|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Organism &References | Motif | Mutations | Effects | Equivalent mutation in human residue |
| *Saccharomyces cerevisiae* [1] | Walker A | K40AK40RK40E | 1. All mutation cause hypersensitivity to DNA damaging agent. Also showed homologous recombinant (HR) defects as Rad50 deletion.

Deficiency in non homologous end joining (NHEJ), defective in ATPase, ATP-dependent, DNA unwinding& ATP stimulated endonuclease activity. | K42AK42RK42E |
| T4 bacteriophage [2] | Walker A(WA)& D-loop | R37AN38AD512ND512AE514QE515A | 1. D512H and N38I are the naturally occuring mutation in the Cystic fibrosis transmembrane conductance regulator (CFTR) which lead to cystic fibrosis.
2. Mutation of D512N and D512A (D-loop) and N38A (WA) results in dramatic reduction in ATP affinity, hydrolysis rate and cooperativity.
3. D loop aspartate (D) fx tu stabilize the WA in a position favorable for catalysis.
4. N38 (asparigine) is crucial for mechanism of ATP hydrolysis by increase affinity to ATP & positioning the γ-phosphate of ATP for catalysis.

E514Q, E515A and R37A has little effects on ATP hydrolysis and nuclease activity of Mre11. | P37AN38AD1238ND1238AE1240QN1241A |
| *Saccharomyces cerevisiae* [3] | ATP binding domain &Walker A | K6ES14PR20ME21KG39D (WA)K40E (WA)V63EQ79KK81IN97DQ99KE915VA930P | 1. G39D and K40E : Both of this mutation confer a phenotype identical to Rad50 null mutation characterized by total defect in formation of viable spore.
2. G39D and K40E is highly conserved residue and analogous changes eliminate ATP hydrolysis (Personage et al. 1987; Sung et al. 1988 amd Reinstein et al. 1988).
3. From 9 residue located at ATPase at N terminal (K6E, S14P, R20M, E21K, V63E, Q79K, K81I, N97D), only K81I and R20M exhibit total phenotype defects in sporulation but still nearly to WT in term of resistance to MMS (DNA damage reagent).
4. Rad50 null mutation (complete deletion 3.9kb) showed 3 mitotic defects; 1) sensitive to MMS 2) defect in normal vegetative growth even in high rich media. 3) failure in DNA damage that arise spontaneously or during DNA replication.

Double mutation: E915V + A930P showed temperature sensitive. | K6ES14PK22MQ23KG41DK42ET65EQ81KR83IS99DV101KK921VS936P |
| *Mus musculus* [4] | ATP binding domain | K6E =K6EScK22M=R20MScR83I=K81Sc | 1. R83I and K6E: embryonic lethality.
2. K22M: Mice were viable, but have partial embryonic lethality, growth defect and cancer predisposition.
3. Cell culture derived from mutant mice showed increase spontaneous apoptosis which reduce chromosome stability. This mechanism lead to hematopoietic & spermatogenic depletion in mice.

Shorten lifespan associated with progressive loss of cells; die within 3 month. | K6EK22MR83I |
| *Deinococcus radiodurans* [5] | Walker A,Walker B& Signature motif | K39R (WA)K39M (WA)D303N (WB)S270R (SM) | 1. K39R=K36RE.coli: Mutation prevent ATP hydrolysis but not nucleotide binding. Shown to be critical for RecF function (Sander et al. 1992; Web et al. 1999).
2. K39R=K36ME.coli: Mutation prevent ATP binding.
3. D303N: In other SMC proteins, this changes traps ATP in its transition state & stabilized dimerization of the homodimer (Smith et al. 2002).

