

**A Human Genetic Marker for Radioresistance  
and Chemoresistance**

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## Abstract

A human homologue of the fission yeast *Schizosaccharomyces pombe rad9* gene, which mediates radioresistance, chemoresistance and cell cycle checkpoint control, has been identified by searching the dBest data base for sequences similar to the yeast gene, and subsequently screening human cDNA and genomic DNA libraries. The cDNA encodes a 391 amino acid long, 42,520-Da protein that is 25% identical and 52% similar to the *S. pombe rad9* gene product. The human cDNA can rescue to different degrees the sensitivity of *S. pombe rad9::ura4* mutant cells to the DNA synthesis inhibitor hydroxyurea, and gamma-rays, as well as the associated checkpoint controls. UV resistance levels remained unaltered in mutant cells expressing *HRAD9*. Thus, most of the yeast *rad9* gene related characteristics appear to be highly conserved throughout evolution. In addition, the *HRAD9* protein was found to interact physically with p16 as well as p53. These results suggest that *HRAD9* participates in regulating the G1/S phases of the human cell cycle via pathways involving p16 and p53. *HRAD9* resides on human chromosome 11q13.1-13.2, which is a region often found altered in cervical carcinoma samples. Evidence is provided that *HRAD9* is very tightly associated with the gene responsible for this type of cancer. Based on all of these characteristics, *HRAD9* can serve as a genetic marker for determining and perhaps predicting the response of cells to radiations and chemicals.

# Alignment of Rad9 Protein Sequences from Different Organisms

	1				50
Human-Rad9	MKCLVTGGNV	KVLGKAVHSL	SRIGDELYLE	PLEDGLSLRT	VNSSRSAYAC
Mouse-Rad9	MKCLVTGGNV	KVLGKAVHSL	SRIGDELYLE	PLKDGSLRT	VNSSRSAYAC
Octos-Rad9	MEFVVSNTNL	RDLSRIFLNL	SRIDDAVNW	INKDQLILT	LNSSRSRGFK
Pombe-Rad9	MEPTVSNVNL	RDLARIFTNL	SRIDDAVNW	INKNQIETC	LNSSRSRGSF
	51				100
Human-Rad9	FLFAPLFFQQ	YQAATPGQDL	.....LRC	KILMKSFLSV	FR.....
Mouse-Rad9	FLFAPLFFQQ	YQAASPGQDL	.....LRC	KILMGAFSLV	FR.....
Octos-Rad9	VTLTKKFFDK	PTFHPDTLFL	TGFVSPTVRL	STQIKPILSI	FRNKIPSTL
Pombe-Rad9	VTLKKAFFDK	YIFQPDVLL	TGLMPTTIRI	RTQVKPILSV	FRNKIPDFIP
	101				150
Human-Rad9	.....	....SLAMLE	KTVEKCCISL	.NGRSSRLV	QLHCKFQVRK
Mouse-Rad9	.....	....SLAIVE	KSVEKCCISL	.SGSHSLV	QLHCKYGVKK
Octos-Rad9	LVNNNLNTNA	GAAESSKKN	VVVENIQMI	TSGKECRVIF	KFNCKHGVVK
Pombe-Rad9	TVVTNSKNG	YGSESASRKD	VIVENVQISI	STGSECRIF	KFLCKHGVK
	151				200
Human-Rad9	THNLSFDCE	SLQAVFDPAS	CPHMLRAPAR	VLGEAVLPFS	PALAEVTLGI
Mouse-Rad9	THNLSFDCE	SLQAVFDPAS	CPHLLRTPAR	VLAEAVLSFP	LALTEVTLGI
Octos-Rad9	TYKIAYEQTQ	TLHAVFDKAS	CHNNWQINSK	ILKDLIEHFG	QRTEELTIQP
Pombe-Rad9	TYKISYEQTQ	TLHAVFDKSL	SHNNFQINSK	ILKDLTEHFG	QRTEELTIQP
	201				250
Human-Rad9	GRGRRVILRS	YHEE...EAD	STAKAMVTEM	CLGEEDFQOL	QAQEGVAITF
Mouse-Rad9	GRGRRVILRS	YQEE...EAD	STSKAMVTET	SIGDEDFQOL	HAPEGIAVTF
Octos-Rad9	VQG.RVLLTS	FTEEVVHNKD	VLKQPTQTTV	SIDGKEFEQV	SINEGIIITL
Pombe-Rad9	LQE.RVLLTS	FTEEVVHNKD	ILKQPTQTTV	SIDGKEFERV	ALNEGVSVTL
	251				300
Human-Rad9	CLKEFRGLLS	FAESANLNL	IHFDPGRPA	IFTI...KDS	LLDGHFVLAT
Mouse-Rad9	CLKEFRGLLS	FAESANLPLT	IHFDPGRPV	IFTI...EDS	LLDAHFLVAT
Octos-Rad9	SLKEFRAAVL	LAESLGTSLA	SYYSVSGKPA	LFTFNKRGKFM	EIEAQFILAT
Pombe-Rad9	SLREFRAAVI	LALGALSSIC	AYYGVPGKPI	LLTFARGKNS	EIEAQFILAT
	301				350
Human-Rad9	LSDTDSH..S	QDLGSPERHQ	PVPQLQAHST	PHPDFA..N	DDIDSYMIAM
Mouse-Rad9	LLQDQSC..S	QGCPSPKPHQ	PVPQKQAHST	PHLDDFT..S	DDIDCYMIAM
Octos-Rad9	VMGPDFDFES	S.LGARWQQS	GTANSSLLVP	ENTSAAPALE	NEAPSASIGW
Pombe-Rad9	VVGSDEQEVS	SMMGNRWQHS	STPASLNFNSV	ERNNSLTAVA	HNPP.GSIGW
	351				400
Human-Rad9	ETTIGNEGSR	VLPSISLSPG	PQPPKSPGPH	SEEEDEAEPS	TVPGTTPPKK
Mouse-Rad9	ETTGGNEGSG	AQPSTSLPPV	SLASHDLAPT	SEE..EAEPS	TVPGTTPPKK
Octos-Rad9	QTNGDAETSR	MFHSTLDIPR	NEEPAAKPSR	QTTDEENHPL	FLEGMPEDETE
Pombe-Rad9	QT.DQSDSR	MPNSALD..R	SDETNGIKEP	STTNDAGQSL	FLDGIPNESE
	401			435	
Human-Rad9	FRSLFF....	GSILAPVRSR	QGSPVLAED	SEGG	
Mouse-Rad9	FRSLFF....	GSILAPVHSP	QGNPVLAED	SDGEG	
Octos-Rad9	LMAFDNDVAD	DAEPGPTQHE	QTYHGIFSQD	DTET.	
Pombe-Rad9	LAAFNNVDND	DAEPGPTQAE	QSYHGIFSQE	DZ...	

