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Function of the Yeast Rad57 Protein Depends on a Conserved Lipid-Binding Motif

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ABSTRACT: The Rad57 protein of the yeast *Saccharomyces cerevisiae* has been extensively studied and is known to have multiple functions critical for vegetative growth and meiosis. Here we present evidence suggesting that these functions are regulated by lipid binding. This binding likely occurs via a GW…W motif conserved in the phosphatidylinositol kinase (PIK) protein family and in a meiosis-specific yeast protein Spo73. Earlier studies indicate these proteins respond to and are regulated by phosphatidylinositol phosphate (PIP) or phosphoinositide signaling molecules. Specific mutations in the GWLVGW motif of Rad57 abolish the ability of lipid binding, and significantly impair Rad57 functions essential for vegetative growth, meiotic recombination and spore germination. These results imply the multiple roles of Rad57 are governed via lipid signaling, and can be modulated by direct binding of specific lipid second messengers to a specific motif in Rad57p. *© 2007 Bull. Georg. Natl. Acad. Sci.*

Key words: *lipid-binding, phospatidylinositol phosphates, Rad57, yeast, spore viability*

INTRODUCTION. Lipid signaling is considered one of the most potent and diverse mechanisms regulating various important cellular processes, such as protein traffic and re-localization, exo- and endocytic vesicle formation, actin nucleation and cytoskeletal assembly. Phosphatidylinositol (PI) and its phosphorylated derivatives (PIP), which act as signaling molecules, have several characteristics which make them ideal for this function: *i*) their low cellular abundance compared to other lipids (e.g., phosphatidylserine, phosphatidylcholine); *ii)* multiple sites of phosphorylation within the inositol ring, providing the ability to generate wide variety of differentially phosphorylated derivatives from a single progenitor (PI); *iii)* rapid interconversion between various PIPs due to activities of multiple lipid kinases and phosphatases [1]. Regulation by lipid signaling often includes modular protein domains that bind specific PIP-molecules [2]. In addition to well-characterized domains (FYVE, PH, PX, C2), there are indications that several (less obvious) motifs can bind lipids as cofactors required for enhancement of their biochemical activity and/or for maintaining protein stability. Lipid-binding may also occur as a secondary function within a domain with a previously known distinct role [3, 4].

In our previous studies, we identified a motif (GWC..W), shared by the PIK-related kinases and a mid-late meiosis-specific protein Spo73 (Tevzadze *et al.*, in preparation). This motif is the only common fragment for Spo73p and TOR-kinases, which are quite disparate in size (14 kDa for Spo73, >200 kDa for TORs) as well as in function: Spo73 is involved in a specific step of spore wall formation, whereas TOR kinases (components of the TORC1 and TORC2 complexes) control wide variety of cellular functions, e.g., translation, transcription, autophagy, and nutrient transport [5-8]. TORs are lipid-kinase homologs, but have only protein kinase activity [9]. Spo73 activity is dependent on a putative phospholipase Spo1, which is thought to produce Lyso-PI second messengers [10-13]. These observations led us to suggest that the motif acts as a sensor of lipid signals, i.e., is involved in direct binding to second messenger molecules produced by upstream activators. Indeed, the GWC..W motif was shown to be critical for protein function, as well as for binding specific lipids both in Spo73 and TOR kinases (Tevzadze *et al.*, in preparation). The GW pair is conserved in the PIK-kinase protein family

and is critical for binding. For Spo73, the presence of either residue is important for meiotic function of the protein and its PIP-binding abilities.

The importance of the GW pair prompted us to examine the distribution of this motif or its variants throughout the yeast genome. In this paper, identification of GWlvGW in the Rad57 protein is reported and its importance demonstrated in PIP-binding and Rad57p function during vegetative growth and meiosis.

MATERIALS AND METHODS

Strains: *E. coli* DH5α strain was used for the maintenance and propagation of plasmids, and BL21(DE3) was employed for expression of the Rad57 protein fragments from an IPTG-inducible promoter in the pGEV2 plasmid ([14]; see also below). The *S. cerevisiae* strains used in this study are listed in Table 1.

