Evolutionary and Functional Analysis of the Invariant SWIM Domain in the Conserved Shu2/SWS1 Protein Family from *Saccharomyces cerevisiae* to *Homo sapiens*

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ABSTRACT The *Saccharomyces cerevisiae* Shu2 protein is an important regulator of Rad51, which promotes homologous recombination (HR). Shu2 functions in the Shu complex with Shu1 and the Rad51 paralogs Csm2 and Psy3. Shu2 belongs to the SWS1 protein family, which is characterized by its SWIM domain (CXC...X_n...CXH), a zinc-binding motif. In humans, SWS1 interacts with the Rad51 paralog SWSAP1. Using genetic and evolutionary analyses, we examined the role of the Shu complex in mitotic and meiotic processes across eukaryotic lineages. We provide evidence that the SWS1 protein family contains orthologous genes in early-branching eukaryote lineages (*e.g.*, *Giardia lamblia*), as well as in multicellular eukaryotes including *Caenorhabditis elegans* and *Drosophila melanogaster*. Using sequence analysis, we expanded the SWIM domain to include an invariant alanine three residues after the terminal CXH motif (CXC...X_n...CXHXXA). We found that the SWIM domain is conserved in all eukaryotic orthologs, and accordingly, *in vivo* disruption of the invariant residues within the canonical SWIM domain inhibits DNA damage tolerance in yeast and protein-protein interactions in yeast and humans. Furthermore, using evolutionary analyses, we found that yeast and *Drosophila* Shu2 exhibit strong coevolutionary signatures with meiotic proteins, and in yeast, its disruption leads to decreased meiotic progeny. Together our data indicate that the SWS1 family is an ancient and highly conserved eukaryotic regulator of meiotic and mitotic HR.

KEYWORDS DNA repair; Shu complex; budding yeast; evolutionary rate covariation; homologous recombination

HOMOLOGOUS recombination (HR) is an error-free DNA repair pathway that is essential both in mitotic cells to ensure DNA stability and in meiotic cells for faithful chromosome segregation. HR begins with double-strand break (DSB) formation and DNA end processing that give rise to 3′ single-stranded DNA (ssDNA) overhangs (Heyer *et al.* 2010). A key step in HR is the formation of RecA-like filaments on these ssDNA ends. During mitosis in the budding yeast *Saccharomyces cerevisiae*, the RecA-like protein Rad51 coats the ssDNA, whereas during meiosis, both Rad51 and another RecA-like protein, Dmc1, form on ssDNA ends (Lin *et al.* 2006; Heyer *et al.* 2010). Formation of RecA-like filaments on the DNA is essential for the homology search and strand-invasion steps that define HR. Therefore, regulation of RecA filament formation is critical for accurate repair of DNA damage and chromosome segregation. Given their importance, both Rad51 and Dmc1 are extremely well-conserved descendants of the archaeal protein RADA (Diruggiero *et al.* 1999; Komori *et al.* 2000; Lin *et al.* 2006; Chintapalli *et al.* 2013).

There are many proteins that both promote Rad51 filament formation and disassemble inappropriate filaments.

doi: 10.1534/genetics.114.173518
Manuscript received December 9, 2014; accepted for publication February 2, 2015; published Early Online February 5, 2015.
Supporting information is available online at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.173518/-/DC1.
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Interestingly, in many organisms, the proteins that stabilize Rad51 filaments themselves share structural homology with Rad51 and evolved from the archaeal RADB homolog (Lin *et al.* 2006). In humans, these Rad51 paralogs include RAD51B, RAD51C, RAD51D, XRCC2, XRCC3, and SWSAP1, whereas in the budding yeast, they include RAD55, RAD57, CSM2, and PSY3 (Schild *et al.* 2000; Takata *et al.* 2001; Martin *et al.* 2006; She *et al.* 2012; Tao *et al.* 2012; Godin *et al.* 2013; Sasanuma *et al.* 2013b). It has been proposed that SHU1 is also a Rad51 paralog (Martin *et al.* 2006). A great deal of work has been done to characterize these proteins *in vitro*, *in vivo*, and phylogenetically (Kawabata *et al.* 2005; Lin *et al.* 2006; Heyer *et al.* 2010; Suwaki *et al.* 2011). Importantly, many human Rad51 paralogs are mutated in cancers and are associated with cancer predisposition (Vaz *et al.* 2010; Suwaki *et al.* 2011; Park *et al.* 2012; Pennington and Swisher 2012; Shamseldin *et al.* 2012; Somyajit *et al.* 2012; Filippini and Vega 2013). Consistent with a critical role in genome maintenance, disruption of the yeast Rad51 paralogs results in a mutator phenotype and in some cases increased chromosomal rearrangements, which are often observed in tumor cells (Huang *et al.* 2003; Shor *et al.* 2005). In budding yeast, Shu1 and the Rad51 paralogs Csm2 and Psy3 form an obligate heterotetramer called the Shu complex (also referred to as the PCSS complex) with a fourth member, Shu2, whose major defining feature is a SWIM domain (Shor *et al.* 2005; Martin *et al.* 2006). The association of divergent Rad51 paralogs with a SWIM domain–containing protein is conserved in fission yeast *Schizosaccharomyces pombe*, where the Rad51 paralogs Rlp1 and Rdl1 interact with a Shu2 homolog Sws1 (Martin *et al.* 2006). Similarly, the human homolog of Shu2, hSWS1, interacts with the Rad51 paralogs RAD51D and XRCC2 (Martin *et al.* 2006; Liu *et al.* 2011). Importantly, hSWS1 functions as an obligate heterodimer with hSWSAP1, which itself resembles a previously unidentified highly divergent hRad51 paralog (Liu *et al.* 2011).

The conserved association between Shu2-like proteins and the Rad51 paralogs promotes Rad51-dependent HR via a largely undetermined mechanism (Godin *et al.* 2013). In all species where Shu2-like proteins have been described, their disruption results in a reduction in Rad51 filament formation and a corresponding decrease in HR (Shor *et al.* 2005; Martin *et al.* 2006; Liu *et al.* 2011; Sasanuma *et al.* 2013b). Strikingly, these defects are similar to disruption of the Rad51 paralogs (Sasanuma *et al.* 2013b). It remains to be determined what the functional significance of the SWIM domain is and why Rad51 paralogs associate with SWIM domain–containing proteins to promote HR throughout several eukaryotic lineages.

To further our understanding of the Shu2/SWS1 protein family, we characterized its evolution across taxonomic groups with a special focus on conservation of the defining SWIM domain. During this process, we confirmed the orthologous relationship of Shu2 and hSWS1 and discovered previously unknown SWS1 orthologs in multiple species, including *Caenorhabditis elegans* and *Drosophila melanogaster*. Interestingly, evolutionary analysis implicates yeast Shu2 and its fly ortholog CG34314 in meiosis because they show strong rate covariation with meiotic proteins—specifically with those that contribute to HR. Through analysis of eukaryotic Shu2/SWS1 orthologs, we found that the SWIM domain is invariant and can be expanded to include an invariant alanine residue after the canonical SWIM domain. Furthermore, we identified in the literature a cancer patient from the COSMIC database who harbors a mutation in this invariant alanine (Shepherd *et al.* 2011). *In vivo* disruption of the invariant SWIM domain residues likely results in protein instability and loss of function. Together our work indicates that the *SHU2* gene is found in all major eukaryotic lineages, where it promotes HR in both mitosis and meiosis.

Materials and Methods

Yeast strains, plasmids, and media

The strains used in this study are listed in Supporting Information, Table S1 and are isogenic to *RAD5*$^+$ *W303*, except for the PJ69-4A and PJ69-4α yeast two-hybrid strains (Thomas and Rothstein 1989; James *et al.* 1996; Zhao *et al.* 1998). The primers used are listed in Table S2. Standard protocols were used for yeast culturing, transformation (LiOAc method), sporulation, and tetrad dissection. The medium was prepared as described previously with twice the amount of leucine (Sherman *et al.* 1986). The yeast two-hybrid (Y2H) plasmid for *hSWS1* was created by PCR amplification using pJ636 (pcDNA3-3HA-*hSWS1*) from Paul Russell as a template with oligonucleotides hSWS1.F and hSWS1.R and subcloned into the *Eco*RI and *Sal*I restriction sites of pGBD-C1 (Martin *et al.* 2006). hSWSAP1 was PCR amplified from the MYC-SWSAP1 vector from Jun Huang with oligonucleotides SWSAP1.F and SWSAP1.R and subcloned into the *Eco*RI and *Sal*I restriction sites of pGAD-C1(Liu *et al.* 2011). Creation of pGBK-*shu2-C114S* (Shu2.C114S.F and Shu2.C114S.R), pGBK-*shu2-C116S* (Shu2.C116S.F and Shu2.C116S.R), pGBK-*shu2-F119A* (Shu2.F119A.F and Shu2.F119A.R), pGBK-*shu2-C176S* (Shu2.C176S.F and Shu2.C176S.R), pGBK-*shu2-H178A* (Shu2.H178A.F and Shu2.H178A.R), pGBK-*shu2-A181T* (Shu2.A181T.F and Shu2.A181T.R), pGBD-*SWS1-C85S* (hSWS1.C85S.F and hSWS1.C85S.R), pGBD-*SWS1-C87S* (hSWS1.C87S.F and hSWS1.C87S.R), pGBD-*SWS1-F90A* (hSWS1.F90A.F and hSWS1.F90A.R), pGBD-*SWS1-C103S* (hSWS1.C103S.F and hSWS1.C103S.R), pGBD-*SWS1-H105A* (hSWS1.H105A.F and hSWS1.H105A.R), and pGBD-*SWS1-A108T* (hSWS1.A108T.F and hSWS1.A108T.R) was accomplished using site-directed mutagenesis of the pGBK-*SHU2* or pGBD-*SWS1* plasmids.