## Comparison of the RAD9 protein sequences from humans, mice and the yeasts *S. pombe* and *S. octosporus*.

The *rad9* genes from humans, mice and the yeasts *S. pombe* and *S. octosporus* have been isolated. The human gene encodes a 391 amino acid long, 42,520-Da protein. It is 82% identical and 88% similar to the mouse protein, 25% identical and 52% similar to *S. pombe rad9*, and 27% identical and 54% similar to the *S. octosporus* protein. Sequences were aligned using the Genetics Computer Group (Madison, WI) programs PILEUP and PRETTY/CONSENSUS. The yellow highlights indicate amino acid positions completely conserved throughout evolution.

### References:

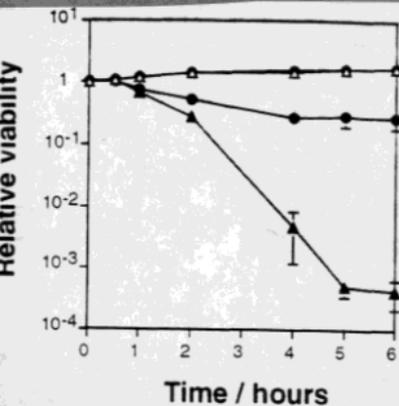
Lieberman, H.B., K.M. Hopkins, M. Laverty, and H.M. Chu. (1992) Molecular cloning and analysis of *Schizosaccharomyces pombe rad9*, a gene involved in DNA repair and mutagenesis. *Mol. Gen. Genet.* 232:367-376.