Plasmids: *Plasmids for bacterial expression of Rad57p internal fragments*. An internal ~100 bp *RAD57*-fragment containing the sequence encoding the ^{298}GWLVGW^{303} domain was PCR-amplified from the yeast genomic DNA. The *Bam*HI and *Xho*I sites, engineered at the 5' and 3' termini, respectively, were used for cloning into the pGEV2 vector. The pPW24C plasmid contains this fragment from *RAD57* (bases 811-909) fused by its N-terminus to the IgG-binding domain of Protein G; it encodes residues 271-303 (the motif is at the C-terminus). Two derivatives of pPW24C contain mutations which alter the ^{298}GWLVGW^{303} motif to ^{298}AWLVAW^{303} (pPW29) or to ^{298}GALVGA^{303} (pPW30) in the otherwise identical fragments encoding residues 271-303.

Plasmids for integration of the rad57 *mutant alleles:* For introduction of the mutant ^{298}AWLVAW^{303} or ^{298}GALVGA^{303} alleles, a 2 kb *Sac*I fragment (containing

Table 1

Yeast Strains Used in this Study

Strain	Relevant Genotype	Source
W303-1A	*MAT***a** *ade2 can1-100r his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein*
W303-1B	*MATα ade2 can1-100r his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein*
GTY484	W303-1A *RAD57:URA3:rad57(5'Δ)*	This study
GTY485	W303-1B *RAD57:URA3:rad57(5'Δ)*	This study
GTY486	W301-1A *rad57-g,g:URA3:rad57(5'Δ)*	This study
GTY487	W303-1B *rad57-g,g:URA3:rad57(5'Δ)*	This study
GTY490	W301-1A *rad57-w,w:URA3:rad57(5'Δ)*	This study
GTY491	W303-1B *rad57-w,w:URA3:rad57(5'Δ)*	This study
GTY494	W303-1A *rad57Δ:TRP1*	This study
GTY495	W303-1B *rad57Δ:TRP1*	This study
GTY502	W303-1A *rad57Δ:KanMX6*	This study
GTY503	W303-1B *rad57Δ:KanMX6*	This study
SK1-A	*MAT***a** *arg4 his4X::LEU2 ho::LYS2 leu2::hisG lys2 ura3*	D. Bishop**
SK1-B	*MATα arg4 his4B::LEU2 ho::LYS2 leu2::hisG lys2 ura3*	D. Bishop**
GTY508	SK1-A *RAD57:URA3:rad57(5'Δ)*	This study
GTY509	SK1-B *RAD57:URA3:rad57(5'Δ)*	This study
GTY512	SK1-A *rad57-g,g:URA3:rad57(5'Δ)*	This study
GTY513	SK1-B *rad57-g,g:URA3:rad57(5'Δ)*	This study
GTY516	SK1-A *rad57-w,w:URA3:rad57(5'Δ)*	This study
GTY517	SK1-B *rad57-w,w:URA3:rad57(5'Δ)*	This study
GTY520	SK1-A *rad57Δ:KanMX6*	This study
GTY521	SK1-B *rad57Δ:KanMX6*	This study

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1 kb of the 3' region of the *RAD57* ORF and 1 kb of the 3' UTR) was PCR-amplified from yeast genomic DNA and cloned into pRS306, a *URA3*-marked integrative vector [15]. The G298A G302A ("g, g") and W299A W303A ("w, w") mutations were introduced by the recombinant PCR method [16], and the amplified fragments were sequenced throughout their length to confirm that no additional mutations were generated due to the PCR errors. To introduce the resulting pPW35 (pRS306-*RAD57*), pPW36 (pRS306-*rad57-g,g*) and pPW39 (pRS306-*rad57-w,w*) plasmids into the yeast genome, they were linearized by the *Bgl*II restriction endonuclease, which has a unique recognition site within the *RAD57* ORF, ~200 bp upstream of the fragments encoding the GWLVGW wild-type or mutant motifs. Integration of these plasmids into the *RAD57* locus created an insertion of the *URA3* marker, flanked by a full-size *Rad57* (wild-type or mutant) allele on one side and a 5' deletion allele of *RAD57* (lacking the 5'UTR and ~250 bp of the 5' ORF) on the other. Proper integration was confirmed by PCR, sequencing and by mating with *rad57Δ* strains (the deletions marked with *TRP1* or *KanMX6* for W303, and *KanMX6* for SK1). Sporulation and tetrad analysis of the heterozygous *rad57Δ* diploids demonstrated segregation of *URA3* opposite to the *rad57Δ*-markers.