Integration of a *C114S* mutation at the endogenous *SHU2* locus was accomplished by creating an integration vector by subcloning with *Eco*RI and *Pst*I from pGBK-*shu2-C114S* in the yiPLAC211 integration vector. Wild-type (WT) yiPLAC-*SHU2* was created by reversing the *C114S* mutation by site-

directed mutagenesis using Shu2.S114C.F and Shu2.S114C.R. yiPLAC211 was subsequently mutagenized to C116S, F119A, C176S, H178A, and A181T with the primers listed earlier (Figure S2). The WT and mutant versions of yiPLAC211-*shu2* were linearized with *Bam*HI and transformed into W9100-2D. Pop-outs were screened on 5-fluoroorotic acid and verified via PCR. All inserts were verified by DNA sequence analysis.

Serial dilutions

The indicated strains were grown to an OD_{600} of 0.5 and then fivefold serially diluted onto rich medium (YPD) or YPD with either 0.006, 0.012, and 0.02% methyl methanesulfonate (MMS) or 50 mM hydroxyurea (HU). For strains harboring pGBK-*shu2* vectors, serial dilutions were performed as earlier with the exception that cells were grown in SC-TRP medium prior to plating onto YPD medium or YPD with 0.02% MMS. Plates were incubated for 2 days at 30° prior to imaging.

Yeast two-hybrids

The GAL4 DNA-activating domain (pGAD) expressing plasmids was transformed into PJ69-4A, and the GAL4 DNA-binding domain (pGBK or pGBD) expressing plasmids was transformed into PJ69-4α. The *SHU1* gene was disrupted with NatNT2 using pFA6A-NatNT2 as described by Janke *et al.* (2004) in both the PJ-694A or PJ69-4α strain backgrounds where indicated. The plasmid containing PJ69-4A and PJ69-4α haploid yeast cells was mated, and diploids were selected by growth on SC-LEU-TRP solid medium. Individual colonies were grown to early log phase to OD_{600} 0.2, and then 5 μl was spotted onto medium to select for the plasmids (SC-LEU-TRP) or onto medium to select for expression of the reporter *HIS3* gene (SC-LEU-TRP-HIS) indicating an interaction. Plates were incubated for 2 days at 30° and photographed. The experiments were done in triplicate.

Homology searching and phylogenetics

Yeast Shu2 homologs from fungi were retrieved from the nonredundant amino acid sequence database at NCBI using PSI-BLAST. Hits with *E*-values below 0.005 were used for subsequent iterations. An additional PSI-BLAST search beginning with the human SWS1 (ZSWIM7) protein led to hits across many eukaryotic taxa and in archaea after three iterations. While Shu2 homologs were found in hundreds of species, a selection representing major lineages and model organisms is shown in Figure 2. Protein-sequence phylogenies were inferred in PhyML using the LG substitution model with four rate classes (Guindon and Gascuel 2003). Branch support values were generated with the approximate likelihood ratio test (aLRT).

Evolutionary rate covariation of Shu2 and fly SWS1 with meiotic and mitotic proteins

Values of evolutionary rate covariation (ERC) were calculated using previously described methods (Clark *et al.* 2012). Briefly, orthologous protein sequences were collected from species with sequenced genomes and aligned in *muscle* (18 fungal species and 12 *Drosophila* species for their corresponding data sets) (Edgar 2004; Clark *et al.* 2012; Findlay *et al.* 2014). For each protein, we then estimated amino acid branch lengths using a fixed-tree topology and the *aaml* program of the PAML package. Branch lengths then were transformed to relative rates using a projection operator (Sato *et al.* 2005; Yang 2007). The ERC value between any two proteins was calculated as the correlation coefficient between their evolutionary rates (Table S3, Table S4, Table S5, Table S6, Table S7, Table S8, Table S9, and Table S10).

The elevation of Shu complex ERC values as a group was tested by comparison with 100,000 random sets of genes of the same size ($N = 4$ proteins). A *P*-value was estimated from the number of random protein sets with mean ERC values equal to or greater than the mean ERC between Shu complex proteins. Sets of mitotic, meiotic, and "recombinase activity" proteins were obtained from the Gene Ontology Annotation Database through the Yeast Mine and FlyBase Query Builder web tools for yeast and *Drosophila*, respectively (Wilson *et al.* 2008; Balakrishnan *et al.* 2012). Statistical significance for ERC between Shu2 and these functional groups was determined by comparing their ERC distributions with the whole-proteome background (all genes) through Wilcoxon rank-sum tests.

Spore viability assay

Diploid yeast strains where both copies of the indicated genotype had been disrupted or mutated at the endogenous locus were sporulated at 30°. The individual spores were tetrad dissected onto rich medium, and spore viability was ascertained. A plate of 22 individual tetrads was analyzed in triplicate, and standard deviations were calculated.

Mitotic recombination assays

Mitotic recombination rates were calculated from WT, *shu1Δ*, and *shu2Δ* cells containing the *leu2-ΔEco*RI::*URA3*::*leu2-ΔBst*EII direct-repeat recombination assay, as described by Alvaro *et al.* (2007). Gene conversion (GC) events were measured by Leu^+ Ura^+ colonies, and single-strand annealing (SSA) recombinants were measured by Leu^+ Ura^- colonies. Nine individual colonies were analyzed for each genotype, and the experiment was performed in triplicate. The mitotic recombination rate and standard deviation were calculated as described earlier (Lea and Coulson 1949).

Results

Characterization of shu2Δ recombination phenotype and its physical interactions with the other Shu complex members

The Rad51 paralogs have a number of defining features that are related to promoting Rad51 filament formation, such as decreased rates of Rad51-mediated gene conversion on disruption (Sung 1997; Alvaro *et al.* 2007; Godin *et al.* 2013). Consistently, we previously found that deleting either *CSM2* or *PSY3*

results in a decrease in gene conversion and a subsequent increase in Rad51-independent repair (Godin *et al.* 2013). To determine if Shu1 or Shu2 has a similar role in promoting Rad51-dependent repair, we performed a heteroallelic recombination assay in WT, *shu1Δ*, and *shu2Δ* cells (Figure 1A). In this assay, a recombination event between two *leu2* heteroalleles with an intervening *URA3*$^+$ gene can generate a *LEU2*$^+$ prototroph through repair by Rad51-dependent sister-chromatid GC (*LEU2*$^+$ *URA3*$^+$) or Rad51-independent intrachromosomal SSA (*LEU2*$^+$ *URA3*$^-$). Similar to *csm2Δ* and *psy3Δ* cells (Godin *et al.* 2013), we found that *shu1Δ* and *shu2Δ* cells have significantly decrease rates of Rad51-dependent GC ($P \leq 0.02$ and $P \leq 0.005$, respectively) with a corresponding increase in rates of error-prone SSA (Figure 1A). These results demonstrate that although Shu2 is not a Rad51 paralog, it exhibits many of the same phenotypes as the other Shu complex members.

The human Shu2 homolog hSWS1 interacts with multiple Rad51 paralogs either directly or indirectly through hSWSAP1 (Martin *et al.* 2006; Liu *et al.* 2011). We asked whether other yeast Shu members similarly bridge the protein-protein interactions between Shu2 and Shu1 or Psy3 (Shor *et al.* 2005; Ball *et al.* 2009). To address this question, we performed Y2H analysis of yeast expressing *SHU2* in the GAL4 DNA-binding domain (pGBK-*SHU2*) and tested its interaction with either *SHU1* or *PSY3* expressed in the GAL4 DNA-activating domain (pGAD-*SHU1*, pGAD-*PSY3*) in a genetic background in which one of the four *SHU* genes is disrupted (Figure 1B and data not shown). We found that loss of *SHU1* disrupts the Shu2-Psy3 Y2H interaction (Figure 1B). Therefore, similar to hSWS1, which interacts with the other hRad51 paralogs through hSWSAP1, the interaction of Shu2 with the Rad51 paralog Psy3 is likely stabilized by Shu1.