Lieberman, H. B., and K. M. Hopkins. (1994) *Schizosaccharomyces malidevorans* and *Sz. octosporus* homologues of *Sz. pombe rad9*, a gene that mediates radioresistance and cell cycle progression. *Gene* 150:281-286.

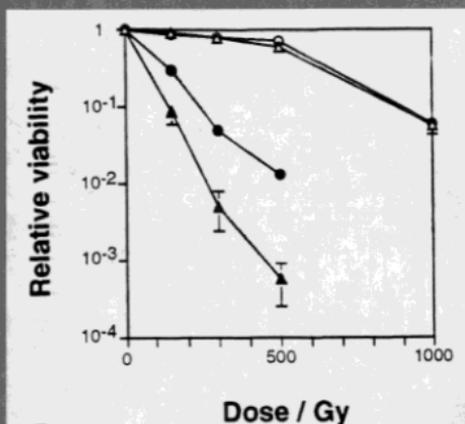
Lieberman, H.B., K.M. Hopkins, M. Nass, D. Demetrick, and S. Davey. (1996) A human homologue of the *Schizosaccharomyces pombe rad9<sup>+</sup>* checkpoint control gene. *Proc. Natl. Acad. Sci. USA* 93:13890-13895.

Hang, H., S.J. Rauth, K.M. Hopkins, S. Davey, and H. B. Lieberman. (1998) Molecular cloning and tissue-specific expression of *Mrad9*, a murine orthologue of the *Schizosaccharomyces pombe rad9<sup>+</sup>* checkpoint control gene. *J. Cell. Physiol.* (In press).

# HRAD9 function in *S. pombe*

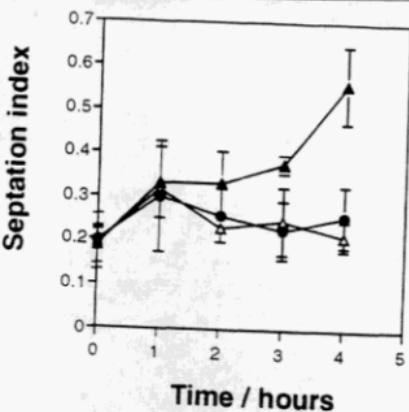


HU Sensitivity

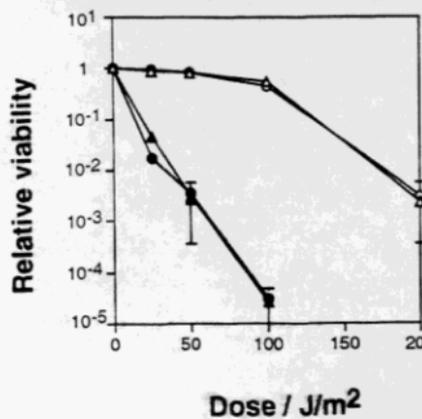


Gamma-Ray Resistance

*rad9*<sup>+</sup> Δ  
*rad9*<sup>+</sup> [pHRAD9] ○  
*rad9::ura4* ▲  
*rad9::ura4* [pHRAD9] ●