Media, Growth, and Sporulation: *E. coli* growth media (LB and SOC) were described previously [17]. The yeast media YPDA, YPA, Synthetic Complete (SC) for growth, and SPII and SPIII for sporulation were described previously [18, 19]. Isolation of genomic DNA for Southern blot analysis was performed as described elsewhere [20]. Transformation of yeast cells was performed by high-efficiency lithium acetate protocol [21, 22]. RbCl-mediated transformation of *E. coli* strain DH5a was performed as described earlier [12, 23]. Sporulation of the SK1 background strains was done as described [24], heteroallelic recombination frequency was assayed based on the number of His+ colonies forming as a result of recombination between the *his4-X* and *his4-B* heteroalleles [24, 25].

Assay of γ-Radiation Sensitivity: The W303 background haploid strains were grown overnight in 5 ml YPDA at 30°C with aeration and diluted to OD600=1.0 in the same volume and media. Cultures were treated with 800 Gy (at 0.5 Gy/sec) in a GammaCell 220 industrial irradiator equipped with the cobalt-60 source (Nordion). After irradiation, cultures were serially diluted in YPDA and 2.5 μl of 10^0 to 10^{-5} dilutions were spotted on YPDA plates together with untreated controls. Plates were incubated at 30°C and 23°C, since the *rad57* null mutants are ts regarding their sensitivity to radiation. While radiation sensitivity for the control *rad57Δ::TRP1* deletion strains was evident at both temperatures, incubation at 23°C did give the most dramatic differences between the isogenic *RAD57* and *rad57Δ* strains.

Lipid-Binding Assays using PIP-strips (Echelon Biosciences Inc., Salt Lake City, Utah, USA) were done as suggested by manufacturer (www.echelon-inc.com), with several modifications. The fragments of Rad57 alleles expressed in the BL21(DE3) bacterial strain (see above) were diluted to 0.5 μg/ml in TBST (100 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.1% Tween 20) containing 0.1% ovalbumin (Sigma) and incubated with PIP-strips or arrays for 1 h at room temperature. The strips were then washed 3 times for 15 min in TBST + 0.1% ovalbumin and incubated again for 1 h at room temperature with horseradish peroxidase-conjugated anti-Protein G polyclonal antibody raised in rabbit (1:10,000 dilution, AbCam). Strips were washed as before and activity of HRP bound to the filter was assayed by ECL (Amersham). Signal intensities for individual lipid spots were calculated using the NIH Image 1.62 software and normalized to the control spots containing no lipids.

RESULTS AND DISCUSSION

Search for the *S. cerevisiae* Proteins Containing the GW…W Motif: The search was done using the Pattern Matching (PatMatch) algorithm provided by the Stanford Genome Database (http://db.yeastgenome.org/cgi-bin/ PATMATCH/nph-patmatch). PatMatch allows to search for the presence of short (<20 residues) peptide sequences in the yeast ORFs. The GW…W motif was found in 33 proteins (Table 2) among the 5869 sequences (i.e., the entire *S. cerevisiae* genome) searched. The more restricted searches specified smaller sets, e.g., 3 hits for GWC..W (Tor1, Tor2, Spo73); 4 hits for GW.Y.W (Ygl262w, Mep1, Mep3, Spo73) and GW..SW (Sup35, Ypl162c, Eaf3, Spo73) each. These variations were chosen since they are present in the Spo73 sequence (GWCSYW), where this motif was initially identified. Finally, a duplication of the GW pair, separated by two other residues (GW..GW), was identified in only two yeast proteins: GWlvGW for Rad57p (residues 298-303 in the 460 aa protein) and GWriGW for Bna3p (278-282, 444 aa).

