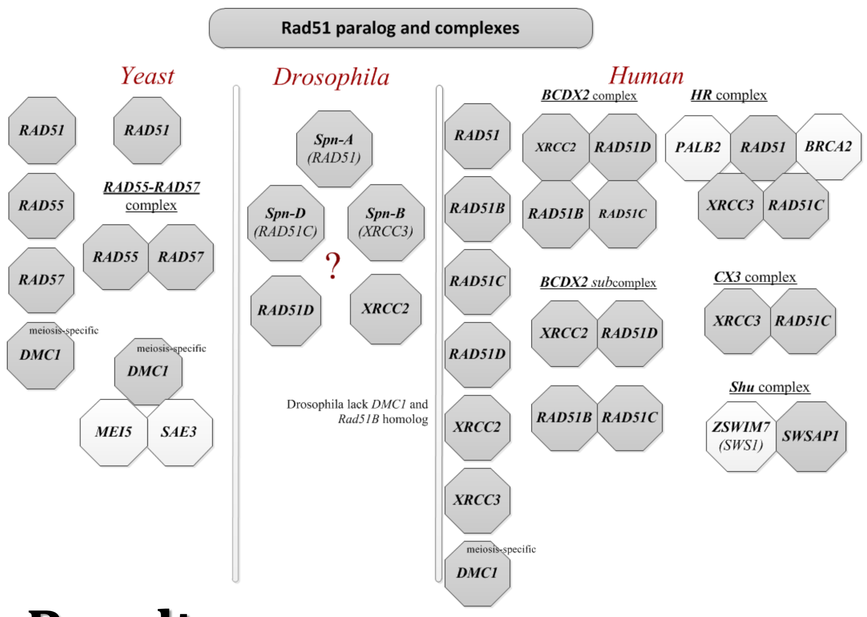
TAGC2020 online APRIL 22-25, 2020 Genetics Society of America

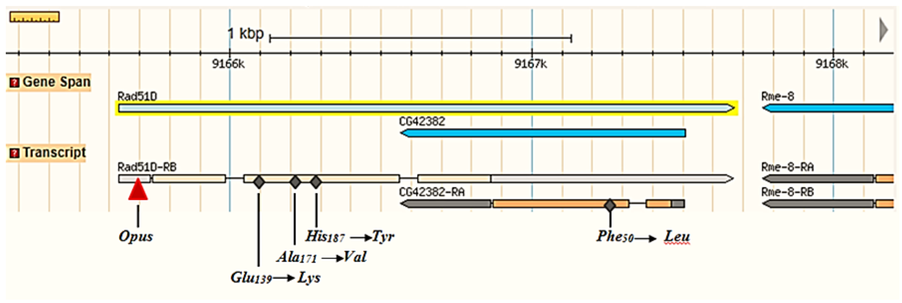
Genetic analysis of the *Rad51D* gene

Alexander Y. Konev*, Y. Ilina, V. Ukraintsev
NRC «Kurchatov Institute» - PNPI
* Correspondence : konev.alexander@gmail.com; konev_AY@pnpi.nrcki.ru

The key event in recombination repair of DNA double strand breaks (DSB) is the formation of the Rad51 nucleoprotein filament, which is necessary for the homology search and exchange of DNA strands. An important role in the regulation of the assembly, stabilization, and disassembly of Rad51 filaments is played by the Rad51 paralogs — proteins structurally similar to Rad51. Recently, an interest to Rad51 paralogs has greatly increased due to their significant role in carcinogenesis. Mammals have six paralogs of the Rad51: Rad51B, Rad51C, Rad51D, XRCC2;, XRCC3 and the SWSAP1 (RadA homolog). The Drosophila genome contains the Rad51 ortholog spnA and four Rad51 paralogs: spnB (XRCC3 homolog), spnD (hRad51C), XRCC2 and Rad51D. However, the individual functions of these proteins remain largely unclear.