HU-Induced Changes in Septation Index

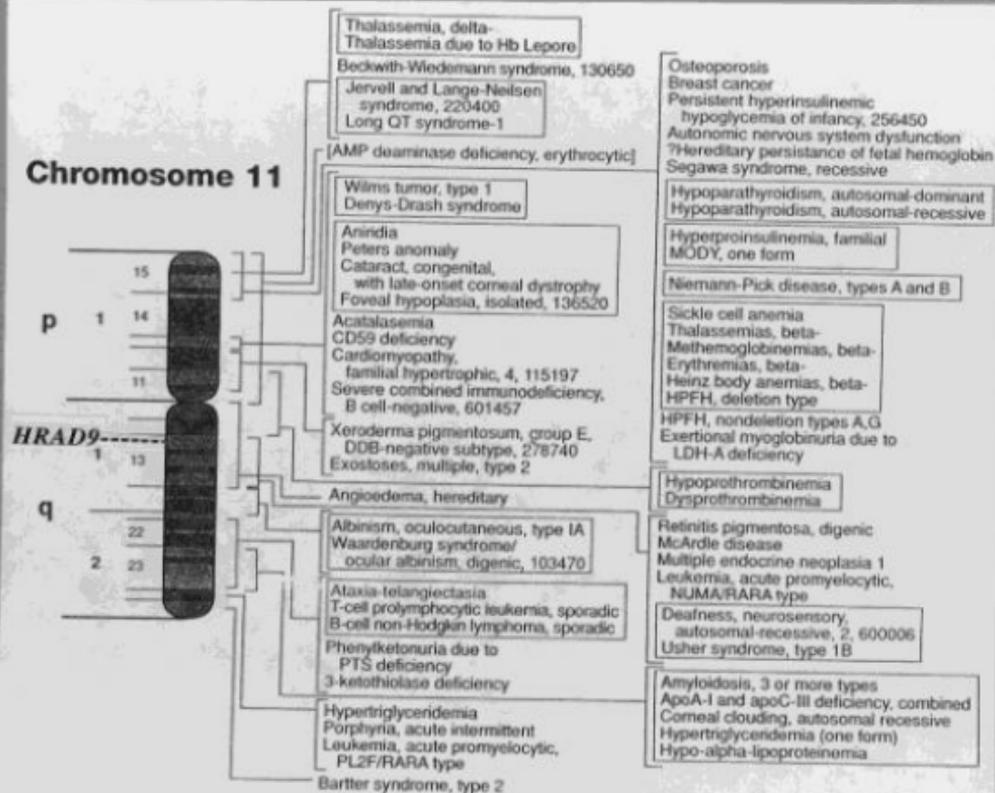


UV Resistance

## Resistance of *S. pombe* cells to hydroxyurea (HU), gamma-rays and UV light, and HU-induced cell cycle delay.

pHRAD9, a plasmid containing the *HRAD9* cDNA in the *S. pombe* expression vector pART1, confers nearly wild-type levels of HU resistance, and moderate gamma-ray resistance to yeast *rad9::ura4* cells. In addition, the plasmid permits mutant cells to delay entering mitosis after HU treatment (i.e., it restores HU-induced checkpoint control), and confers a partial delay on mutant cells after exposure to gamma-rays (data not shown). pHRAD9 did not confer UV resistance upon mutant cells, nor did it have any detectable effect on the resistance of *rad9+* cells to these agents. Therefore, *rad9* gene function is at least partially conserved from yeasts to humans.

## Chromosome 11



***HRAD9* is located on human chromosome 11q13.1-13.2.**

The map location of *HRAD9* was determined by performing PCR experiments on a panel of human X rodent somatic cell hybrid DNAs using *HRAD9* specific primers, and by fluorescence in situ hybridization using a fragment of the corresponding human genome as a probe.