The *BNA3* locus encodes arylformamidase, involved in biosynthesis of nicotinic acid from tryptophan via kynurenine pathway [26]. Studies of this protein were considered beyond the scope of this paper (and our expertise). Since the presence of the GW..GW motif in Rad57 raised an intriguing possibility of meiotic recombination being regulated by lipid signaling, here we concentrated on the importance of the GWlvGW motif in Rad57 function.

The *RAD57* gene encodes a 52 kDa-protein that stimulates strand exchange by stabilizing Rad51p binding to single-stranded DNA via forming a heterodimer with Rad55 [27]; it is involved in the recombinational repair of double-strand breaks in DNA during vegetative growth [28] and meiosis [29], and has a minor role

Table 2

Proteins with the GW…W Motif in the *S. cerevisiae* Genome

Locus/Gene*	Motif	Starts	Ends**	Locus Information (*Saccharomyces* Genome Database Annotation)
YAL048C/GEM1	GWLAQW	390	395	Mitochondrial membrane GTPase, regulating mitochondrial morphology
YBR021W/FUR4	GWFQRW	592	597	Uracil permease, localized to the plasma membrane
YBR136W/MEC1	**GWLPFW**	**2363**	**2368**	Checkpoint protein and PI kinase superfamily member; monitors and participates in meiotic recombination
YBR155W/CNS1	GWISKW	369	374	TPR-containing co-chaperone
YCR026C/NPP1	GWRSAW	36	41	Hypothetical protein
YCR101C	GWGVVW	53	58	Hypothetical protein
YDR004W/RAD57	**GWLVGW**	**298**	**303**	Protein that stabilizes the binding of Rad51p to single-stranded DNA; forms heterodimer with Rad55p
YDR093W/DNF2	GWTGIW	1394	1399	Non-essential P-type ATPase, localizes to the plasma membrane, late exocytic or early endocytic membranes
YDR172W/SUP35	GWYLSW	306	311	Translation termination factor eRF3; altered protein conformation creates the [PSI(+)] prion
YDR234W/LYS4	GWFLKW	640	645	Homoaconitase, catalyzes a step in the lysine biosynthesis pathway
YDR335W/MSN5	GWFSVW	219	224	Karyopherin involved in nuclear import and export; cargo dissociation involves binding to RanGTP
YER046W/SPO73	**GWCYSW**	**108**	**113**	Meiosis-specific protein, required for spore wall formation but dispensible for both nuclear divisions
YGL262W	GWKYYW	82	87	Hypothetical protein
YGR121C/MEP1	GWAYQW	164	169	Ammonium permease
YIR028W/DAL4	GWFQRW	594	599	Allantoin permease
YJL016W	GWVKVW	205	210	Cytoplasmic protein of unknown function
YJL060W/BNA3	GWRIGW	277	282	Arylformamidase, involved in biosynthesis of nicotinic acid from tryptophan via kynurenine pathway
YJL133W/MRS3	GWKGFW	278	283	Mitochondrial iron transporter of the mitochondrial carrier family (MCF), functionally redundant with Mrs4p
YJR066W/TOR1	**GWCPFW**	**2465**	**2470**	PIK-related protein kinase and rapamycin target; subunit of TORC1
YKL203C/TOR2	**GWCPFW**	**2469**	**2474**	PIK-related protein kinase and rapamycin target; subunit of TORC1 and TORC2
YKR052C/MRS4	GWKGFW	268	273	Mitochondrial iron transporter of the mitochondrial carrier family (MCF), functionally redundant with Mrs3p
YLR088W/GAA1	GWLPCW	587	592	Subunit of the GPI-protein transamidase complex, removes the GPI-signal, attaches GPI to proteins in the ER
YLR195C/NMT1	GWKKDW	128	133	N-myristoyl transferase, acts on several proteins involved in cellular growth and signal transduction
YMR078C/CTF18	GWLRQW	140	145	Subunit of a complex with Ctf8; is required for sister chromatid cohesion and the DNA damage checkpoint
YMR266W/RSN1	GWIFFW	157	162	Membrane protein of unknown function

Table 2 (continued)