S270R: Equivalent mutation interfers with ATP dependent dimerization (Moncalian et al. 2004), | K42RK42MD1231NS1202R |
| *Thermotoga maritima* [6]*Saccharomyces cerevisiae* | ATPase binding domain&Walker B (WB)&Signature motif (SM) | K115EK175EK182ER94EK95ER765EE798Q (WB)S768R (SM)K99EK108EK109EK110ER125EK103EK104ER131ER1201EN190DS1205E+E1235Q | ***In vitro: Thermotoga maritima***1. Coiled-coil: K175E, K182E; substantially reduced DNA binding, but K115E almost abolish DNA interaction in vitro.
2. R94E & K95E: Important for DNA binding.
3. R765E: Significantly diminished DNA binding without affecting dimer formation in vitro.
4. E798Q: Showed strong DNA binding in the present of ATP and slightly low affinity to DNA compared to WT in the presence of AMPPNP.
5. S768R: Significantly reduced DNA binding in the presence of AMPPNP.

***In vivo:*** ***Saccharomyces cerevisiae***1. S1205R (SM)+E1235Q (WB): unable to rescue the impaired DNA damage response; showed importance function of ATP binding & ATP hydrolysis in the double strand break (DSB) repair activity.
2. K103E, K104E & R131E: Strongly affected DNA binding in vitro but little influence on the DSB repair fx. in vivo.
3. K110E & R125E: Insignificant reduction in plasmic recovery; do not involved in end joning process.
4. K103E, K104E & R131E: Moderate reduction in telomere length.
5. K103E+R131R & R1201E: Significantly reduced telomere length.
6. S1205R: Significantly reduced telomere length.
7. E1235Q: Fully proficient in telomere maintenance.

N190D: No effect on telomere | K132ET191EC221EK105ES106EG1199EE1232QS1202RE110KE126KK127EK122EK126EK105ES106EK132ER1198ET191DS1202R+E1232Q |
| *Mus musclulus* [7] | Zinc hook | S679RP682EV683R | 1. Double mutation S679R and P682E showed reduced zinc affinity and dimerization efficiency. Also showed lethality in mice. Same finding for V683R. Crossbreed with WT showed hydrocephalus, defects in primitive hematopoietic & gametogenic cells.

Therefore, this hook domain strongly influences Mre11 complex dependent DDR signaling, tissue homeostasis and tumorigenesis. | S679RP682EV683R |
| *Homo sapiens* [8] | Zinc hook | C680GC681GC684GR686AC681G+C684GC680G+C681G+C684G | 1. 6 mutation has been created (point mutatio, double and triple mutation)

Cystein in hook domain do not required for human Rad50 interaction with itseft and no significant effect on DNA binding.  | C680GC681GC684GR686A |
| *Saccharomyces cerevisiae* [9] | Zinc hook  | C684NC685AP686AV6871C688RQ689S | 1. Mutation 1: C684N + C685A + P686A + V6871 + C688R + Q689S

Mutation 2: C685A + C688A1. In vitro: mutant unable to bind to double strand break compred to WT.

In vivo: mutant also defective to be recriuted to chromosomal double strand break.1. Mutant phenotype as severe as Rad50 null mutant.

Rad50 hook mutant severely defects in various DNA damage response includes ATM activation, homologous recombinant, sensitive to irradiation and ATR activation. | C680NC681AP682AV683IC684RQ685S |
| T4 bacteriophage [10] | Zinc hook & coiled coil domain (ATPase domain) | C288SC291SC312SK211PK351PS183C | 1. Double mutation of C288S and C291S in T4 is lethal; indicate the ability to bind zinc2+ is critical for the functioning of MR complex.
2. Point mutation of zinc hook or coiled coil domain do not affect Mre11 and DNA binding, but their activation of Rad50 ATPase activity is abolish.
3. Deletion of coiled-coil and zinc hook eliminate Mre11 binding & ATPase activation but do not affect basal activity.

Therefore coiled coil enhance ATPase activity, major conduit for communication between Mre11 and Rad50 as well as support nucleoide excision. | C681SC684SC990SK256PN1028PM208C |
| *Saccharomyces cerevisiae* [11] | Zinc hook&coiled coil domain (ATPase domain) | S685RY688EY688RL689RI680VK700QL703FV285AN607YN873IS193F | 1. 3 mutation has been created in zinc hook.