Shu2 orthologs are conserved across eukaryotes

Although Shu2 homologs were identified in fission yeast and humans (Shor *et al.* 2005; Martin *et al.* 2006), their phylogenetic orthology with budding yeast Shu2 has not been demonstrated, nor have Shu2 orthologs been identified in other major eukaryotic lineages, including important model organisms. To produce a complete picture of the SWS1 family, we searched for Shu2 homologs across eukaryotic and outgroup archaeal lineages. PSI-BLAST queried with yeast Shu2 yielded hits across fungal species and metazoans. PSI-BLAST queried with human SWS1 (ZSWIM7) led to hits across eukaryotes, including plants, and archaeal proteins containing a SWIM domain. In addition, we identified putative SWS1 orthologs in several early-branching eukaryote lineages, including Diplomonadida (*Giardia lamblia*), Euglenozoa (*Leishmania* and *Phytomonas*), green algae (*Coccomyxa* and *Bathycoccus*), Stramenopiles (*Aphanomyces*, *Albugo*, *Phaeodactylum*, and *Ectocarpus*), Alveolata (*Paramecium*, *Plasmodium*, and *Oxytricha*), and Ichthyosporea (*Capsaspora*) (sequences in File S1). These sequences clustered with known SWS1 orthologs in phylogenetic trees separated from archaea outgroup sequences. This deep diversity, in addition to known fungal, metazoan, and plant orthologs, suggests an ancient origin of the SWS1 protein family. PSI-BLAST recovered a single-protein

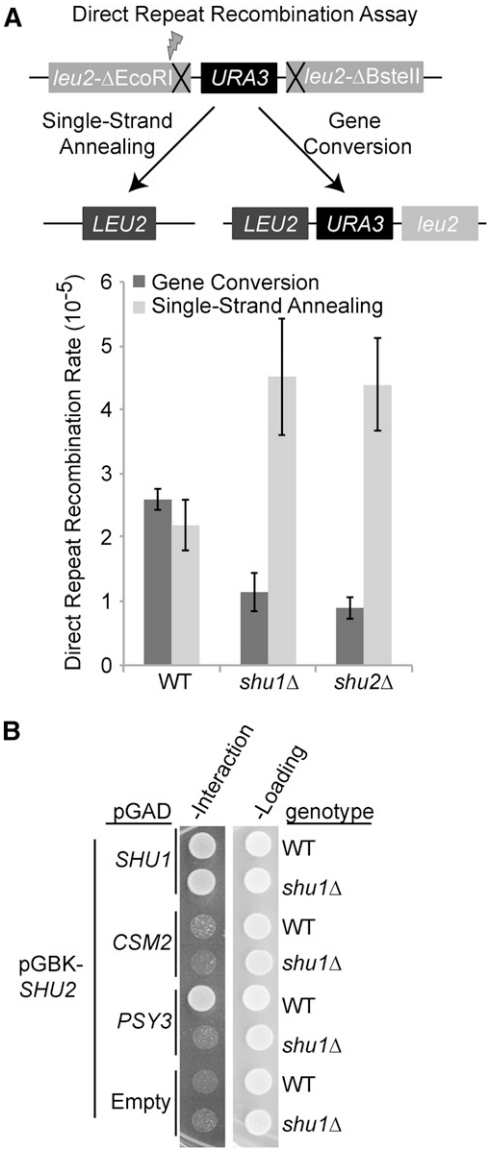

Figure 1 *SHU2* mutants have phenotypes similar to the Rad51 paralogs. (A) Disruption of *SHU1* or *SHU2* leads to decreased rates of Rad51-dependent repair. Diagram of direct repeat recombination assay (*leu2-ΔEco*RI::*URA3*::*leu2-ΔBst*EII) used to measure rates of direct-repeat recombination by Rad51-dependent GC (right side of diagram, Ura$^+$Leu$^+$ colonies) or Rad51-independent SSA (left side of diagram, Ura$^-$Leu$^+$ colonies). The rates of GC and SSA events in *shu1Δ* or *shu2Δ* cells were compared with WT and standard deviations shown. (B) Shu1 bridges the interaction between Shu2 and Psy3. Yeast two-hybrid analysis of pGBK-*SHU2* (containing a GAL4-binding domain) with pGAD-*SHU1*, pGAD-*CSM2*, pGAD-*PSY3*, and pGAD-C1 empty plasmid (containing a GAL4-activating domain) in the presence or absence of the endogenous *SHU1* gene. Interaction is indicated by growth on medium lacking histidine. Growth on medium lacking leucine and tryptophan selects for the individual plasmids and serves as a loading control.

sequence from each species, suggesting that there were few or no Shu2/SWS1 family duplications and that these orthologs are well conserved.

To define a comprehensive Shu2/SWS1 protein family, we constructed a phylogeny using Shu2 homologs from

major eukaryotic and archaeal lineages (Figure 2A). In the resulting phylogeny, eukaryotic sequences were cleanly separated from the archaeal sequences with high branch support (aLRT = 0.98). Moving forward from the archaeal root, the well-supported branches between eukaryotic sequences were in agreement with accepted speciation events, thus supporting the orthology of these Shu2/SWS1 sequences. Although the short alignment (219 amino acids) of Shu2/SWS1 did not provide sufficient power to infer all branch nodes with strong support, these sequences appear to be true orthologs. Notably, this tree revealed Shu2 orthologs in *D. melanogaster* and *C. elegans*, which we will refer to as dmSws1 and ceSws-1 hereafter, as well as an *Arabidopsis thaliana* ortholog, AT4G33925. The archaeal SWIM domain–containing proteins served only to root the tree, and we are not able to comment on the orthology or specific function of those sequences.

A defining feature of the Shu2/SWS1 protein is the SWIM domain, a zinc-binding feature (Figure 2B) (Martin *et al.* 2006). To further ensure orthology between yeast Shu2 and human SWS1 (also known as ZSWIM7) in exclusion of other SWIM domain–containing proteins in humans (ZSWIM1–6 and ZSWIM8), *C. elegans* (pqn-55), and *D. melanogaster* (CG34401), we constructed a phylogeny with all SWIM domain proteins from these species and all putative eukaryotic SWS1 orthologs (Figure 2C). Homology between these proteins is limited to the SWIM domain, so their alignment is limited to a region of 30 highly conserved amino acids. As expected for a small alignment, many branching nodes were not well resolved; however, the branching pattern cleanly separates the SWS1 orthologs from the other SWIM domain proteins with moderate support (aLRT = 0.70). This topology further supports the putative orthology of the sequences in Figure 2A (*i.e.*, they descended from a single common ancestor).

Shu2 and its orthologs have evolutionary histories strongly correlated with recombination and meiosis-related proteins

To better define the biologic function of the Shu complex, we performed coevolutionary analysis in both budding yeast and *Drosophila*. This analysis exploits the observation that functionally related proteins tend to have covarying rates of evolution because they experience shared evolutionary pressures. This property can be quantified as ERC, reflecting the degree to which two proteins have rates of sequence evolution that covary between species (Clark *et al.* 2009; Clark and Aquadro 2010; Clark *et al.* 2012). ERC for a protein pair is quantified as a correlation coefficient (ranging between −1 and 1) for which higher values reflect stronger rate covariation. ERC values are typically elevated between functionally related proteins genome-wide in yeasts and *Drosophila*, as well as between meiosis and DNA repair proteins in mammals (Clark *et al.* 2012, 2013; Findlay *et al.* 2014). Hence an elevated ERC value for a protein pair suggests cofunctionality between them.