Results



Opus insertion

```
w. t.   № bp      1          11--//--72          82 83
5' --/ / CAT TTTGGGCGGCC--/ /-- GTCCACATTAT AAATAATCGATAACAGTGCGGCTAAATTTAAATTTCACCAATCAGGAAA / /-- 3'
          scRad51D

radD1                                82 83
5' --/ / TAGTTTGTCCACATTAT AAATAATCGATAACAGTGCGGCTAAATTTAAATTTCACCAATCAGGAAAATAAATTGAAATGC / /--

radD4                                82 83
5' --/ / GATAGTTTGTCCACATTAT AAATAATCGATAACAGTGCGGCTAAATTTAAATTTCACCAATCAGGAAAATAAATTGAAAT / /--

DmOpus          AGT TAAGAACCC -- /7587 bp/ -- CTTGCGTTAACT

radD2                                82                                  83
5' --/ / TTTGTCCACATTATAGTTAAGAACCC --/7581 bp /-GTGGAACTTGCGTTAACTAAATAATCGATAACAGTGCGGCTAAA / /--3

radD3                                82                          83
5' --/ / AGTTTGTCCACATTATAGTTAAGAACCC--/ 7596 bp /--ACTAAATAATCGATAACAGTGCGGCTAAATTTATATTTCACC / /--3
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Selection of rad201 revertants

Rad201G1 has only γ-sensitivity without MMS-sensitivity

Radiosensitivity Reversal

The huge number of ***rad201G1*** breeding the third larval instar

20 Gy

≈ 100% pupae lethality

single survivors

pre-revertant line ***rad*** $^{\gamma+}$

≈ 97-98% lethality after 20 Gy

♀ ***CyO / Pla*** x ♂ *individual* ***rad*** $^{\gamma+}$ ***/ CyO***

№	Sublines	γ-sensitivity
1	***radD1***	**no**
2	*radD2*	yes
3	*radD3*	yes
4	***radD4***	**no**
5	*rad201G1*	yes
6	*Oregon R*	no

Isolation of individual chromosomes
Testing for ɣ-rays sensitivity

Rad51D

DmRad51D_2 exon (w. t.)
Lys Arg ***Glu*** $_{139}$ Phe Ser Cys Val Gln ***Ala*** $_{171}$ Ala Thr Gly Phe Asp ***His*** $_{187}$ Gln Leu Thr
--/ / AAGCGGGAATTCTCCTGT--/ / /--GTGCAGGCTGCCACTGGT--/ / /--TTTGATCATCAATTGACC / /--

DmRad51D_2 exon (radD1)
Lys Arg ***Lys*** $_{139}$ Phe Ser Cys Val Gln ***Val*** $_{171}$ AlaThr Gly Phe Asp ***His*** $_{187}$ Gln Leu Thr
--/ / AAGCGGAAATTCTCCTGT--/ / /--GTGCAGGTTGCCACTGGT--/ / /--TTTGATCATCAATTGACC / /--

DmRad51D_2 exon (radD2)
Lys Arg ***Lys*** $_{139}$ Phe Ser Cys Val Gln ***Val*** $_{171}$ Ala Thr Gly Phe Asp ***Tyr*** $_{187}$ Gln Leu Thr
--/ / AAGCGGAAATTCTCCTGT--/ / /--GTGCAGGTTGCCACTGGT--/ / /--TTTGATTATCAATTGACC / /--

DmRad51D_2 exon (radD3)
Lys Arg ***Lys*** $_{139}$ Phe Ser Cys Val Gln ***Val*** $_{171}$ Ala Thr Gly Phe Asp ***Tyr*** $_{187}$ Gln Leu Thr
--/ / AAGCGGAAATTCTCCTGT--/ / /--GTGCAGGTTGCCACTGGT--/ / /--TTTGATTATCAATTGACC / /--

DmRad51D_2 exon (radD4)
Lys Arg ***Lys*** $_{139}$ Phe Ser Cys Val Gln ***Val*** $_{171}$ Ala Thr Gly Phe Asp ***Tyr*** $_{187}$ Gln Leu Thr
--/ / AAGCGGAAATTCTCCTGT--/ / /--GTGCAGGTTGCCACTGGT--/ / /--TTTGATTATCAATTGACC / /--

CG42382

DmCG42382_2 exon (w. t.)
