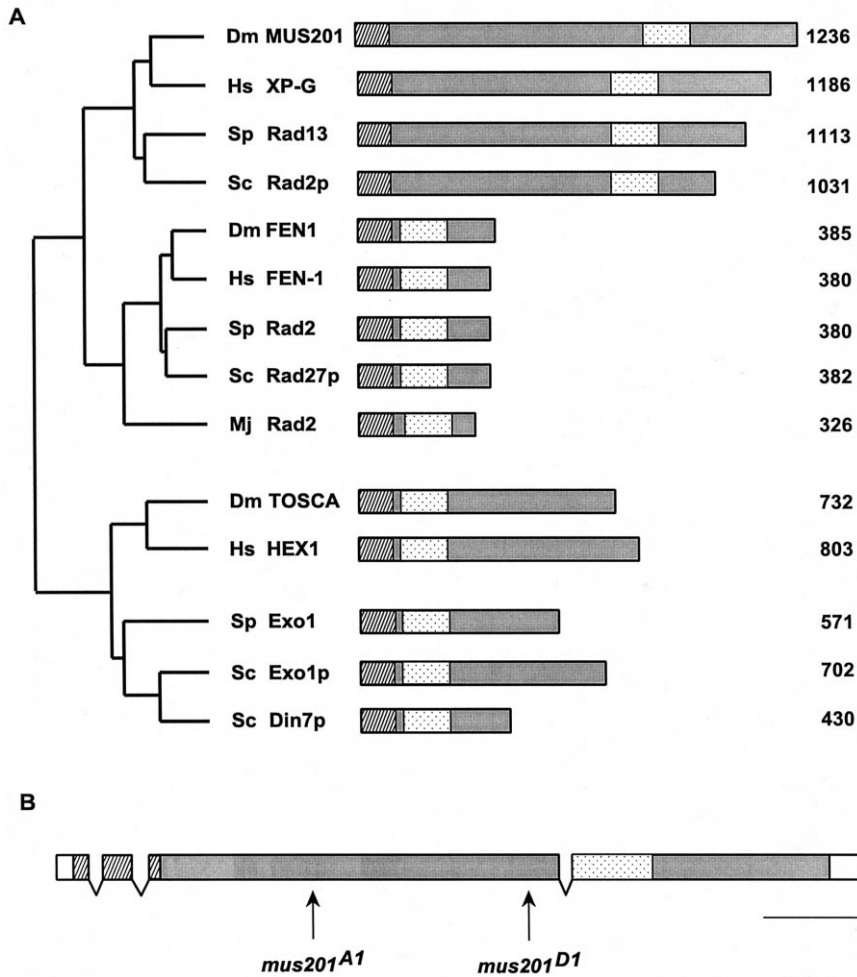


Fig. 1. (A) The eukaryotic FEN-1 family. The dendrogram on the left depicts the relationships between FEN-1 family members from humans, *D. melanogaster*, *S. cerevisiae*, and *S. pombe*. A homologue from the archeon *Methanococcus jannaschii* is also indicated. To the right are schematics of the corresponding protein structures. The N and I boxes are indicated with hatching and stippling, respectively. Total number of amino acid residues predicted for each protein is given at the right. (B) Schematic of the *mus201* gene structure. Boxes represent exons; connecting lines represent introns. Unfilled regions are untranslated; hatched and stippled sections represent regions encoding the N and I boxes. The bar indicates 500 bp. The positions of the *mus201<sup>A1</sup>* and *mus201<sup>D1</sup>* frameshift mutations are indicated.

that also contains the *gurken* gene [29], whose 5' end is within 5 kb of the 5' end of XPG<sup>Dm</sup> coding region, transcribed divergently.

We isolated and sequenced several cDNAs encoding XPG<sup>Dm</sup>. Comparison of these sequences to the sequence of DS02110 allowed us to determine the genomic structure of the gene (Fig. 1B). Transcripts comprise four exons; the conserved N region is interrupted by two introns, and a third intron separates the conserved I region from the spacer.