Mutation Rad5046: S685R + Y688E; Rad5048: S685R + Y688R; Rad5047: L689R1. Sensitivity test: Rad5046 and Rad5047 shoewd partial sensitivity to MMS, hyroxyurea & camptothesin.

Rad5048 showed minimal sensitivity to MMS.1. Interaction: Rad5046; Rad50-Mre11 interaction was strongly impaired. Rad5047 and Rad5048 not clearly affected compared to WT.
2. Viabiliy: Sporulation efficiency and viability were severely impaired in Rad5046 followed by Rad5047. Rad5048 close to WT.
3. Rad5046 (S685R + Y688E ) also showed partial supression of telomere and meiotic defects.

Mre11 interaction was also partially improved in I680V, K700Q, V285A, N607Y, N873I and S193F. L703F allele restored the interaction, consistent with its pronounced effect on MMS resistance. | S679RP682EP682RV683RL673VL694QV697FM293AS603YQ886IQ194F |
| *Homo sapiens*[12]  | Zich hook | S635G  | 1. Characterised by chromosomal instability and defective ATM-dependent signalling.
2. The study found that ATM phosphorylates Rad50 at a single site (S635) that plays an important role in signalling for cell cycle control and DNA repair.
3. Even a Rad50 phosphosite-specific mutant (S635G) supported normal activation of ATM in Rad50 deficient cells, but it was defective in correcting DNA damage induced signalling through the ATM-dependent substrate SMC1.
4. This mutant also failed to correct radiosensitivity, DNA double strand break (DSB) repair and an S phase checkpoint defect in Rad50 deficient cells.

Demonstrated that the Rad50 S635 phosphorylation site plays a role in mediating the intra-S checkpoint after irradiation, and it is hypothesised to play a role as an ATM adaptor to regulate downstream ATM signalling. | S635G |
| *Pyrococcus furiosus* [13] | Signature motif &ATPase domain | R797GR805ER805WL802WL806FK155A | 1. L802W: Decrease dimerization in ATP & hydrolysis; also decrease in cleavage site
2. R805E: Increase dimerization and do not have significant proteolysis with WT.
3. In vivo; L802E=I1214WSc grown well in camptothecin (CPT) and showed rapid DNA repair after induced with HO endonuclease.

R805E=R1217ESc showed poorly grown in CPT; inability to repair endogenous DNA damage by HR. Also showed defect in resection which stll required 25kb for repair. And showed defect in resection in HO induced.1. I1214W + R1217E: Robust in promoting NHEJ; same as WT.
2. R1217E improve survival significantly compared WT and I1214W; after generating DSBs across genome.
3. R1217E: separation of function such that the resection are specifically impaired but NHEJ activity are intact& hyperactive; consistent with results in vitro.

I1214W: Nearly WT for all assays. | K1206GR1214ER1214WL1211WL1215FQ174A |
| *Schizo-saccharomyces pombe* [14] | Signature motif | K1187AK1187ER1195AR1195E | 1. K1187A: sensitive in higher dose of clastogens.
2. K1187E, R1195A & R1195E: significantly sensitive to clastogen agents and are deleterious as Rad50 null mutation.
 | K1206AK1206ER1214AR1214E |
| *Pyrococcus furiosus* [15] | Signature motif & Q loop | S793RQ140H | 1. S793R=S1202R H.sapiens: Analogs to the mutation in CFTR gene that results cystic fibrosis.
2. S793R: This mutation prevent ATP binding & disrupts the communication among the other ATP loops. This structural changes, in turn, altrs th interaction among rad50 monomers and thus prevents Rad50 dimerization.
3. S1202R: In human genes; mutant protein did form MRN complex but significantly dificient in all ATP-dependent enzymatic activities.
4. S1205R S. cerevisiae: Failed to complement Rad50 deletion strain in DNA repair assay in vivo.