Ser Ala Ser Gln Ala ***Phe*** $_{50}$ Ser Gln Gly
--/ / AGTGCTAGCCAAGCTTTTTCCCAAGGA / /--

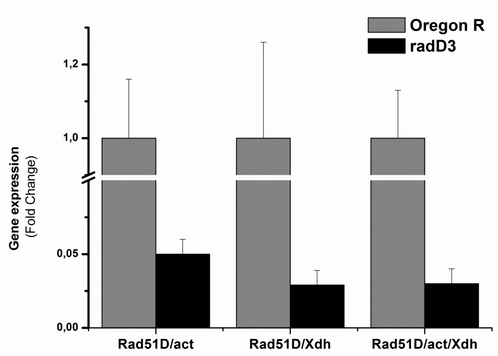
DmCG42382_2 exon (radD1)
Ser Ala Ser Gln Ala ***Leu*** $_{50}$ Ser Gln Gly
--/ / AGTGCTAGCCAAGCTCTTTCCCAAGGA / /--

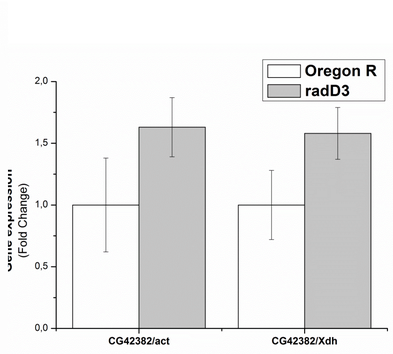
DmCG42382_2 exon (radD2)
Ser Ala Ser Gln Ala ***Leu*** $_{50}$ Ser Gln Gly
--/ / AGTGCTAGCCAAGCTCTTTCCCAAGGA / /--

DmCG42382_2 exon (radD3)
Ser Ala Ser Gln Ala ***Leu*** $_{50}$ Ser Gln Gly
--/ / AGTGCTAGCCAAGCTCTTTCCCAAGGA / /--

DmCG42382_2 exon (radD4)
Ser Ala Ser Gln Ala ***Leu*** $_{50}$ Ser Gln Gly
--/ / AGTGCTAGCCAAGCTCTTTCCCAAGGA / /--

Expression of *Rad51D* and *CG42382 genes in 4-6 hrs embryos*



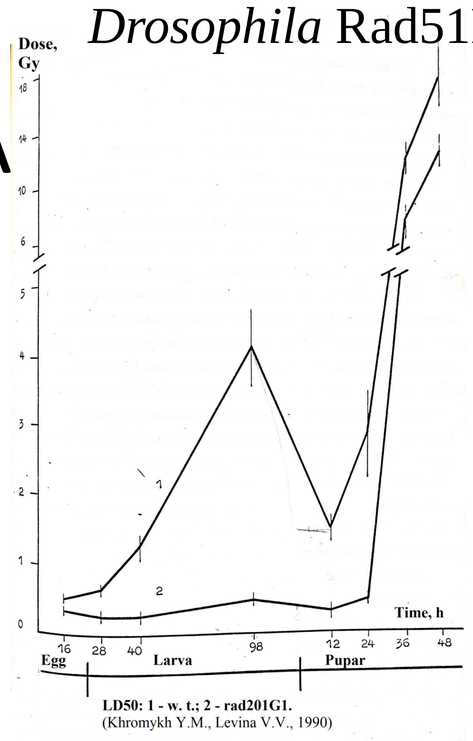


Radiation sensitive Drosophila strain was isolated from a natural Krymean population in 1973 (Sechkina, Zakharow, 1973). The rad201G1 mutation was identified in this strain by the hypersensitivity to ionizing irradiation and it was mapped to 2- 59.9 (Khromykh, Zakharow, 1981). The rad(2)201 locus was mapped to thin region 45B1-3 using deletions isolated as a result of cytogenetic analysis of the chromosome region containing radiosensitivity gene (Konev et al., 1994). Cloning of the DNA from this region of the mutant chromosome had shown that the "rad201[1]" chromosome contains a number of lesions in two different genes of 45B1-3 region (Khromykh, et al., 2008). These are: F50L in the CG42382 annotation, and K61E, V93A and Y108H in the CG2412 annotation (corresponding to the Rad51D gene). In addition, an Opus element is inserted near the Rad51D promoter. Because the mutant allele was isolated from a natural population, it was unknown which of the lesions are neutral and which cause the mutant phenotype.