We analyzed the XPG<sup>Dm</sup> coding region from the two *mus201* mutants. Each chromosome carried, in addition to several silent polymorphisms, a frameshift mutation predicted to severely truncate the XPG<sup>Dm</sup> protein (Fig. 1B). The *mus201<sup>A1</sup>* chromosome has a deletion of five base pairs and an insertion of a T–A base pair at 1212–1216 (relative to the cDNA sequence deposited in GenBank record AF144092), creating a predicted stop codon that would cause a truncation after residue 376 of 1236. The *mus201<sup>D1</sup>*

Table 1  
Deficiency mapping of *mus201*

Deficiency	Region deleted	Allele	no MMS		+MMS	
			Cy	Cy <sup>+</sup>	Cy	Cy <sup>+</sup>
<i>Df(2L)N22-14</i>	29C1-2; 30C8-9	<i>mus201<sup>A1</sup></i>	233	156	161	0
		<i>mus201<sup>D1</sup></i>	61	48	49	0
<i>Df(2L)N22-5</i>	29D1-2; 30C4-D1	<i>mus201<sup>A1</sup></i>	310	220	314	256
		<i>mus201<sup>D1</sup></i>	236	195	133	71

chromosome has a deletion of two T–A base pairs out of the six from 2258 to 2263, predicting a frameshift that would result in a truncation after residue 726. Based on the deficiency mapping and the identification of frameshift mutations in two different *mus201* alleles, we conclude that *mus201* does indeed encode the *Drosophila* XPG orthologue.

### 3.3. Identification of the *Drosophila* ERCC1 homologue

The NER 5' endonuclease consists of a heterodimer between XPF (ERCC4) and ERCC1 in vertebrates, and Rad1p and Rad10p in *S. cerevisiae* [4,5,7]. In *Drosophila*, the gene encoding the XPF homologue has been identified as the product of the *mei-9* meiotic recombination gene [23]. We found the putative ERCC1/Rad10p homologue (ERCC1<sup>Dm</sup>) through database searches, which detected EST GM10122 (accession number AA735926). We sequenced this cDNA as well as the encompassing genomic DNA to determine the complete *Ercc1* sequence and genomic structure (Fig. 2B). The predicted *Drosophila* protein is 44% identical and 55% similar to human ERCC1, with very little similarity in the first 60 residues. Sijbers et al.