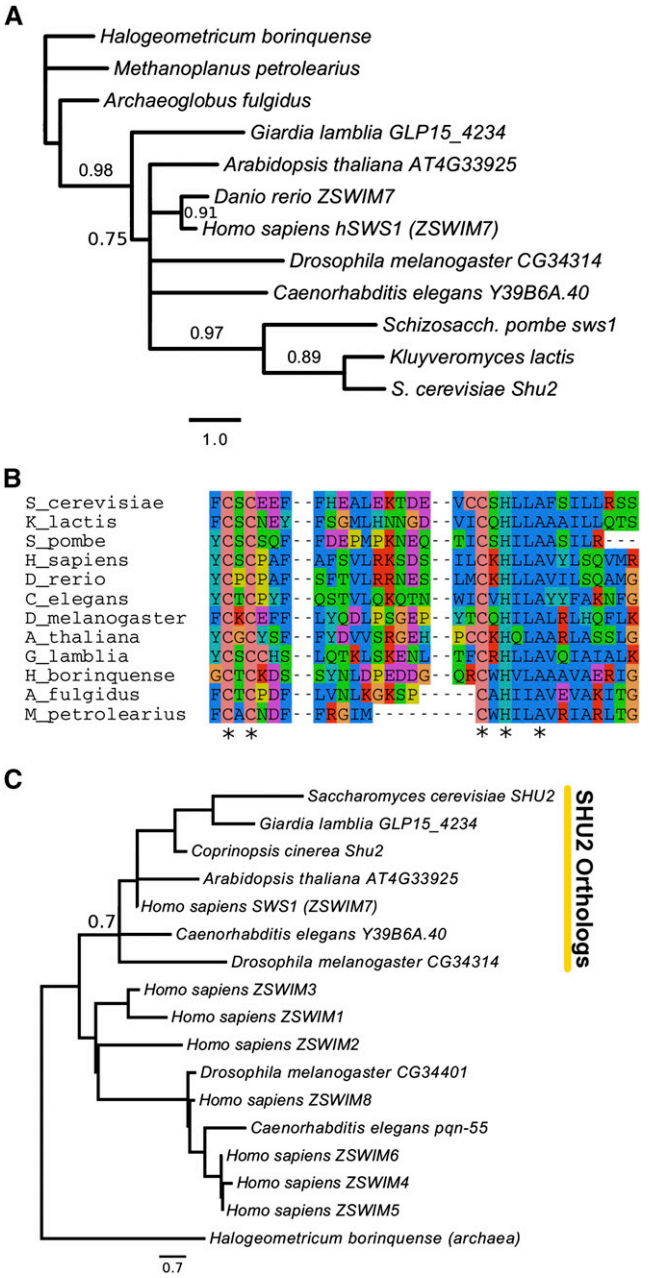

Figure 2 The SWS1 protein family extends across eukaryotes. (A) A phylogeny based on amino acid sequences shows the deep conservation of the SWS1 protein from *Giardia lamblia*, an early branching eukaryote lineage, to plants, fungi, and metazoans. The short sequence length did not allow resolution of some interior branches, but all well-supported nodes support the orthology of these sequences. (B) A partial multiple alignment shows the highly conserved SWIM domain. Absolutely conserved residues (*) include the defining CXC…X_n…CXH motif. Double-gap columns (–) indicate trimmed regions of low sequence identity. (C) The Shu2-SWS1 protein family is a monophyletic clade in this phylogeny of all proteins with SWIM domains from humans, *D. melanogaster*, *C. elegans*, and yeast. The alignment for this phylogeny contained only the 30 most highly conserved residues in the SWIM domain. These relationships further support the newly discovered Shu2-SWS1 orthologs.

We first demonstrated that members of the yeast Shu complex have significantly covarying rates with each other, as we might predict given their cofunctionality (Figure 3A);

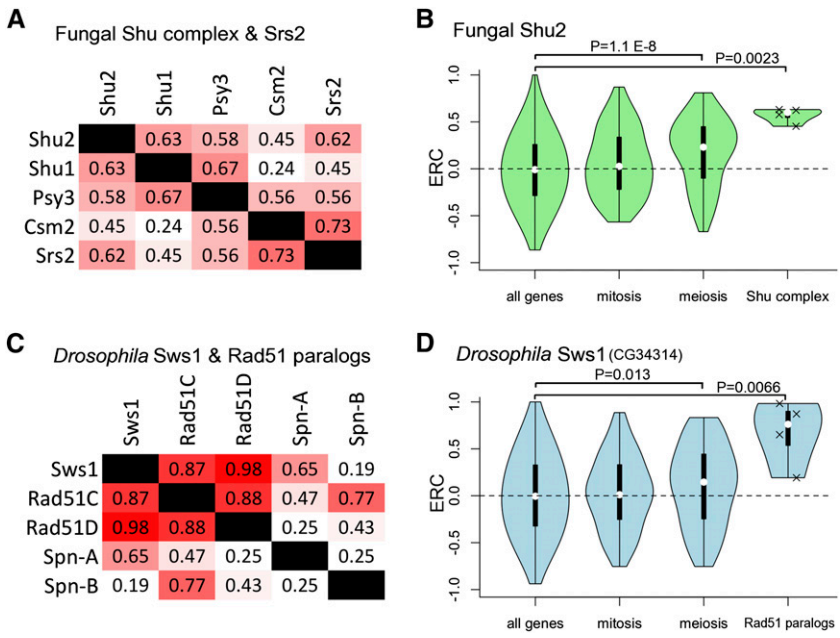

Figure 3 Coevolutionary signatures indicate that Shu2/SWS1 has highly conserved functional relationships with Rad51 paralogs and meiosis proteins. (A) Evolutionary rate covariation (ERC) values between members of the Shu complex are highly elevated ($P < 1 \times 10^{-5}$). The degree of red shading in a cell indicates a higher ERC for that protein pair compared with the null expectation of zero. (B) Violin plots depict the distributions of ERC values between Shu2 and proteins from various functional classes. Violin width is proportional to the density at that ERC value. The genome-wide distribution (all genes) of ERC with Shu2 is centered at zero, as is Shu2 with mitosis genes ($N = 107$ genes). Meiosis ($N = 126$) and Shu complex genes ($N = 4$) show a strong enrichment of high ERC values with Shu2, consistent with cofunctionality between them. *P*-values (horizontal bars) strongly reject similarity between those distributions and the genome-wide distribution. Each of the four values in the Shu complex is also plotted with an X. (C) *Drosophila* Sws1 (dmSws1), ortholog of fungal Shu2, similarly shows elevated ERC values with Rad51 paralogs. (D) DmSws1 also has high ERC values with meiosis proteins ($N = 116$) and Rad51 paralogs ($N = 4$) but not with mitosis proteins ($N = 129$).

their pairwise ERC values were highly elevated as a group (mean ERC = 0.52, permutation test $P < 0.00001$), whereas the expected mean ERC for a random gene set is zero. These ERC values were calculated across a phylogeny of 18 fungal species, including *S. cerevisiae*. We also found elevated ERC values between the Shu complex members and Srs2 (Figure 3A), consistent with a conserved physical and/or genetic interaction between Shu2 and Srs2 (a DNA helicase that disassembles Rad51 filaments) in both budding and fission yeast (Ito *et al.* 2001; Martin *et al.* 2006; Bernstein *et al.* 2011).

To determine the coevolutionary relationship of the Shu complex with broader functional groups within fungi, we studied Shu2's ERC values with mitotic and meiotic proteins. Shu2 did not show significant rate covariation with mitotic proteins, but ERC values between Shu2 and meiotic proteins were significantly elevated, suggesting strong coevolution between them ($P = 1.1 \times 10^{-8}$) (Figure 3B and Table S3 and Table S4). Importantly, these results were unchanged when recombination-related genes and genes shared by the two sets were removed from analysis, so the association is not limited to HR proteins (mitosis $P = 0.27$; meiosis $P = 1.1 \times 10^{-6}$). Similar to Shu2, Psy3 also exhibits significantly increased ERC values with meiotic but not mitotic proteins (Figure S1A and Table S5 and Table S6). In contrast to Shu2 and Psy3, both Shu1 and Csm2 show significant rate covariation with both mitotic and meiotic proteins (Figure S1, B and C, and Table S7, Table S8, Table S9, and Table S10). In addition, the *D. melanogaster* Shu2 ortholog dmSws1 (CG34314) also showed rate covariation with meiotic proteins ($P = 0.013$) and with Rad51 paralogs ($P = 0.0066$) but not with mitotic proteins as a class (Figure 3D). The *Drosophila* dmSws1 results also remained unchanged after removing recombination-related and shared genes (mitosis $P = 0.62$; meiosis $P = 0.0238$). Most notably, Rad51C and Rad51D showed extremely high rates of covariation, whereas the other two Rad51 paralogs, Spn-A and Spn-B, showed modestly elevated levels (Figure 3C). Finally, we tested for coevolutionary associations of meiotic and mitotic genes with the mammalian Shu2 ortholog SWS1 (ZSWIM7). Contrary to results in fungi and *Drosophila*, ERC values between mammalian SWS1 and meiotic and mitotic genes were not generally elevated. Overall, results from fungi and *D. melanogaster* suggest that the Shu complex has conserved meiotic and mitotic roles in eukaryotic species.

Expansion of the SWIM domain to include an invariant alanine three amino acids downstream of the canonical CXC...X_n...CXH motif

The SWIM motif was originally defined as a zinc-binding motif that contains the canonical CXC...X_n...CXH sequence (Liu *et al.* 1995; Makarova *et al.* 2002; Banerjee *et al.* 2004). However, on analysis of our evolutionarily deep alignment, we identified an invariant alanine located three amino acids downstream from the CXH motif (Figure 4A and Figure S2). Interestingly, in humans, this invariant alanine in hSWS1 is mutated to a threonine (A108T) in a cancer patient from the COSMIC database (Shepherd *et al.* 2011). To determine whether alanine 108 may be functionally important, we constructed a Y2H vector containing hSWS1 mutagenized to include this mutation, SWS1-A108T, and mutations in the canonical SWIM domain residues (C85S, C87S, C103S, and H105A)(Figure 4B). Suggesting that these residues are functionally important, disruption of the canonical SWIM domain or the invariant alanine (A108) results in a reduced Y2H interaction with hSWS1's obligate binding partner hSWSAP1 (Figure 4B). Interestingly, another highly conserved residue, F90, does not result in reduced Y2H

interaction when mutated to an alanine (Figure 4B, SWS1-F90A). These results suggest that the SWIM domain in hSWS1 including the invariant alanine is likely important for hSWS1 function.