Q159H & S1202R: Mutant exhibited normal exonuclease & endonuclease activity, while all of the ATP-dependent activities of the complex were completely abrogated. | S1202RQ159H |
| T4 bacteriophage [16] | Signature motif | S471AS471RS471ME472GE474QK475M | 1. S471A, S471R &S471M: Reduced the level of DNA activation of ATPase suggesting this residues involved in the allosteric transmission between DNA & ATP binding sites. Decreased ATP cooperativity is vary depend on the side chain alteration; indicated that signature motif involved in communication between ATP site.
2. Same finding for E474Q and K475M.

E472G: No effect on DNA activation. | S1202AS1202RS1202MA1203GQ1205EK1206M |
| *Saccharomyces cerevisiae**Pyrococcus furiosus**Homo sapiens*[17] | Signature motif | S1205RS793RS1202R | 1. Mutation at conserved SM (S1202R) reduced adenylate kinase activity for DNA tethering reaction but proficient in ATP hydrolysis. This mutant resembles Rad50 null strain in term of meiosis and telomere maintenance in S. cerevisiae
2. S793R: Found dificient in ATP-dependent dimer formation & ATP binding (Hofner et al. 2004). Previous finding; low affinity to ATP (Moncalian et al. 2004).
3. S793R: This mutation was modelled after an analogous mutation in CFTR (S549R) that results in cystic fibrosis (Kerem et al. 1989).
4. Compared to P. furiosus; equivalent mutation in S1202R H. sapiens and S1205 S. cerevisiae do not inhibit ATPase activity.
5. S1202R & S1205R: Level of adenylate kinase significantly (10-12 fold) lower than WT.
6. S1205R: Its phenotype equivalent to null Rad50 mutation in yeast which respect to MMS & bleomycin resistance (Moncalian et al. 2004). This mutant also fails to complement the sequence in assays of NHEJ in deletion strain in vivo (Zhang & paull 2005). This mutation also do not support spore viability (Bhaskara et al. 2007). Last; the telomere were severely shortened as in Rad50 deletion strain.

S1205R: Mutation showed high level of DNA tethering, but not stimulated by ATP. In contast mutation in Walker A; K40A S. cerevisiae (K42AHS) completely block all DNA tethering, consistent with prevous report by Chen et al. 2005. | S1202R |
| *Homo sapiens* [18] | ATPase domain | R1093X | 1. Nijmegen Breakage Syndrome like disorder (NBSLD). Event where a patient diagnosed with smaller head (microcephaly), mental retardation, ‘bird like’ face and short stature due to Rad50 dificiency.
2. ToFurther studies of this patient’s fibroblasts and lymphoblastoid cells (LCL) revealed that this patient had RAD50 protein deficiency (Waltes et al., 2009). Sequencing of the RAD50 cDNA of this patient revealed compound heterozygosity for mutations that appeared to have hypomorphic effect.