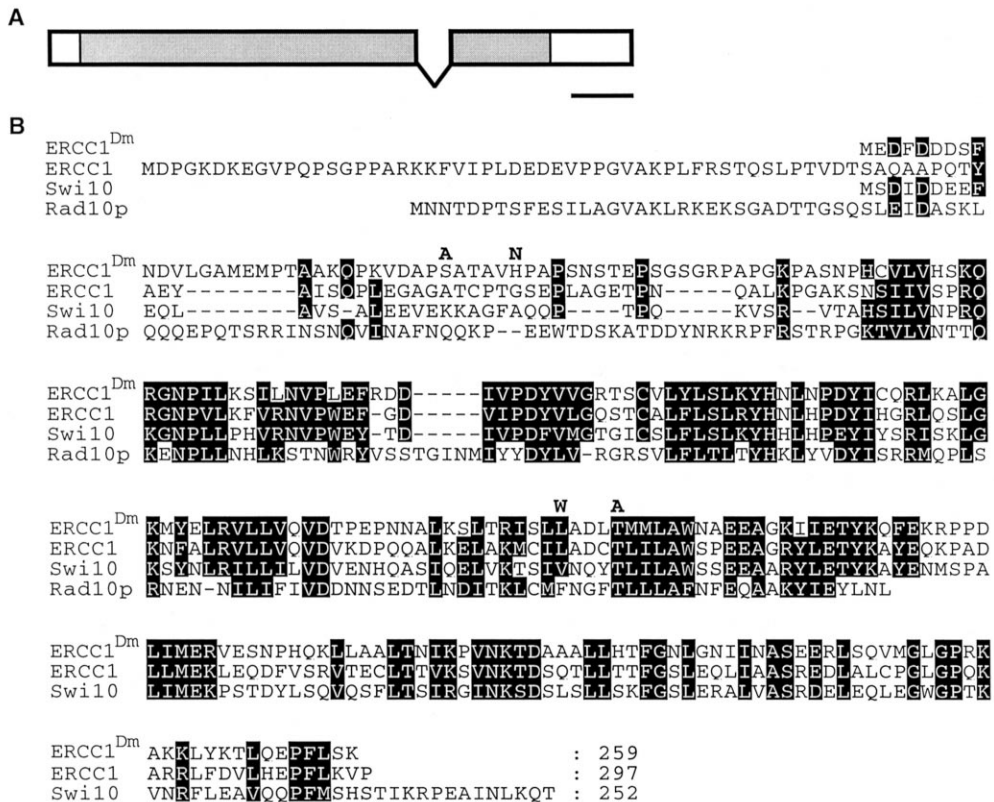


Fig. 2. (A) Schematic of the *Drosophila* *Ercc1* gene structure. Boxes represent exons; connecting lines represent the single intron. The protein-coding region is shaded. The bar indicates 100 bp. (B) Alignment of the predicted ERCC1<sup>Dm</sup> sequence with its human, *S. pombe*, and *S. cerevisiae* homologues. Known polymorphisms are indicated above the ERCC1<sup>Dm</sup> sequence.

[30] have pointed out similarities between the carboxy-terminal region of ERCC1 and bacterial UvrC proteins. This region is lacking in *S. cerevisiae* Rad10p, but is present in the predicted *Drosophila* protein.

### 3.4. ERCC1<sup>Dm</sup> interacts physically with MEI-9

We used the yeast two-hybrid (Y2H) system to determine whether MEI-9 and ERCC1 interact physically. Approximately 100 residues at the carboxy terminus of XPF is sufficient to show a strong interaction with ERCC1 in a Y2H assay, and the carboxy-terminal 75 residues of ERCC1 are sufficient to show a strong interaction with XPF [31]. We expressed MEI-9 residues 252–926 as a fusion with the Gal4 DNA-binding domain (GBD-MEI-9), and ERCC1<sup>Dm</sup> residues 4 to 259 as a fusion with the Gal4 transcriptional activation domain (GAD-ERCC1<sup>Dm</sup>). Constructs were transformed either singly or together into strain PJ69-4A, which contains a *GAL1-HIS3* reporter gene and a *GAL2-ADE2* reporter gene [25]. The *GAL1-HIS3* reporter was sometimes weakly activated in the presence of GAD-ERCC1 alone, but GBD-MEI-9 alone did not activate this reporter, and neither activated the more stringent *GAL2-ADE2* reporter. Transformants expressing both the MEI-9 fusion and the ERCC1 fusion, however, showed strong activation of both reporter genes (Fig. 3 and data not shown). Colonies on plates lacking adenine were white, indicating a high level of activation of the *GAL2-ADE2* reporter, indicative of a strong physical interaction between the GBD-MEI-9 and GAD-ERCC1 fusion proteins.

### 3.5. *Drosophila* *Ercc1* maps to 51D1-2

We mapped the gene encoding ERCC1<sup>Dm</sup> by probing a blot of genomic P1 clones (Genome Systems). GM10122 hybridized to clones DS06145, DS06422, and DS09161. DS09161 has been localized to 51D1-2; the other two clones have not been localized, but each is positive for an STS sequence derived from opposite ends of DS09161. Thus, *Ercc1* maps to the region of overlap between these three clones.