The SWIM domain is important for Shu2's functionality in vivo

To investigate the role of the SWIM domain and the invariant alanine, we created Y2H vectors harboring the analogous mutations in the budding yeast *SHU2* SWIM domain (Figure 5A). Via Y2H analysis, we found that mutating the canonical SWIM domain residues (C114S, C116S, C176S, and H178S) in *shu2* results in an undetectable Y2H interaction with Shu2's binding partners Shu1 and Psy3 (Figure 5A). Interestingly, mutating alanine 181 to a threonine leads to a reduced Y2H interaction with Psy3 but not Shu1. Furthermore, while the *hSWS1-F90A* mutant maintains its Y2H interaction with *hSWSAP1*, we found that the corresponding mutation in yeast *shu2*, F119A, results in a reduced Y2H interaction with Psy3 but not Shu1 (Figure 5A). These results suggest that the canonical SWIM domain in yeast Shu2 is likely important for function and that the conserved alanine and phenylalanine also may be components of this domain.

To determine whether the impaired Y2H interactions of the SWIM domain mutants result in diminished DNA damage tolerance in yeast, we complemented *shu2Δ* cells with a WT *SHU2*- or a mutant *shu2*-expressing plasmid. We found that the WT *SHU2* plasmid complements the MMS sensitivity of *shu2Δ* cells, while mutations in the canonical SWIM domain (C114S, C116S, C176S, and H178A) do not (Figure 5B). Interestingly, despite the altered protein-protein interactions observed in both *shu2-F119A* and *shu2-A181T*, both mutant plasmids complement the MMS sensitivity of a *shu2Δ* cell (Figure 5B). These results indicate that the interaction between Shu2 and Psy3 may be dispensable for Shu2's mitotic function. These findings were confirmed when we stably integrated the canonical SWIM domain mutants (C114S, C116S, C176S, and H178A) as well as F119A at the endogenous *SHU2* locus (Figure S3). Unfortunately, we were unable to integrate A181T into our yeast strains. Because the *Shu* genes were originally characterized by their ability to suppress the sensitivity of a *top3Δ* or *sgs1Δ* strain to MMS or HU treatment, we also examined whether the integrated SWIM domain alleles also would rescue *sgs1Δ* DNA damage sensitivity such as disruption of *SHU2* and found that the canonical SWIM domain mutants do (Figure 5C). Similar findings also were observed with a SWIM domain mutation in the fission yeast gene *sws1-C152S* (Martin *et al.* 2006). Thus our analysis of the four canonical SWIM domain mutants demonstrates that the conserved SWIM domain is important for this protein family's function in the repair of MMS-induced DNA lesions.

Given the conserved nature of the Shu complex in meiotically dividing yeast, we asked how disruption of the SWIM domain would affect meiotic outcome in *S. cerevisiae*.

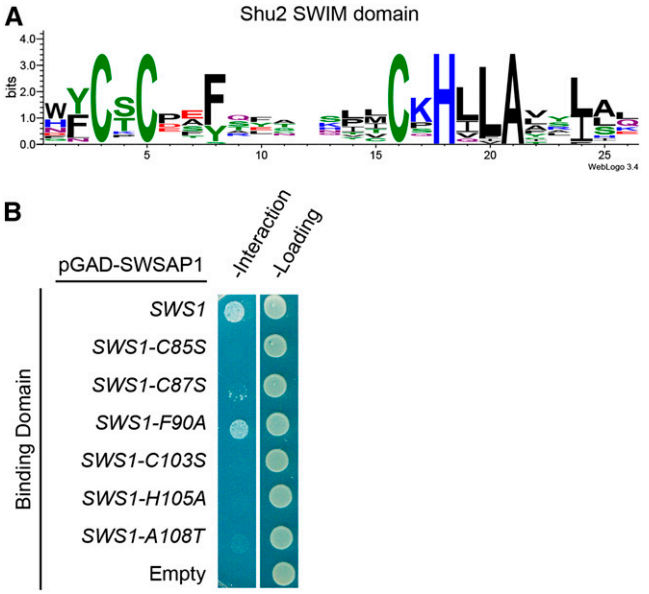

Figure 4 Analysis of the highly conserved SWIM domain residues in the human SWS1 protein. (A) Sequence logo of the SWIM domain reveals a highly conserved phenylalanine two residues after the CXC motif and an invariant alanine two residues after the CXH motif. (B) Mutating the SWIM domain in human SWS1 impairs its interaction with hSWSAP1. Y2H analysis of pGAD-hSWS1 with mutations in the SWIM domain (C85, C87, C103, H105), as well as F90 and A108, were assayed for interaction with human SWSAP1 (pGBD-SWSAP1) as described in Figure 1B.

To test this, we sporulated diploids homozygous for deletion of a single Shu complex member and screened for viability of the resulting offspring. In agreement with two recent reports (Hong and Kim 2013; Sasanuma *et al.* 2013b), we found that loss of any member of the Shu complex causes a marked decrease in spore viability, with Csm2 and Psy3 resulting in a more severe defect ($P \leq 0.02$ for all) (Figure 5D). Next, we tested whether mutations of the SWIM domain similarly would lead to decreased meiotic progeny. We found that the SWIM domain mutants C114S, C116S, C176S, and H178A are also defective for spore viability compared with WT yeast ($P \leq 0.05$ for all) (Figure 5E). In contrast, the *shu2-F119A* allele spore viability was similar to that of WT yeast (Figure 5E). These results demonstrate that the canonical SWIM domain is necessary for Shu2's role during meiosis.

Discussion

Here we describe how the SWS1 protein family has evolved throughout major eukaryotic lineages to interact with the Rad51 paralogs to promote HR. One defining feature of the SWS1 protein family is its invariable SWIM domain (consisting of the CXC. . .X_n. . .CXH motif, where X is any amino acid and n is a variable number of amino acids), and we show that this domain is important for protein functionality. Furthermore, our analysis indicates that the SWIM domain contains an invariant alanine two residues after the CXH motif that would expand the domain to