Here we show that the rad201G1 mutation is a Rad51D allele. We isolated 2 spontaneous reversions of the rad201 radiation sensitivity phenotype. Both reversions are associated with the precise excisions of the Opus transposon, while the nucleotide substitutions in Rad51D and CG42382 genes are dispensable for the phenotype. In the rad201G1 mutant embryos the Rad51D transcription is 30-fold reduced by contrast with the wild type or revertants, while the level of the CG42382 transcription is only slightly changed relatively to the wt strain. Because Rad51D is a very low expressing gene, rad201G1 mutation is an amorph or strong hypomorph allele.

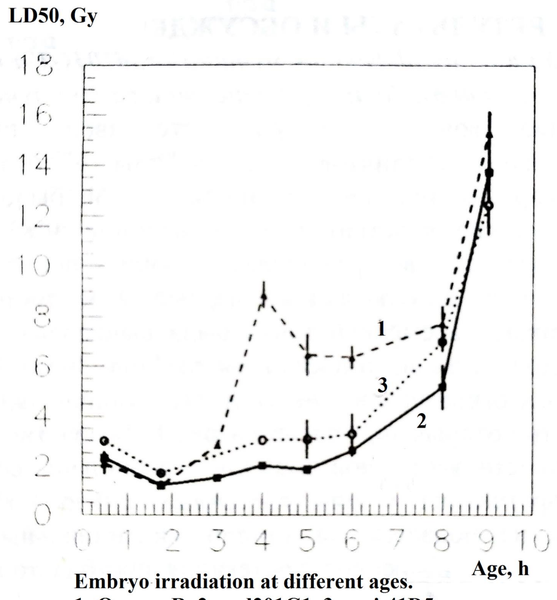
Drosophila Rad51D is required for repair of radiation – induced DNA damages in somatic cells

A



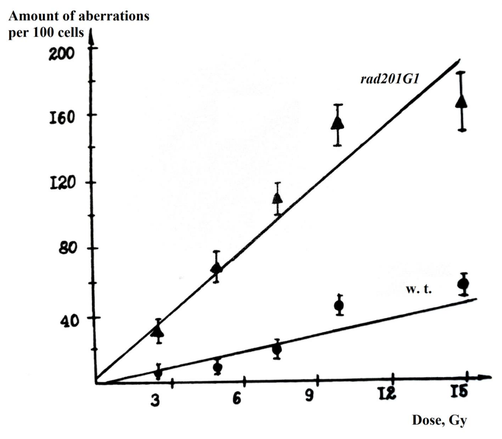
LD50: 1 - w. t.; 2 - rad201G1.
(Khromykh Y.M., Levina V.V., 1990)

B



Embryo irradiation at different ages.
1- Oregon R, 2- rad201G1, 3- mei-41D5.
(Sarantseva S. V. et al, 1999)

C

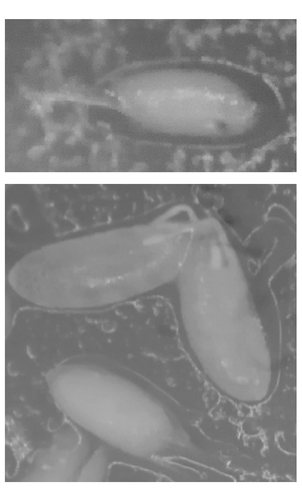


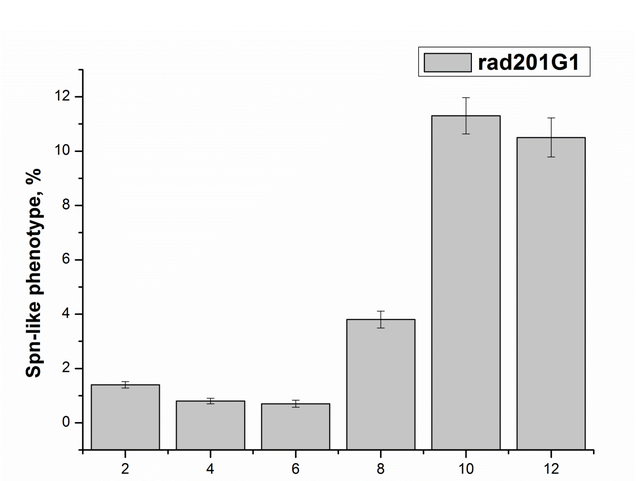
The amount of aberrations as a function of irradiation dose. (Levina V. V. et al, 1992)

Radiation sensitivity of *Rad51D*^{rad201} mutant at different stages of development (A,B) at organismal level.