Locus/Gene*	Motif	Starts	Ends**	Locus Information (*Saccharomyces* Genome Database Annotation)
YNL172W/APC1	GWPDLW	663	668	Largest subunit of the Anaphase-Promoting Complex/Cyclosome (APC/C)
YOR376W-A	GWTILW	6	11	Identified by fungal homology and RT-PCR
YPL162C	GWSDSW	191	196	Hypothetical protein
YPL257W	GWFLIW	75	80	Hypothetical protein
YPR023C/EAF3	GWKSSW	83	88	Esa1p-associated factor, nonessential component of the NuA4 acetyltransferase complex
YPR127W	GWVKHW	281	286	Hypothetical protein
YPR138C/MEP3	GWAYQW	163	168	Ammonium permease of high capacity and low affinity
YPR193C/HPA2	GWQRLW	21	26	Tetrameric histone acetyltransferase; acetylates histones H3 and H4 *in vitro*, exhibits autoacetylation activity

* Proteins relevant for the present and related studies (Rad57, Spo73, PIK-kinase family proteins) are shown **in bold**
** Amino acid residue coordinates where the motif **Starts** and **Ends** are shown for each protein.

Note: several other PIK-kinase protein family members contain the GW.PF. variant of the motif: Tel1 (GWSPFY, 2782-2787), Mec1 (GWLPFW, 2363-2368), Sch9 (GWSPFF, 608-613), Tor1 (GWCPFW, 2465-2470), Tor2 (GWCPFW, 2469-2474). The Cbk1 serine-threonine protein kinase contains GWPPFC (607-612).

in mating efficiency [30]. Rad57 participation in the assembly of recombinational complexes was also demonstrated by immunocytological methods [30]. Null alleles of *rad57* exhibit cold-sensitive phenotype, consistent with the role of Rad57 in stabilization of the DSB repair/recombination complexes [31]. This notion is further supported by the fact that *rad57* mutant phenotype can be suppressed by high-copy *RAD51* [32] or by specific *rad51* mutant alleles with the enhanced abilities of DNA-binding [33]. Most likely, suppression occurs due to the increased stabilization of Rad51-binding to DNA, thus rendering Rad57 function obsolete. Cold-sensitive *rad57* alleles have decreased fidelity of DSB repair, as demonstrated by double-strand gap (DSG) repair studies: significant portion of plasmid-borne DSGs are not restored accurately, indicating the mechanism of re-circularization of a "DSG-linearized" plasmid is ligation of cohesive ends, rather than recombinational repair [34]. Cold-sensitivity is also evident in the enhanced sensitivity of *rad57* mutants towards ionizing radiation (IR), with more dramatic phenotype at 23°C compared to 30°C. Furthermore, *rad57* haploids exhibit higher IR-sensitivity compared to isogenic diploids [35].

Based on our recent findings that the GW…W motif is important for PIP-binding of Spo73 and the PIK-related kinase protein family (Tevzadze *et al.*, in preparation), we proposed that Rad57 also binds specific phosphatidylinositols and binding ability is defined by the presence of the GW...GW domain. This paper reports that: 1) Rad57 binds specific phoshatidylinositol phosphates (PIPs), but not unphosphorylated PI; 2) The strongest binding is detected for PI(3,4)P_2 and PI(3,4,5)P_3; 3) mutations in the GW..GW domain reduce (Gly/Ala) or abolish (Trp/Ala) binding; 4) The Trp/Ala (i.e., the motif GALVGA), but not Gly/Ala (AWLVAW) mutations, generate loss-of-function alleles. Loss of function was demonstrated both in vegetative growth and sporulation by assaying γ-radiation sensitivity, spore viability and meiotic recombination efficiency.

A Fragment of Rad57 with the C-Terminal GWlvGW Motif Binds PIPs: In previous studies [12], our approach has been to test lipid-binding abilities of a protein by expressing its relatively small fragment (encoded by ~100-110 bp) as a fusion with Protein G in a bacterial system [14, 36], purify the expressed protein and examine its binding to various groups of lipids spotted on a nitrocellulose filter. This approach allows to relatively quickly test lipid-binding and, due to a small size of the fragment, does not generally create solubility problems. To further employ this strategy, lipid-binding of Rad57 was examined for a 33 aa fragment (residues 271-303) containing GWlvGW at its extreme C-terminus. This fragment exhibits strong binding to PIPs with a significant preference towards PI(3,4)P_2 and PI(3,4,5)P_3 (Fig. 1, left panel).