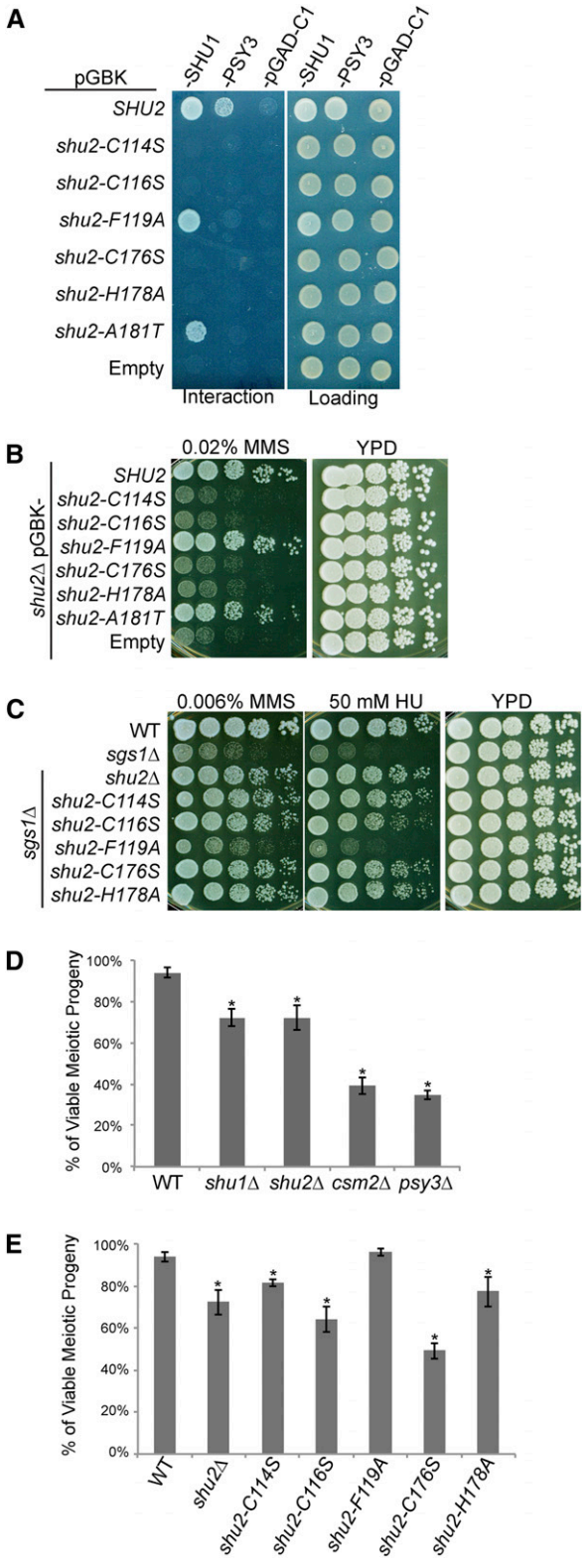

Figure 5 The SWIM domain is important for the function of Shu2. (A) Mutating the SWIM domain in *SHU2* impairs its interaction with *SHU1* and/or *PSY3*. Y2H analysis of pGBK-*SHU2* or pGBK-*shu2* mutant interactions with pGAD-*SHU1* or pGAD-*PSY3* as described in Figure 1B. (B) Canonical SWIM domain mutants are nonfunctional. *shu2Δ* cells harboring the indicated pGBK plasmids were fivefold serially diluted onto YPD medium or YPD medium containing 0.02% MMS and incubated at 30°C for 2 days. (C) Disruption of the canonical SWIM domain suppresses *sgs1Δ* DNA damage sensitivity. WT, *sgs1Δ*, *sgs1Δ shu2Δ*, *sgs1Δ shu2-C114S*, *sgs1Δ shu2-C116S*, *sgs1Δ shu2-F119A*, *sgs1Δ shu2-C176S*, and *sgs1Δ shu2-H178A* cells were fivefold serially diluted onto YPD medium or YPD medium containing 50 mM HU or 0.006% MMS and incubated at 30°C for 2 days. (D) Loss of the Shu complex results in reduced spore viability. Diploid yeast with the indicated mutations were sporulated, and individual spores were tetrad dissected onto rich medium. Viable spore colonies from 22 individual tetrads were quantitated, and the average number of viable meiotic progeny was calculated. Standard deviations are shown from three experiments, and significance was determined by *t*-test ($P < 0.05$). (E) Same as D except that the *shu2* SWIM domain containing mutants were analyzed.

CXC. . .X_n. . .CXHXXA. Specifically, in budding yeast, mutation of the canonical SWIM domain residues reduces Rad51-dependent repair, meiotic viability, and protein-protein Y2H interactions. Additionally, we show further evidence that the Shu complex has an important meiotic function and that this function is evolutionarily conserved. We demonstrate that the SWS1 family is found across eukaryotes and are the first to identify orthologs of SWS1 in important model organisms such as *D. melanogaster* and *C. elegans*, as well as in additional fungal and ancient eukaryotic lineages.

Regulation of meiotic recombination is critical for maintenance of chromosome copy number and genetic diversity. Consistent with a central role for the yeast Shu complex during meiosis, loss of Shu2 or its binding partners reduces the viability of meiotic offspring, implicating this complex as a critical meiotic regulator (Figure 5, D and E). It was demonstrated recently that the Shu complex has an important function in the recruitment of Rad51 to meiotic DSB sites and that Shu1 promotes homolog bias (Hong and Kim 2013; Sasanuma *et al.* 2013b). In agreement with these findings, patterns of coevolution also highlight the importance of the Shu complex in meiosis because we found that all the Shu complex members, including Shu2, are coevolving with other meiotic proteins, as shown by elevated ERC values (Figure 3 and Figure S1). Importantly, we did not observe elevated ERC values between Shu2 and Csm2 with mitotic proteins, unlike the other Shu complex members, Shu1 and Psy3 (Figure 3 and Figure S1).

On further analysis, we identified the *D. melanogaster* SWS1 ortholog, which is also likely to function during meiosis. dmSws1 is expressed primarily in the ovaries, which are the site of meiotic crossing over in flies (St. Pierre *et al.* 2014; B. R. Graveley, G. May, A. N. Brooks, J. W. Carlson, L. Cherbas, C. A. Davis, M. Duff, B. Eads, J. Landolin, J. Sandler, K. H. Wan, J. Andrews, S. E. Brenner, P. Cherbas, T. R. Gingeras, R. Hoskins, T. Kaufman, S. E. Celniker, personal communication). In contrast, dmSws1 is not significantly expressed in other adult tissues, including the testes, which do not produce recombinant gametes in *Drosophila*. Consistent with dmSws1's ovarian expression, we found that dmSws1 also exhibits strong co-evolutionary signatures (ERC) with meiotic proteins, just as observed for its budding yeast ortholog Shu2 (Figure 3,

B and D). Also similar to Shu2, which physically interacts with the Rad51 paralogs, we found that dmSws1 strongly coevolves with the Rad51 paralogs dmRad51D and dmRad51C (Figure 3, A and C), which are also expressed primarily in the ovaries. Despite this conserved evolutionary pattern, we were unable to detect a physical Y2H interaction between dmSws1 and dmRad51D or between ceSws-1 and Rfs-1. However, this may be due to the lack of a third unidentified binding partner, as is required for Y2H interaction between hSWS1 and its hRAD51 paralogs. For example, we only detected a Y2H interaction between hSWS1 and its obligate binding partner hSWSAP1 despite the physical interaction of hSWS1 with other Rad51 paralogs (Martin *et al.* 2006; Liu *et al.* 2011). Alternatively, the strong ERC values between dmSws1 and the Rad51 paralogs may be explained by a conserved genetic but not physical interaction. Altogether, our work suggests that the SWS1 protein family likely consists of conserved pro-recombinogenic factors for meiotic HR in multiple eukaryotic lineages. In the future, direct experimental evidence to examine the role of dmSws1 in meiotic and mitotic Rad51 filament formation will be necessary to confirm this hypothesis.

While our work indicates that the SWS1 family of proteins functions in meiosis, it is clear that these proteins also promote mitotic HR (Shor *et al.* 2005; Mankouri *et al.* 2007; Ball *et al.* 2009; Bernstein *et al.* 2011; Godin *et al.* 2013; Xu *et al.* 2013). Multiple groups including our own have demonstrated that loss of *SHU2* confers sensitivity to replication fork–damaging MMS in various budding yeast strains, and consistent with these findings, previous reports have indicated that loss of SWS1 results in a sensitivity to replication fork–blocking agents in both fission yeast and human HeLa cells (Martin *et al.* 2006; Liu *et al.* 2011). Furthermore, we showed here that the SWIM domain in Shu2 is important for the resistance of the Shu complex to MMS-induced DNA damage. The role and importance of Shu2 and the Shu complex in promoting mitotic HR are not completely understood, although they appear to act in part by regulating the activity of Srs2.

Srs2 is a DNA helicase that destabilizes Rad51 filaments *in vitro*, which, in turn, regulate HR *in vivo* (Rong *et al.* 1991; Krejci *et al.* 2003; Veaute *et al.* 2003; Burgess *et al.* 2009). Previous reports have shown that Shu2 physically interacts with Srs2 (Ito *et al.* 2001; Martin *et al.* 2006) and that loss of Shu1 results in increased Srs2 occupancy at DSB sites (Bernstein *et al.* 2011). Together these findings support a model in which the Shu complex may promote Rad51 filament formation by inhibiting Srs2 in mitosis. However, a new report indicates that Srs2 has an additional important function in regulating Rad51-dependent repair during meiosis (Hong and Kim 2013; Sasanuma *et al.* 2013b). In contrast to its role in mitosis, the function of Srs2 in meiosis is pro-recombinogenic. Furthermore, disruption of *SRS2* does not rescue or alter the meiotic defect observed in a Shu complex mutant, suggesting that the genetic interaction between the Shu complex and Srs2 is different during mitosis and meiosis (Sasanuma *et al.* 2013a, b). Despite the differing mitotic and meiotic roles of Srs2, the conserved physical interaction between Shu2 and Srs2 and the strong evolutionary covariation clearly indicate that these proteins have a functionally important relationship (Figure 3A). In the future, the strong ERC observed between Shu complex members could be exploited to identify additional protein modifiers of its HR function, as demonstrated in a recent ERC study (Findlay *et al.* 2014). Moreover, it remains uninvestigated whether hSWS1 or its binding partner, hSWSAP1, retains this physical interaction with the putative Srs2 homologs such as PARI or RTEL or whether they promote meiotic HR. In conclusion, the SWS1 protein family is an important factor in both mitotic and meiotic HR, and future work will shed light on its unique regulatory mechanisms for Rad51.

Acknowledgments

We thank Rodney Rothstein for providing materials and reagents, Paul Russell and Jun Huang for expression constructs of the human proteins used to make the yeast two-hybrid vectors, and Shahm Raslan for experimental assistance. This work was supported by grants from the National Institutes of Health (GM-088413 and ES-0244872), a V Foundation Scholar Award to K.A.B., and a Charles E. Kaufman New Investigator Award from the Pittsburgh Foundation and a CURE grant from the Commonwealth of Pennsylvania Department of Health to N.L.C.