The mutations *mus(2)201<sup>G1</sup>* and *mus210<sup>B1</sup>* appeared to be candidates for mutations in *Drosophila*

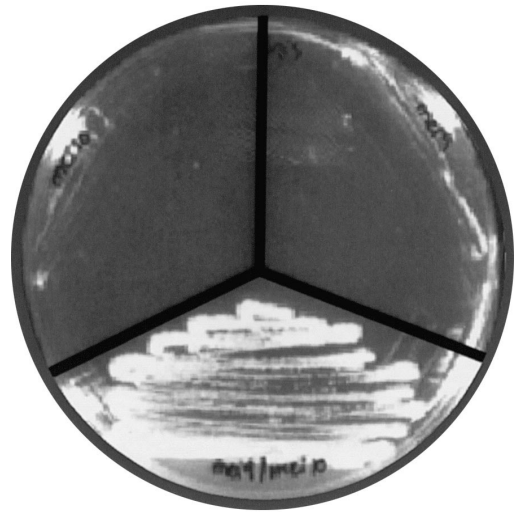


Fig. 3. Physical interaction between MEI-9 and ERCC1<sup>Dm</sup> in a yeast two-hybrid assay. Cultures expressing GBD-MEI-9 (upper left), GAD-ERCC1<sup>Dm</sup> (upper right) or both (lower) were streaked onto a minimal medium plate lacking adenine. Growth indicates activation of the *GAL2-ADE2* reporter gene. All transformants grew well on plates containing adenine (not shown).

*Ercc1*. Both mutations confer hypersensitivity to MMS and ultraviolet light, and both map to the medial region of chromosome arm 2R [28,32]. Further, both mutations were reported to impair excision of pyrimidine dimers, indicating a role in NER [27,28]. We found that these mutations fail to complement one another for hypersensitivity to MMS (data not shown); accordingly, we rename *mus(2)201<sup>G1</sup>* to *mus210<sup>G1</sup>*. We used deficiencies to map *mus210*. According to the published breakpoints of these deficiencies, *mus210* maps to 51D3 to 51F13 (Table 2, but see Fig. 4), consistent with the possibility that *mus210* might encode ERCC1<sup>Dm</sup>. We sequenced the region encoding ERCC1<sup>Dm</sup> from *mus210<sup>B1</sup>* and *mus210<sup>G1</sup>*, although the progenitor chromosomes were unavailable for comparison. The *mus210<sup>B1</sup>* chromosome differs at three positions from the cDNA sequence. Two of these are silent polymorphisms, but the third predicts a change of a threonine residue to alanine. This threonine is absolutely conserved in each of 10 homologues currently in GenBank. Although this seemed to be a good candidate for the *mus210<sup>B1</sup>* mutation, we were unable to find any potential mutation in the ERCC1<sup>Dm</sup> coding sequence from *mus210<sup>G1</sup>*.

Table 2  
Deficiency mapping of *mus210*

Deficiency	Breakpoints <sup>a</sup>	Allele	no MMS + MMS			
			Cy	Cy <sup>+</sup>	Cy	Cy <sup>+</sup>
<i>Df(2R)knSA3</i>	51B5-11; 51C1-D1	<i>mus210</i> <sup>G1</sup>	323	299	284	277
		<i>mus210</i> <sup>C1</sup>	218	272	194	201
<i>Df(2R)Jp1</i>	51C3; 52F8-9	<i>mus210</i> <sup>G1</sup>	161	120	115	0
<i>Df(2R)XTE-18</i>	51D3-E1; 52D1	<i>mus210</i> <sup>G1</sup>	218	193	348	0
<i>Df(2R)Jp4</i>	51F13; 52F8-9	<i>mus210</i> <sup>G1</sup>	351	280	310	251
<i>Df(2R)Jp5</i>	52A13; 52F11	<i>mus210</i> <sup>G1</sup>	173	142	133	115

<sup>a</sup>Previously published breakpoints are listed. For revised breakpoints, see Fig. 4.