Next, we inquired whether introducing mutations in the GW..GW residues will alter lipid-binding abilities of Rad57. Replacing both glycines to alanines caused a significant reduction in PIP-binding (Fig. 1, central panel). The effect of replacing the tryptophans to alanines was even more dramatic, completely abolishing lipid-binding for *rad57-w,w* (Fig. 1, right panel).

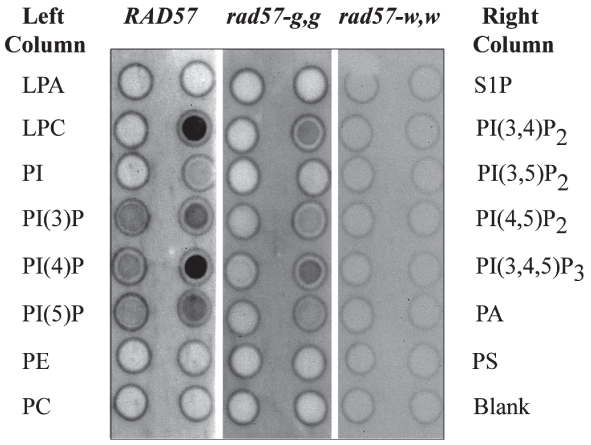

Fig. 1. Typical results of lipid-binding overlay assays, using PIP-strips (Echelon Biosciences Inc.), for the Rad57 fragments containing the wild-type motif GWLVGW (*RAD57*, left panel) or mutated AWLVAW (*rad57-g,g*; central panel) and GALVGA (*rad57-w,w*; right panel) motifs. The strips were successively incubated first with the fusion proteins purified on IgG sepharose columns (Amersham) and then with anti-Protein G antibody conjugated with horseradish peroxidase (AbCam) to detect the protein bound to lipid spots. The PIP-strips contain 100 pmol spots of 15 different lipids and a control blank spot. The positions of test lipids on the left and right sides of the strips are shown on both sides of the test samples: LPA, Lysophosphatidic acid; LPC, Lysophosphocholine; PI, phosphatidylinositol (unphosphorylated inositol ring); PI(3)P, phosphatidylinositol-3-monophosphate; PI(4)P, phosphatidylinositol-4-monophosphate; PI(5)P, phosphatidylinositol-5-monophosphate; PE, phosphatidylethanolamine; PC, phosphatidyl-choline; S1P, sphingosine-1-phosphate; $PI(3,4)P_2$, phosphatidylinositol-3,4-biphosphate; $PI(3,5)P_2$, phosphatidylinositol-3,5-biphosphate; $PI(4,5)P_2$, phosphatidylinositol-4,5-biphosphate; $PI(3,4,5)P_3$, phosphatidylinositol-3,4,5-triphosphate; PA, phosphatidic acid; PS, phosphatidylserine; the lowest spot on the right does not contain any lipids.

One possible explanation of the mutant alleles' inability to bind PIPs is a low stability of the mutated proteins. However, the abundance and stability of bacterially expressed wild-type and mutant proteins were comparable (monitored on PAGE gels by Coomassie staining, data not shown), indicating that decreased (for "g,g") or abolished (for "w,w") ability to bind PIPs genuinely reflects a change in Rad57 function(s), rather than low levels of the mutant proteins.

Thus, the GWlvGW motif in Rad57 is important for binding the PIP-molecules, which is indicative of regulation via lipid signaling. To further explore this possibility, we inquired how the Gly/Ala and Trp/Ala mutations within GWlvGW affect the diverse functions of Rad57p.

