**1. Protein function: involved pathways, pathologies, and interaction partners**

RAD51 (UniProt Q06609) is a human DNA repair recombinase involved in homologous recombination of double-strand DNA breaks. In the KEGG homologous recombination pathway (map03440), RAD51 is localized in the nucleus within the presynaptic filament assembled on single-stranded DNA together with its key partners BRCA2, PALB2, RAD51C, and XRCC3, which collectively ensure high-fidelity repair synthesis. The diagram also indicates that RAD51 activation predominantly occurs during the S–G2 phases of the cell cycle, when accurate repair is essential during DNA replication.

In the KEGG Fanconi anemia pathway (map03460), RAD51 is positioned downstream of the interstrand crosslink (ICL) recognition module, which includes FANCM and the monoubiquitinated FANCD2/FANCI complex. The scheme shows that after initial ICL processing within the Fanconi anemia system, the lesion is transferred to the homologous recombination module, where RAD51 acts as the final effector completing accurate DNA restoration. This pathway topology explains the Fanconi anemia type R phenotype caused by RAD51 mutations.

In KEGG maps of general cancer pathways (map05200) and pancreatic adenocarcinoma (map05212), RAD51 is part of a DNA repair gene cluster functioning downstream of oncogenes such as KRAS and EGFR and in parallel with p16, p53, SMAD4, and BRCA2. In the pancreatic carcinogenesis scheme, RAD51 is shown at the convergence point of BRCA2 and p53 defects with KRAS activation, highlighting the role of homologous recombination defects involving RAD51 in the progression from PanIN lesions to invasive pancreatic ductal adenocarcinoma (PDA).

**Protein partners, interaction network, and diseases**

The STRING protein–protein interaction network for RAD51 reveals a densely connected cluster with RAD51 as the central node, surrounded by BRCA2, BRCA1, RAD51B, RAD51C, RAD51D, XRCC2, XRCC3, PALB2, and the helicase BLM. High combined scores, reflected by thick interaction edges, indicate experimentally validated and functionally predicted interactions, primarily in the context of homologous recombination and chromosome stability maintenance. The co-occurrence of RAD51 and BLM/RecQ helicases within the same subcluster emphasizes coordination between recombinase activity and DNA remodeling at damage sites.

Clinical disease maps based on COSMIC, MalaCards, GeneCards, and DISEASES summarize the association of RAD51 with dozens of disorders, predominantly solid tumors. Specific ClinVar variants are annotated as FANCR and are linked to characteristic genomic loci, reflecting the particular clinical significance of deleterious RAD51 mutations. Integrated disease maps demonstrate RAD51 involvement both in loss-of-function conditions (Fanconi anemia) and in dysregulation or overexpression in malignant tumors of the breast, ovary, pancreas, and other organs.

**2. Nucleotide sequence: chromosomal location, ORFs, and functional mutations**

The NCBI Gene representation for RAD51 (Gene ID 5888) shows a single dominant open reading frame (ORF) corresponding to the full-length protein, as well as alternative transcripts that do not alter the conserved recombinase core. ORFfinder identifies the main ORF on the forward strand with a length corresponding to 339 amino acids, consistent with UniProt annotation and confirming the absence of additional long alternative reading frames. ClinVar variant

tables detail a spectrum of missense, nonsense, and splice-site mutations, among which certain alleles are classified as FANCR and cause complete loss of protein function.

### 3. Amino acid sequence: domain structure, conserved residues, functional sites

InterPro analysis classifies RAD51 as a member of the RecA/RadA/Rad51 family, characterized by a RecA-like ATPase core. The domain architecture reveals a large central P-loop NTPase domain containing Walker A and Walker B motifs, as well as a RAD51/RadA-specific segment responsible for nucleoprotein filament formation and stabilization. The N-terminal and C-terminal regions are predicted to be less ordered but are functionally important for regulation and interaction with BRCA2 and other cofactors.

Structural analyses highlight the L1 and L2 loops on the protein surface, which directly contact DNA. Key aromatic and hydrophobic residues within these loops include Phe97, Phe248, and Met251. High-resolution structures of RAD51 presynaptic filaments on ssDNA obtained by cryo-electron microscopy, together with crystal structures of full-length archaeal Rad51, indicate that conformational changes in these loops and ATP-binding motifs determine the transition between active and inactive filament states.