To aid in clarifying the relationship between *mus210* and *Drosophila Ercc1*, we isolated two additional alleles of *mus210* (see Section 2), *mus210*<sup>C1</sup> and *mus210*<sup>C2</sup>. We sequenced ERCC1<sup>Dm</sup> from each of these alleles in *trans* to *Df(2R)Jp1*, which uncovers the MMS hypersensitivity of *mus210* mutations (Table 2). We found no obvious mutations in *Ercc1*. Furthermore, examination of the chromatograms revealed that the PCR product was heterozygous at four sites within the gene, indicating that *Df(2R)Jp1* does not remove the coding region for ERCC1<sup>Dm</sup>. Subsequent complementation mapping between these deficiencies and P-element insertions with known cytological locations suggests revisions of the deficiency breakpoints (Fig. 4). Ac-

cordingly, *mus210* maps to 51E-F, and therefore does not encode the ERCC1<sup>Dm</sup>. Another candidate for *mus210* is *Xpcc*, which encodes the *Drosophila* NER protein XPC [33]. This gene is contained on BACR33K06, which maps to 51F (Berkeley *Drosophila* Genome Project, unpublished). Sequencing of *Xpcc* from *mus210* mutants revealed nonsense mutations in all four alleles (JJS, unpublished data). We subsequently determined that the alteration *mus210*<sup>B1</sup> resulting in the conserved threonine being replaced by alanine is a naturally occurring polymorphism, without apparent effect on the function of ERCC1<sup>Dm</sup> (Table 2 and data not shown).

*Df(2R)knSA3* deletes *Ercc1* (Fig. 4 and data not shown) but does not uncover *mus210*. We used this deficiency to screen other unmapped chromosome 2 mutations that confer hypersensitivity to MMS. *Df(2R)knSA3* complements *mus202*<sup>A1</sup>, *mus204*<sup>A1</sup>, *mus205*<sup>A1</sup>, and *mus206*<sup>A1</sup>. Thus, we were unable to find extant mutations in *Ercc1*. Screens are underway to generate such mutations.

#### 4. Discussion

We determined that *mus201* encodes the *Drosophila* homologue of XPG/Rad2p. The two *mus201* alleles we sequenced carry frameshift muta-

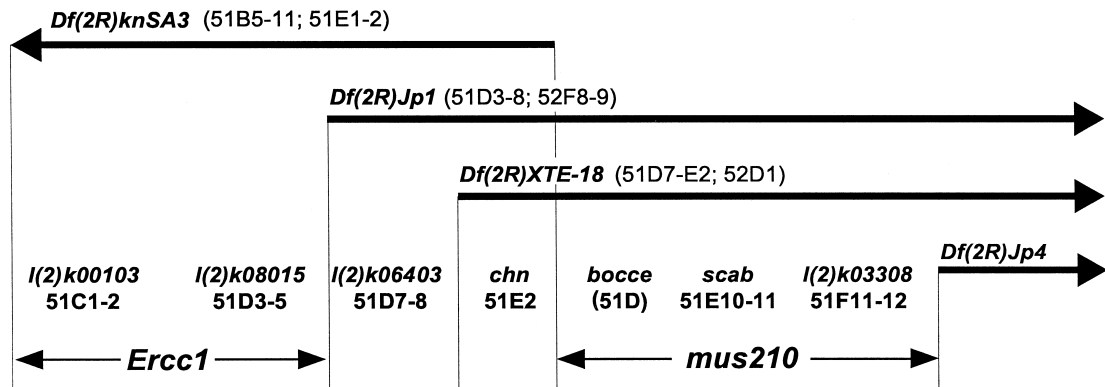


Fig. 4. Genetic map of the 51C-F region. Regions deleted in various deficiencies are indicated with black lines, with arrowheads indicating that the deletion extends beyond the region depicted. In cases where breakpoints have been revised, new breakpoints are given. Lethal P-element insertions shown were positioned genetically by complementation tests. Their positions on the polytene chromosome map, determined by *in situ* hybridization [38] are indicated. *ms(2)loc* was reported to be located in 51D [39], but on the sequenced contig spanning 51E-F it is to the right of *chn* (Berkeley *Drosophila* Genome Project, unpublished). *Ercc1* maps to 51D1-2 and is deleted by *Df(2R)knSA3*; *mus210* maps to 51E-F.

tions. The truncated proteins predicted by these mutations would lack the conserved I region as well as the carboxy-terminal nuclear localization signal. The I region in *Pyrococcus furiosus* FEN-1 is involved in DNA binding [34], and thus these *mus201* alleles are most likely complete losses of function.