The Tryptophans in the GWlvGW Motif Are Also Essential for Rad57 Function. The effect of both mutants on Rad57 function was examined using two approaches: first, their sensitivity to IR was assayed in vegetatively growing haploid strains and compared to wild-type and deletion mutants. Haploid strains were chosen since they are more sensitive to IR than isogenic diploids [35]. Second, the effect on sporulation was monitored by determining spore viability (in the W303 and SK1 backgrounds) and heteroallelic recombination at the *his4-X/his4-B* hot-spot in SK1 [25].

Tests of radiation sensitivity clearly show that the *rad57-w,w* allele renders the cells IR-sensitive at levels similar to the deletion allele (Fig. 2, compare row 5 to rows 7 and 8), whereas the *rad57-g,g* allele is almost identical to wild-type (Fig. 2, compare rows 3 and 4).

Meiotic recombination in strains carrying the *rad57-w,w* alleles is impaired as well: while the frequency of His+ colonies (forming as a result of recombination between the *his4-X* and *his4-B* heteroalleles) is 200-500 per 10^4 viable cells for wild-type and *rad57-g,g* strains, the *rad57-w,w* diploids accumulate only 40-60 His+ colonies per 10^4 viable cells. This defect, however, is not as dramatic as the one for *rad57Δ*, where only 4-8 His+ colonies per 10^4 viable cells are detected. Thus, the efficiency of meiotic recombination, monitored at the *his4* loci, is significantly decreased in *rad57-w,w* mutants compared to wild-type and *rad57-g,g*.

Consistently, spore germination efficiency is lower in *MAT***a**/*MAT*α *rad57-w,w* diploids than in the isogenic *RAD57* and *rad57-g,g* strains, derived both from W303 and SK1 backgrounds, although the survival for *rad57-w,w* is higher than for *rad57Δ* strains (Table 3). Interestingly, while the viability of spores derived from *RAD57/rad57Δ* and *rad57-g,g/rad57Δ* heterozygotes is almost indistinguishable from *RAD57* homozygous diploids, spore viability of *rad57-w,w/rad57Δ* is dramatically reduced even compared to *rad57-w,w* homozygotes, although still higher than for *rad57Δ* diploids. This result indicates that while the "w,w" mutant allele function is severely impaired, it's not completely abolished. Possibly, some residual function of Rad57p, acting indepen-

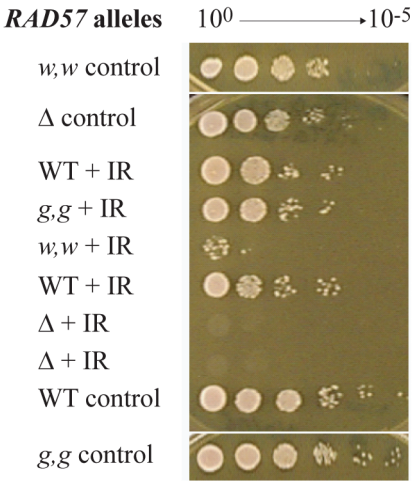

Fig. 2. Sensitivity to γ-irradiation of the wild-type Rad57 and various mutant alleles. Growth of each strain after treating with 800 Gy (denoted as "+ IR") is compared to the untreated control. Serial dilutions from 10^0 to 10^{-5}, spotted on YPDA media, are shown for each strain.

Table 3

Spore Germination in *RAD57* and Mutant Strains

Strain, Relevant Genotype	No. Tetrads Dissected	No. Complete Tetrads Survived	% Spore Viability
W303:			
a/α *RAD57*	11	11	100 (44/44)
a/α *rad57-g,g*	11	10	98 (43/44)
a/α *rad57-w,w*	33	12	73 (96/132)
a/α *rad57Δ*	11	0	5 (2/44)
a/α *RAD57/rad57Δ*	5	5	100 (20/20)
a/α *rad57-g,g/rad57Δ*	6	5	92 (22/24)
a/α *rad57-w,w/rad57Δ*	27	4	64 (69/108)
SK1:			
a/α *RAD57/rad57Δ*	5	5	100 (20/20)
a/α *rad57-g,g/rad57Δ*	6	5	92 (22/24)
a/α *rad57-g,g*	11	9	93 (41/44)
a/α *rad57-w,w*	11	0	47 (21/44)
a/α *rad57-w,w*,liquid	22	0	27 (24/88)
a/α *rad57Δ*,liquid	22	0	0 (0/88)
a/α *rad57-g,g/rad57Δ*	11	11	100 (44/44)
a/α *rad57-w,w/rad57Δ*	22	0	14 (12/88)

Sporulation was done on solid media (SPIII) at 30°C for 5 days unless noted otherwise.