### 4. Three-dimensional structure and docking hypothesis

Three-dimensional structures of RAD51 obtained by X-ray crystallography and cryo-EM reveal the helical organization of the RAD51–ATP(ADP) nucleoprotein filament on single-stranded DNA, as well as a double-filament configuration in which two filaments interact with each other. Such double filaments explain the dynamics of RAD51 assembly and disassembly during homologous

recombination, as transitions between ADP- and ATP-bound states are accompanied by rearrangements of inter-filament contacts.

Schematics also illustrate known small-molecule modulators of RAD51. RS-1 is shown as a chemical stimulator that enhances filament assembly and may exploit RAD51 overexpression in tumor cells. Compounds B02 and DIDS are described as inhibitors that disrupt homologous pairing and strand exchange, partly through interactions with the L1/L2 loops. RI-1 is presented as a covalent inhibitor that modifies cysteine residues in RAD51 and blocks homologous recombination in human cells. CAM833 targets the BRCA2–RAD51 interaction interface, modulates filament assembly, and enhances cell death following DNA damage.

**5. BLAST analysis and identification of potential off-target ligands**

BLAST comparisons of RAD51 with other proteins reveal a group of highly homologous paralogs (RAD51B, RAD51C, RAD51D) and more distant homologs from archaea and bacteria (RadA, RecA). Closely related eukaryotic paralogs show high sequence identity within the catalytic core and substantial divergence in peripheral regions, indicating a shared ATP-dependent recombinase mechanism with specialization of regulatory elements. RadA/RecA homologs exhibit lower overall identity, but conservation of the P-loop NTPase core and DNA-binding motifs underscores the evolutionary conservation of homologous recombination mechanisms.

**6. Docking and visualization with selected ligands**

RS-1 is positioned in a manner that stabilizes the RAD51–ssDNA presynaptic filament: the molecule occupies a pocket between adjacent monomers, forming π–π stacking and hydrophobic contacts with aromatic residues Phe97, Phe248, and Met251 within the L1/L2 loops. This binding mode is consistent with the reported mechanism of filament assembly stimulation and exploitation of RAD51 overexpression in cancer cells.

The figure presents results of blind molecular docking of the RS-1 ligand to the RAD51 surface using automated pocket detection. Pocket 2, selected for detailed analysis, exhibits the lowest calculated binding energy (Score ≈ −9.2 kcal/mol), indicating thermodynamically favorable complex formation and identifying this site as the most promising candidate for functional interpretation.

## Sources


Shin DS, Pellegrini L, et al. (2003). Full-length archaeal Rad51 structure and mutants: mechanisms for RAD51 assembly and control by BRCA2. EMBO J. 22(17):4566-76.

Short JM, Liu Y, et al. (2016). High-resolution structure of the presynaptic RAD51 filament on single-stranded DNA by electron cryo-microscopy. Nucleic Acids Res. 44(19):9017-30.

Xu J, Zhao L, et al. (2018). Two distinct conformational states define the interaction of human RAD51-ATP with single-stranded DNA. EMBO J. 37(7):e98162.

Luo J, et al. (2023). A RAD51–ADP double filament structure unveils the mechanism of filament dynamics in homologous recombination. Nat Commun. 14(1):4885.

Jayathilaka K, Sheridan SD, et al. (2008). A chemical compound that stimulates the human homologous recombination protein RAD51. Proc Natl Acad Sci USA. 105(41):15848-53.

Mason JM, Logan HL, et al. (2014). The RAD51-stimulatory compound RS-1 can exploit the RAD51 overexpression that exists in cancer cells and tumors. Cancer Res. 74(13):3546-55.

Huang F, Mazina OM, et al. (2011). Identification of specific inhibitors of human RAD51 recombinase using high-throughput screening. ACS Chem Biol. 6(6):628-35.

Ishida T, et al. (2009). DIDS, a chemical compound that inhibits RAD51-mediated homologous pairing and strand exchange. Nucleic Acids Res. 37(10):3367-76.

Budke B, Logan HL, et al. (2012). RI-1: a chemical inhibitor of RAD51 that disrupts homologous recombination in human cells. Nucleic Acids Res. 40(15):7347-57.

Scott DE, et al. (2021). A small-molecule inhibitor of the BRCA2-RAD51 interaction modulates RAD51 assembly and potentiates DNA damage-induced cell death. Cell Chem Biol. 28(6):835-847.