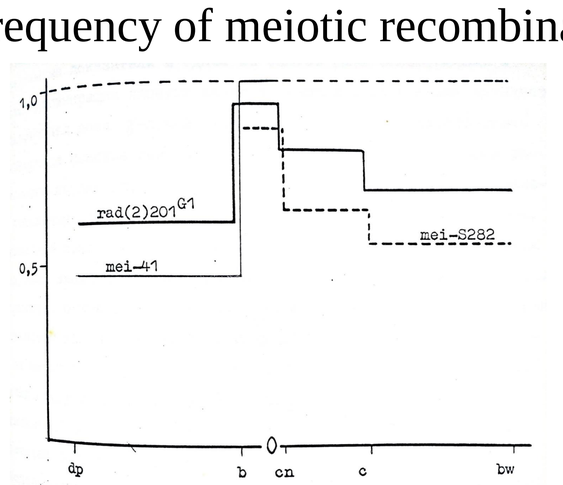
Rad51D^{rad201} mutation has a very mild effect on spontaneous chromosome instability but dramatically increases the frequency of radiation induced chromosome aberrations in larval neuroblasts (C).

Rad51D^{rad201} mutant has a weak *Spn* phenotype



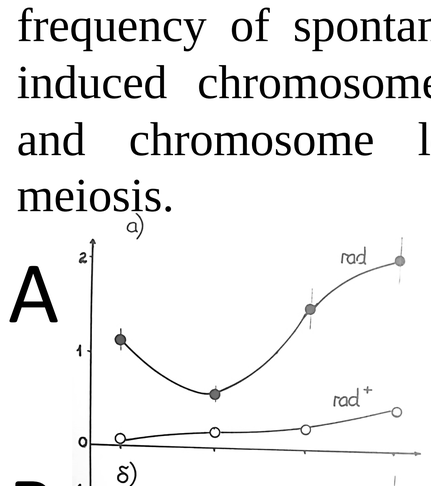


Rad51D^{rad201} mutant decreases the frequency of meiotic recombination



(Khromykh Y. M., 1982)

Rad51D^{rad201} mutant increases the frequency of spontaneous and radiation - induced chromosome nondisjunction (A) and chromosome loss (B) in female meiosis.



(Varentsova E.R, 1982)

Rad51D-spnA interactions

№	Genotype	Abnormal tergites (AT) flies, % ♂	♀	∑AT (∑ flies)
1	*CyO; spnA/TM6B*	13,5	46,0	31,1 (209)
2	*radD2/CyO; TM6B*	1,7	7,4	4,9 (267)
3	*radD2; TM6B*	9,1	44,4	32,8 (67)
4	*radD2/CyO; spnA/TM6B*	47,2	65,9	57,4 (237)
5	*radD2; spnA/TM6B*	37,7	43,9	41,7 (192)
6	*radD2/CyO; spnA*	70	83,9	77,7 (157)
7	*radD2; spnA*	51,6	72,5	63,4 (142)
8	*radD3/CyO; TM6B*	4	30,4	19,3 (119)
9	*radD3; TM6B*	25,5	75,8	53,8 (117)
10	*radD3/CyO; spnA/TM6B*	57,1	88,2	74,2 (217)
11	*radD3; spnA/TM6B*	60,7	70,6	65,4 (214)
12	*radD3; spnA*	59,3	75,5	66,7 (108)

Conclusions

- *Drosophila Rad51D* (*rad201*) is the first genetically characterized Rad51 paralog in higher eukaryotes
- *Rad51D* is not an essential gene in *Drosophila*
- *Rad51D*^{rad201} mutant is very sensitive to ionizing radiation, but not sensitive to MMS
- *Drosophila Rad51D* is required for mitotic DNA damage repair
- *Rad51D* is not strictly essential, but it is important for meiotic DSB repair