Liquid sporulation was carried out in SPII at 30°C for 48 hours for **a**/α *rad57-w,w* and **a**/α *rad57Δ* SK1 homozygous strains, since the deletion strains formed no asci on solid media.

dently from the GWlvGW motif, still exists in these strains.

From these data, we infer that replacement of conserved tryptophans to alanines in the GWLVGW motif, while causing a dramatic decrease of lipid-binding, also affects Rad57 function as demonstrated by increased radiosensitivity in vegetatively growing cells, lower frequency of heteroallelic recombination, and decreased ascospore viability in meiosis. The mutant carrying the replacement of glycines to alanines, causing only a moderate effect on lipid-binding, is not defective for either of the assayed functions of Rad57, i.e., radiation resistance, induction of meiotic recombination and ascospore viability.

In conclusion, this paper presents the initial studies of a previously unexplored facet of Rad57 function, specifically, examining the possibility that Rad57 is governed by lipid signal transduction and can be controlled via direct binding of PIPs, potent second messenger molecules. Precise role(s) of PIP-binding for Rad57 is yet to be elucidated and might include, for example, 1) increasing protein activity via PIP-binding, or 2) facilitating the interaction of Rad57 with the Rad55 protein. Strong binding by $PI(3,4)P_2$ and $PI(3,4,5)P_3$ (and no binding by $PI(3,5)P_2$) is also indicative of specific binding of PIPs phosphorylated both in the third and fourth positions of the inositol ring. Quantitative analysis of Rad57-binding to various PIP-derivatives, and examining its dependence upon key enzymes regulating intracellular PIP concentration will further elucidate physiological relevance of Rad57 regulation by lipid second messengers and its involvement in lipid signaling networks.

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მოლეკულური ბიოლოგია

საფუარის Rad57 ცილის ფუნქცია დამოკიდებულია ცხიმების მბმელ კონსერვატულ მოტივზე

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Saccharomyces cerevisiae **საფუარის ცილა Rad57 საფუძვლიანად არის შესწავლილი და გააჩნია როგორც ვეგეტატიური ზრდისათვის, ასევე მეიოზისათვის აუცილებელი ფუნქციები. სტატიაში წარმოდგენილი შედეგების საფუძველზე შესაძლოა ვიფარაუდოთ, რომ ამ ფუნქციების რეგულირება ხდება სასიგნალო მოლეკულებთან (ფოსფატიდილინოზიტიდებთან ან ფოსფატიდილინოზიტოლთან) ბმის გზით. ეს ბმა ხორციელდება GW…W მოტივის საშუალებით, რომელიც კონსერვირებულია როგორც ფოსფატიდილ-ინოზიტოლ კინაზების (PIK) ცილების ოჯახში, ისევე მეიოზისათვის სპეციფიურ Spo73 ცილაში. ადრინდელი შედეგების საფუძველზე მიჩნეულია, რომ ამ ცილების ფუნქცია ცხიმოვან სასიგნალო მოლეკულებთან ურთიერთმოქმედებაზეა დამოკიდებული. Rad57-ის GWLVGW მოტივის მუტირება სპობს ცხიმებთან ბმას და მნიშვნელოვნად აზიანებს ამ ცილის სხვადასხვა ფუნქციას, რომლებიც ვეგეტატიური ზრდის, მეიოზური რეკომბინაციისა და სპორების გაღივებისთვისაა აუცილებელი. ამ შედეგების ერთობლიობა მიუთითებს, რომ Rad57-ს როლი საფუარის უჯრედის ზრდასა და გაყოფაში იმართება ამ ცილის ცხიმოვან სასიგნალო მოლეკულებთან უშუალო ბმის მექანიზმით.**

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