## SSCP Analysis of *HRAD9*

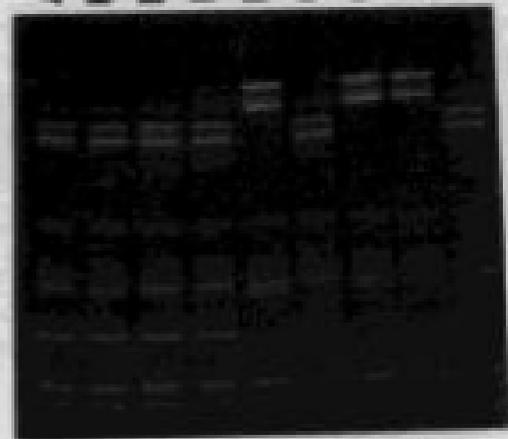


— 0.1 kb



## SSCP for Fragment B

AG1522  
HTB31  
HTB32  
HTB34  
HTB35  
CRL-1550  
CRL-1594  
HeLa  
CRL-7920

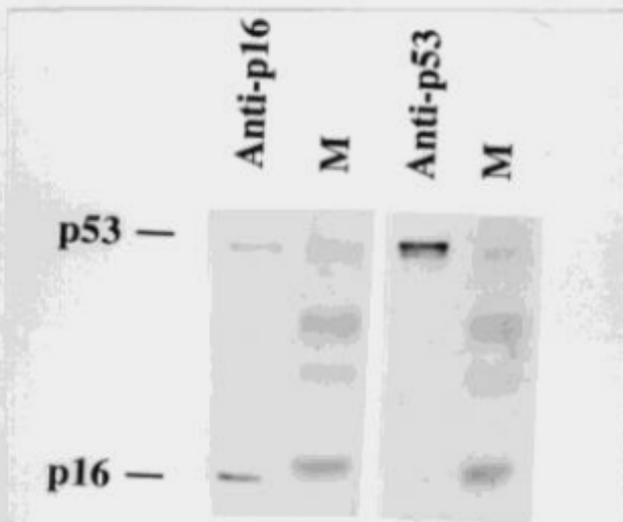


### **A polymorphism within *HRAD9* associated with cervical carcinoma.**

The status of *HRAD9* in cervical carcinoma cells and tissue biopsies was examined because the gene is located in a region of chromosome 11 found by other investigators to often be rearranged in this type of cancer cell. Three techniques were used together to address this issue. 1. RT-PCR, using RNA from normal and cervical carcinoma samples, in conjunction with primers that flank three overlapping regions of the *HRAD9* cDNA, was used to make a cDNA copy of *HRAD9* RNA, and subsequently amplify three regions of the cDNA (labeled A, B, C above); 2. SSCP, where the amplified fragments of DNA were digested with restriction enzymes and examined for alterations (especially of interest is fragment B, which was digested with *AvaII*); and 3. DNA sequence analysis was used to identify the base pair change(s) suggested by positive results from the SSCP studies.

**Results:** As illustrated in the gel above, AG1522 represents the normal SSCP profile for fragment B of *HRAD9*. Of the other 8 cervical carcinoma cell populations examined and shown, 3 had an altered profile for this fragment. These fragments were subcloned and their DNA sequence was determined. All 3 had the same polymorphism, a T to C transition in codon 338 (GGT to GGC), which did not alter the encoded glycine amino acid. Additional studies indicated that the polymorphism was absent in two other noncancer cell lines and in blood samples from 16 patients with different cancers. Paraffin-embedded tissues were also examined for this base-pair alteration. The identical polymorphism was found in 2 of 2 cases examined that involve cervical cancer tissue, but absent in 15 other normal sample controls. Since the base-pair change does not affect the encoded amino acid-i.e., glycine remains as glycine in codon 338, these results suggest that *HRAD9* is not the cervical carcinoma gene but is closely associated with it on chromosome 11. An unlikely but still viable alternative is that the polymorphism changes the level of expression of *HRAD9* through an alteration in methylation, which is known to often modulate transcription levels. This possibility would potentially make the function of *HRAD9* more directly related to cervical carcinoma, and this hypothesis is currently being tested.

## HRAD9 Protein Interacts Physically with p16 and p53



Western blot analysis indicating the physical interactions between HRAD9 and p16 or p53. A plasmid encoding a FLAG-HRAD9 fusion protein was transiently expressed in human fetal kidney 293T cells, which were harvested and immunoprecipitated with anti-FLAG antibodies. Extracts were then analyzed by western blotting for the presence of other proteins. The blot to the left was probed with anti-p16 antibodies, and the one on the right with anti-p53 antibodies. M labels marker lanes. Confirming results were obtained when anti-HRAD9 antibodies, instead of those against FLAG, were used as the initial precipitating agent. Furthermore, when the precipitations were carried out with either anti-p16 or anti-p53 antibodies, and subsequently analyzed by western blotting with anti-FLAG or HRAD9 antibodies, FLAG-HRAD9 could be visualized, indicating that the reverse co-immunoprecipitation was also successful. Interestingly, HRAD9 appeared as three large bands during this analysis, as well as in HRAD9 overproducing cells, suggesting that the protein is alternatively processed after translation (data not shown).

## Conclusions:

A human structural homologue of the fission yeast *S. pombe rad9* gene has been isolated. The yeast gene is a key player in promoting radioresistance, chemoresistance and regulating early S phase and G2/M checkpoint controls. Preliminary characterization of *HRAD9*, the human cognate, suggests that it has retained many of the same functions through evolution. Studies are underway to further characterize the function of *HRAD9* in the context of human cells, and to generate a mouse *Mrad9* knockout model, to develop the gene as a tool for predicting responses to radiation or chemical exposure.