The mutation (c.3277C/T; p.R1093X) on exon 21 was maternally inherited. This mutation created a premature termination codon, thus producing a truncated RAD50 protein. The second mutation on exon 25 (c.3939A/T) was inherited paternally. This mutation changed the stop codon of RAD50 to a tyrosine codon, thereby producing a larger RAD50 peptide. How these mutations affect the low level of RAD50 protein expression in this patient remains to be elucidated (Waltes et al., 2009). | R1093X |
| References1. Chen L, Trujillo KM, Van Komen S, Roh DH, Krejci L, Lewis LK, et al. Effect of amino acid substitutions in the rad50 ATP binding domain on DNA double strand break repair in yeast. J Biol Chem. 2005;280: 2620–2627. doi:10.1074/jbc.M4101922002. De La Rosa Metzere Bierlein, and Scott W. Nelson. An Interaction between the Walker A and D-loop Motifs Is Critical to ATP Hydrolysis and Cooperativity in Bacteriophage. J Biol Chem. 2011;286: 26258–26266. doi:10.1074/jbc.M111.2563053. Alani E, Padmore R, Kleckner N. Analysis of wild-type and rad50 mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell. 1990;61: 419–436. doi:10.1016/0092-8674(90)90524-I4. Bender CF, Sikes ML, Sullivan R, Huye LE, Le Beau MM, Roth DB, et al. Cancer predisposition and hematopoietic failure in Rad50S/S mice. Genes Dev. 2002;16: 2237–2251. doi:10.1101/gad.10079025. Koroleva O, Makharashvili N, Courcelle CT, Courcelle J, Korolev S. Structural conservation of RecF and Rad50: implications for DNA recognition and RecF function. EMBO J. 2007;26: 867–77. doi:10.1038/sj.emboj.76015376. Rojowska A, Lammens K, Seifert FU, Direnberger C, Feldmann H. Structure of the Rad 50 DNA double-strand break repair protein in complex with DNA. EMBO J. 2014;33: 2847–2859. doi:10.15252/embj.2014888897. Roset R, Inagaki A, Hohl M, Brenet F, Lafrance-Vanasse J, Lange J, et al. The Rad50 hook domain regulates DNA damage signaling and tumorigenesis. Genes Dev. 2014;28: 451–462. doi:10.1101/gad.236745.1138. Cahill D, Carney JP. Dimerization of the Rad50 protein is independent of the conserved hook domain. Mutagenesis. 2007;22: 269–274. doi:10.1093/mutage/gem0119. He J, Shi LZ, Truong LN, Lu CS, Razavian N, Li Y, et al. Rad50 zinc hook is important for the Mre11 complex to bind chromosomal DNA double-stranded breaks and initiate various DNA damage responses. J Biol Chem. 2012;287: 31747–31756. doi:10.1074/jbc.M112.38475010. Barfoot T, Herdendorf TJ, Behning BR, Stohr BA, Gao Y, Kreuzer KN, et al. Functional analysis of the bacteriophage T4 Rad50 Homolog (gp46) Coiled-coil Domain. J Biol Chem. 2015;290: 23905–23915. doi:10.1074/jbc.M115.67513211. Hohl M, Kocha??czyk T, Tous C, Aguilera A, KrEzel A, Petrini JHJ. Interdependence of the Rad50 Hook and Globular domain functions. Mol Cell. 2015;57: 479–492. doi:10.1016/j.molcel.2014.12.01812. Gatei M, Jakob B, Chen P, Kijas AW, Becherel OJ, Gueven N, et al. ATM protein-dependent phosphorylation of Rad50 protein Regulates DNA repair and cell cycle control. J Biol Chem. 2011;286: 31542–31556. doi:10.1074/jbc.M111.25815213. Deshpande RA, Williams GJ, Limbo O, Williams RS, Kuhnlein J, Lee JH, et al. ATP-driven Rad50 conformations regulate DNA tethering, end resection, and ATM checkpoint signaling. EMBO J. 2014;33: 482–500. doi:10.1002/embj.20138610014. Williams GJ, Williams RS, Williams JS, Moncalian G, Arvai AS, Limbo O, et al. ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. Nat Struct Mol Biol. 2011;18: 423–431. doi:10.1038/nsmb0911-1084c15. Moncalian G, Lengsfeld B, Bhaskara V, Hopfner KP, Karcher A, Alden E, et al. The Rad50 Signature Motif: Essential to ATP Binding and Biological Function. J Mol Biol. 2004;335: 937–951. doi:10.1016/j.jmb.2003.11.02616. Herdendorf TJ, Nelson SW. Functional evaluation of bacteriophage T4 Rad50 signature motif residues. Biochemistry. 2011;50: 6030–6040. doi:10.1021/bi200184w17. Bhaskara V, Dupr A, Lengsfeld B, Hopkins BB, Chan A, Lee JH, et al. Rad50 Adenylate Kinase Activity Regulates DNA Tethering by Mre11/Rad50 Complexes. Mol Cell. 2007;25: 647–661. doi:10.1016/j.molcel.2007.01.02818. Waltes R, Kalb R, Gatei M, Kijas AW, Stumm M, Sobeck A, et al. Human RAD50 Deficiency in a Nijmegen Breakage Syndrome-like Disorder. Am J Hum Genet. 2009;84: 605–616. doi:10.1016/j.ajhg.2009.04.010 |