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Communicating editor: J. Sekelsky

GENETICS

Supporting Information
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.173518/-/DC1

Evolutionary and Functional Analysis of the Invariant SWIM Domain in the Conserved Shu2/SWS1 Protein Family from *Saccharomyces cerevisiae* to *Homo sapiens*

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DOI: 10.1534/genetics.114.173518

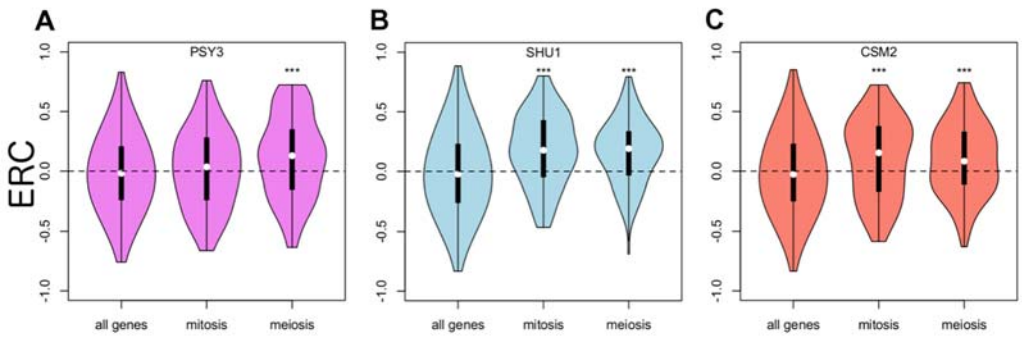

Figure S1 Distributions of ERC with Shu complex members PSY3 (A), SHU1 (B), and CSM2 (C). Violin plots show the median (dot) and upper and lower quartiles (black box) of ERC values of meiosis and mitosis proteins versus Shu complex members. Similarly, the violin curves depict the density of observed values. Both mitosis and meiosis groups were tested for their departure from the null (all genes) distribution. For these tests "***" indicates a P-value less than 0.001.

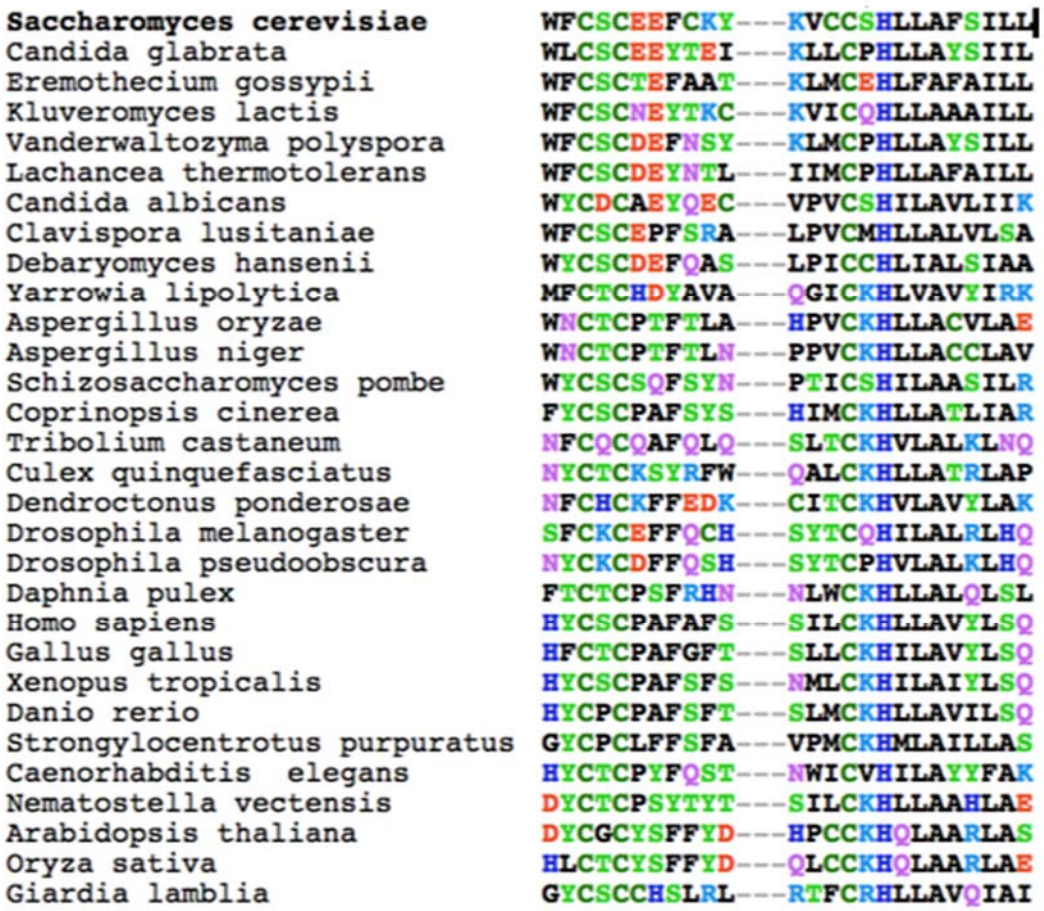

Figure S2 A deep evolutionary alignment of Shu2/SWS1 orthologs clearly reveals conserved residues of the SWIM domain. The first block contains the invariant and canonical CXC motif and the highly conserved F/Y residue just upstream. The second block contains the expanded CXHXXA motif.

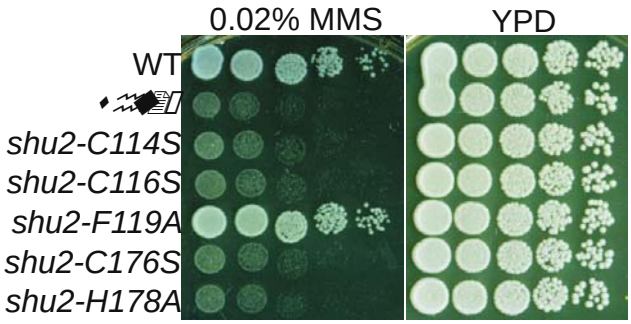

Figure S3 The SWIM domain is important for Shu2's function. **(A)** Canonical SWIM domain mutants are non-functional. The indicated mutants were stably integrated into the endogenous *SHU2* locus and 5-fold serially diluted onto YPD medium or YPD medium containing 0.02% MMS and incubated at 30˚C for 2 days.

Table S1 Strains and plasmids

Name	Description
W9100-2D	*MAT***α** *ADE2 leu2-3,112 his3-11,15 ura3-1 TRP1 lys2*Δ *RAD5*
KBY159-1C	*MAT***α** *LYS2 shu1::Hph4*
KBY162-7C	*MAT***a** *trp1-1 lys2*Δ *shu2::NAT*
KBY107-5C	*MAT***a** *leu2-3,112 trp1-1 LYS2 csm2::KanMX*
KBY108-1C	*MAT***a** *bar1::LEU2 trp1-1 LYS2 psy3::KanMX4*
KBY213-2A	*MAT***α** *rad55::NatMX*
PJ69-4A	*MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ*
PJ69-4**α**	*MAT***α** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ*
KBY403	*MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ shu1::natMX4*
KBY475	*MAT***α** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2::GAL1-HIS3 met2::GAL7-lacZ shu1::natMX4*
W3770-4D	*MAT***a** *leu2*-ΔEcoRI*::URA3::leu2*-ΔBstEII
KBY13-2C	*MAT***a** *leu2*-ΔEcoRI*::URA3-HO::leu2*-ΔBstEII *shu1::HIS3*
KBY90	*MAT***a** *leu2*-ΔEcoRI*::URA3-HO::leu2*-ΔBstEII *shu2::TRP1*
KBY469	*MAT***α** *shu2-C114S*
KBY834-6A	*MAT***α** *shu2-C116S trp1-1 LYS2 sgs1::HIS3*
KBY835-3B	*MAT***α** *shu2-F119A trp1-1 lys2*Δ *sgs1::HIS3*
KBY836-5C	*MAT***α** *shu2-C176S sgs1::HIS3 trp1-1 LYS2*
KBY856-1C	*MAT***α** *shu2-H178A sgs1::HIS3 trp1-1 LYS2*

KBY161-2A	*MAT***a** *trp1-1 LYS2 shu2::HphNT1*
KBY51-3B	*MAT***a** *sgs1::HIS3 trp1-1 LYS2 MET22*
KBY484-2B	*MAT***α** *sgs1::HIS3 shu2::NatMX4*
KBY483-1B	*MAT***a** *sgs1::HIS3 trp1-1 shu2-C114S*
KBY138	*MATa/MAT***α** *ade2-1/ADE2 TRP1/trp1-1 lys2Δ/LYS2*
W9100-11B	*MATa ade2-1*
W9100-12C	*MAT***α** *ADE2 trp1-1 LYS2*
KBY457	*MAT***a**/*MAT***α** *ade2-n/ade2-I-*SceI *shu1::HIS3/shu1::HIS3 met22::KLURA3/MET22 his3::NatMX4/his3::hphMX4 LYS2/lys2::GAL-IsceI*
KBY21-3D	*MAT***a** *ade2-n shu1::HIS3 LYS2 met22::KLURA3 his3::NatMX4 RAD5*
KBY24-13A	*MAT***α** *ade2-IsceI shu1::HIS3 lys2::GAL-IsceI ura3-1 MET22 his3::hphMX4*
KBY341	*MAT***a**/*MAT***α** *shu2::TRP1/shu2::TRP1 leu2ΔEcoRI::URA3-HO::leu2ΔBstEII/ura3-1 LYS2/LYS2 trp1-1/trp1-1 ade2/ADE2*
KBY92-1A	*MAT***α** *shu2::TRP1 LYS2 trp1-1*
KBY488	*MAT***a**/*MAT***α** *ade2-1/ADE2 trp1-1/TRP1 LYS2/lys2Δ leu2ΔEcoRI::URA3-HO::leu2ΔBstEII/ura3-1 shu2-C114S/shu2-C114S*
KBY474-1B	*MAT***a** *ade2-1 trp1-1 LYS2 leu2ΔEcoRI::URA3-HO::leu2ΔBstEII shu2-C114S*
KBY474-1A	*MAT***α** *ADE2 TRP1 lys2Δ ura3-1 shu2-C114S*
KBY342	*MAT***a** *ADE2/ade2-1 bar1::LEU2/leu2-3,112 TRP1/trp1-1 csm2::KanMX4/csm2::KanMX4 ura3-1/leu2ΔEcoRI::URA3-HO::leu2ΔBstEII*
KBY107-2C	*MAT***a** *ADE2 bar1::LEU2 TRP1 csm2::KanMX4 ura3-1*
KBY38-6D	*MAT***α** *csm2::KanMX4 leu2ΔEcoRI::URA3-HO::leu2ΔBstEII his3-11 trp1-1 ade2-1*
KBY343	*MAT***a** *psy3::KanMX4/psy3::KanMX4*