There is only one known function for the enzymatic activity of XPG — in making the 3' incision during NER. In humans, inherited mutations in *XP-G* that result in loss of the catalytic activity of the protein cause defects in NER and the disease xeroderma pigmentosum (XP). Both *mus201* alleles confer extreme hypersensitivity to the alkylating agent MMS, as well as to *N*-acetyl-2-aminofluorene and benzo[*a*]pyrene [27], which causes the addition of bulky adducts of the type typically repaired by the NER pathway.

The MMS hypersensitivity of *mus201* mutants is extreme, with an LD<sub>50</sub> around 0.03% (approximately 3 μM; this is the concentration of a 250 μl dose added to approximately 10 ml of medium on which larvae are feeding, and thus the effective concentration is presumably lower). The mutation *mus210<sup>B1</sup>*, a nonsense mutation in the gene encoding the NER protein XPC (JJS unpublished data) causes a similar degree of sensitivity to MMS [35]. However, a double mutant between *mus201<sup>D1</sup>* and *mus210<sup>B1</sup>* is substantially more sensitive than either single mutant, with an LD<sub>50</sub> of less than 0.01% MMS [35]. This result suggests that *mus201* has one or more functions outside of the canonical NER pathway.

The only described function for XPG outside of NER is in repair of oxidative base damage, where the protein apparently plays a structural role, rather than an enzymatic one [18,19]. The XPG protein is required for transcription-coupled repair of oxidative damage, and stimulates global genome repair of oxidative lesions. It is believed that defects in these processes underlies the finding that patients carrying severe truncation mutations in *XP-G* exhibit characteristics of Cockayne syndrome [18,36]. Similarly, defects in repair of oxidative base damage have been proposed to account for the growth failure and short life span of *xpg<sup>-/-</sup>* mice [37].

The two *mus201* alleles we sequenced should disrupt both the enzymatic and structural functions of the protein, and are therefore more like the CS

alleles of *XP-G* than the NER alleles. As is the case for fibroblasts isolated from *xpg<sup>-/-</sup>* mice [37], we were unable to detect any increased sensitivity of *mus201* larvae to H<sub>2</sub>O<sub>2</sub> added to the medium (unpublished data). However, both alleles cause a mild hypersensitivity to ionizing radiation [27], suggesting a possible role in repair of oxidative damage. Mice that are *xpg<sup>-/-</sup>* exhibit defects in postnatal growth and short life span [37]. In contrast, flies homozygous or hemizygous for either *mus210* allele are fully viable and fertile in the absence of exogenous DNA damaging agents, possibly because of the lower cell number and shorter life span of *Drosophila*.

We were unable to find any candidates for mutations in *Drosophila Ercc1*, but efforts are underway to induce such mutations. *Ercc1<sup>-/-</sup>* mice die before weaning, and exhibit chromosomal abnormalities [9,10]. Although there have not been any reports of a separation of function between XPF and ERCC1, it is not known with certainty whether XPF is also an essential gene. Nonsense mutations in *mei-9* that do not produce detectable protein are not lethal, though they do exhibit severe defects in meiotic recombination, concomitant with high levels of meiotic chromosome nondisjunction (JJS, unpublished). It will be of interest to determine whether defects in *Ercc1* result in lethality and/or a meiotic phenotype like that of *mei-9* mutants.

## Acknowledgements

We thank Randy Mottus for providing the *mus210<sup>B1</sup>* stock, and Pelin Cayirlioglu and Bob Duronio for providing EMS-mutagenized chromosome 2 lines. JJS was supported in part by a fellowship from the Cancer Research Fund of the Damon Runyon-Walter Winchell Foundation, DRG 1355.

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