	leu2ΔEcoRI::URA3-HO::leu2ΔBstEII his3-11/ura3-1 ade2-1/ADE2 trp1-1/trp1-1 LYS2/LYS2
KBY57-1B	*MAT***a** *psy3::KanMX4 leu2ΔEcoRI::URA3-HO::leu2ΔBstEII ade2-1 trp1-1 LYS2*
KBY106-12B	*MAT***α** *trp1-1 LYS2 psy3::KanMX4*
KBY875	*MAT***a/α** *ADE2/ADE2 TRP1/trp1-1 lys2Δ/LYS2 shu2-C116S/shu2-C116S*
KBY861-2C	*MAT***a** *ADE2 TRP1 lys2Δ shu2-C116S*
KBY861-1D	*MAT***α** *ADE2 trp1-1 LYS2 shu2-C116S*
KBY886	*MAT***a/α** *ADE2/ADE2 trp1-1/trp1-1 lys2Δ/LYS2 shu2-F119A/shu2-F119A*
KBY835-3D	*MAT***a** *ADE2 trp1-1 lys2Δ shu2-F119A*
KBY835-5B	*MAT***α** *trp1-1 LYS2 shu2-F119A*
KBY878	*MAT***a/α** *ade2-1/ADE2 TRP1/trp1-1 LYS2/LYS2 shu2-C176S/shu2-C176S*
KBY863-5A	*MAT***a** *ade2-1 TRP1 LYS2 shu2-C176S*
KBY863-5C	*MAT***α** *ADE2 trp1-1 LYS2 shu2-C176S*
KBY876	*MAT***a/α** *ade2-1/ADE2 TRP1/trp1-1 lys2Δ/LYS2 shu2-H178A/shu2-H178A*
KBY864-1A	*MAT***a** *ade2-1 TRP1 lys2Δ shu2-H178A*
KBY864-1D	*MAT***α** *ADE2 trp1-1 LYS2 shu2-H178A*
pWJ1479	*pGBDK-SHU2 (TRP, KAN^R)*
pWJ1474	*pGAD-SHU1 (LEU, AMP^R)*
pWJ1477	*pGAD-CSM2 (LEU, AMP^R)*
pWJ1476	*pGAD-PSY3 (LEU, AMP^R)*
pGAD-C2	*pGAD-C2 (LEU, AMP^R)*
pKB108	*pGBK-Shu2-C114S (TRP, KAN^R)*
pKB318	*pGBK-Shu2-C116S (TRP, KAN^R)*
pKB330	*pGBK-Shu2-F119A (TRP, KAN^R)*
pKB319	*pGBK-Shu2-C176S (TRP, KAN^R)*
pKB320	*pGBK-Shu2-H178A (TRP, KAN^R)*

pKB321	*pGBK-Shu2-A181T (TRP, KAN^R)*
pKB52	*pGAD-SWSAP1 (LEU, AMP^R)*
pKB78	*pGBD-SWS1 (TRP, AMP^R)*
pKB93	*pGBD-Sws1-C85S (TRP, AMP^R)*
pKB94	*pGBD-Sws1-C87S (TRP, AMP^R)*
pKB322	*pGBD-Sws1-F90A (TRP, AMP^R)*
pKB316	*pGBD-Sws1-C103S (TRP, AMP^R)*
pKB317	*pGBD-Sws1-H105A (TRP, AMP^R)*
pKB142	*pGBD-Sws1-A108T (TRP, AMP^R)*
pGBD-C1	*pGBD-C1(TRP, AMP^R)*
pKB13	yiPLAC211-Shu2-C114S
pKB315	yiPLAC211-Shu2
pKB327	yiPLAC211-Shu2-C116S
pKB323	yiPLAC211-Shu2-F119A
pKB328	yiPLAC211-Shu2-C176S
pKB324	yiPLAC211-Shu2-H178A

All yeast strains are W303 background derivatives and *RAD5* (23) W1588 (24) except for PJ69-4A and PJ69-4**α** (25). The KBY403 and KBY475 strains were constructed in PJ69-4**α** and PJ69-4A backgrounds, respectively. The strains are listed in the order they appear in the figures and text.

Table S2 Primers Used in this Study

Name	Sequence
hSWS1.F	GCGGAATTCATGGCCGTAGTGTTGCCGGCGGTTG
hSWS1.R	GCGGTCGACCTA CTTAAAGGTGGACTGCAGCTC
SWSAP1.F	**'GCGGAATTCATGCCTGCCGCCGGACCGCCTTTG**
SWSAP1.R	**GCGGTCGACTCAGGGCTGGCCTCCAGAGCTTGAA C**
hSWS1.C85S.F	**'GCTTCTTGTCATTACTCTTCATGTCCTGCATTTG**
hSWS1.C85S.R	**CAAATGCAGGACATGAAGAGTAATGACAAGAAGC**
hSWS1.C87S.F	**CTTGTCATTACTGTTCATCTCCTGCATTTGCATTCT C**
hSWS1.C87S.R	**GAGAATGCAAATGCAGGAGATGAACAGTAATGACA AG**
hSWS1.F90A.F	**CTGTTCATGTCCTGCAGCCGCATTCTCAGTGCTAC**
hSWS1.F90A.R	**GTAGCACTGAGAATGCGGCTGCAGGACATGAACAG**
hSWS1.C103S.F	**GACAGCATCCTGTCCAAGCATCTCTTGG**
hSWS1.C103S.R	**CCAAGAGATGCTTGGACAGGATGCTGTC**
hSWS1.H105A.F	**CAGCATCCTGTGCAAGGCCCTCTTGGCAGTTTAC**
hSWS1.H105A.R	**GTAAACTGCCAAGAGGGCCTTGCACAGGATGCTG**
hSWS1.A108T.F	**GCAAGCATCTCTTGACAGTTTACCTGAGTC**
hSWS1.A108T.R	**GACTCAGGTAAACTGTCAAGAGATGCTTGC**
Shu2.C114S.F	**CGCACACTGGTTCTCCTCATGTGAAGAG**
Shu2.C114S.R	**CTCTTCACATGAGGAGAACCAGTGTGCG**
Shu2.S114C.F	**CGCACACTGGTTCTGCTCATGTGAAGAG**
Shu2.S114C.R	**CTCTTCACATGAGCAGAACCAGTGTGCG**
Shu2.C116S.F	**CACACTGGTTCTGCTCATCCGAAGAGTTTTGTAAA**

	TAC
Shu2.C116S.R	**GTATTTACAAAACTCTTCGGATGAGCAGAACCAGT GTG**
Shu2.F119A.F	**GGTTCTGCTCATGTGAAGAGGCCTGTAAATACTTTCA TGAAG**
Shu2.F119A.R	**CTTCATGAAAGTATTTACAGGCCTCTTCACATGAGCA GAACC**
Shu2.C176S.F	**CAAATTTGATAAAGTTTGTTCCTCGCATCTACTGG CGTTCTC**
Shu2.C176S.R	**GAGAACGCCAGTAGATGCGAGGAACAAACTTTATC AAATTTG**
Shu2.H178A.F	**GTTTGTTGTTCGGCTCTACTGGCGTTC**
Shu2.H178A.R	**GAACGCCAGTAGAGCCGAACAACAAAC**
Shu2.A181T.F	**GTTCGCATCTACTGACGTTCTCCATTTTGC**
Shu2.A181T.R	**GCAAAATGGAGAACGTCAGTAGATGCGAAC**

Tables S3-S10

Available for download as Excel files at
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.173518/-/DC1

Table S3 Shu2 Mitosis

Table S4 Shu2 Meiosis

Table S5 Psy3 Mitosis

Table S6 Psy3 Meiosis

Table S7 Shu1 Mitosis

Table S8 Shu1 Meiosis

Table S9 Csm2 Mitosis

Table S10 Csm2 Meiosis

File S1

SWS1 family sequences

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.173518/-/DC1