**Mutation Table**

02/03/2015

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| **SIRTUIN** | **Position** | **Functions** | **Ref.** |
| **ySir2** | H364Y | * Abolishes both its enzymatic activity *in vitro* and its silencing functions *in vivo*
 | Tanny, J.C., Dowd, G.J., Huang, J., Hilz, H., and Moazed, D. (1999). An enzymatic activity in the yeast Sir2 protein that is essential for gene silencing. Cell 99, 735–745. |
| N345A | * Abolishes its enzymatic activity and the minor ADP-ribosyltransferase activities
 | Imai, S., Armstrong, C.M., Kaeberlein, M., and Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD dependent histone deacetylase. Nature 403, 795–800. |
| F274N | * Decrease in deacetylation activity in vitro and transcriptional silencing *in vivo.*
 | Armstrong, C.M., Kaeberlein, M., Imai, S.I., and Guarente, L. (2002). Mutations in Saccharomyces cerevisiae gene SIR2 can have differential effects on*in vivo* silencing phenotypes and in vitro histone deacetylation activity. Mol. Biol. Cell 13, 1427–1438. |
| F33 | * The invariant flexible loop residue Phe33 appears to play a critical role both in the initial reaction steps
* The orientation of Phe33 is also likely to be a key mediator of the nicotinamide exchange reaction
* In this orientation (the side chain of Phe33 is shifted from its position in the ternary complex above the ribose oxygen to a position adjacent to the ribose C1’ position), Phe33 occludes the nicotinamide bound in the hydrophobic C pocket from the O-acetyl ADP ribose bound in the NAD+.
* This positioning has been postulated to block the cleaved nicotinamide for reacting with the O-alkylamidate intermediate
 |
| **HST2** | S36A | * Significantly reduce NAD-NAM exchange and deacetylation activities
 | Min, J. Landry, J. Sternglanz, R., Xu, R.M. (2001) Crystal structure of sir2 homolog-NAD-complex. Cell 105, 269-279. |
| Q115A |
| H135A |
| E186A |
| S36A | * Map to the B or the C NAD binding site.
* Enzymatic activity is severely affected by mutations
* Conserved residues at the C site are directly involved in catalysis.
 |
| Q115A |
| H135A |
| N345A |
| H135A | * Acts as a general base and activate the 2’-OH for nucleophilic attack on the C-1’ iminium intermediate
* His-135 and the 2’-OH of the nicotinamide ribose are not essential for the first step of the chemical mechanism, cleavage of the nicotinamide-ribosyl C-N bond.
 | Jackson, M. D., et al (2003) Mechanism of nicotinamide inhibition and transglycosideation by Sir2 histone/ protein deacetylases, J. Bio. Chem. 278, 50985-50998.) |
| H135A | * Conserved histidine (His-135 in HST2) activates the ribose 2’-hydroxyl for attack on the a -1’-O-alkylamidate.
 | Smith, B and Denu, J.M. (2006) Sir2 protein deacetylases: evidence for chemical intermediates and functions of a conserved histidine. Biochemistry 45, 272-282 |
| G32A | Km(NAD)uM | Km(peptide) uM | Kcat, min-1 | Decrease in affinity for NAD and a role in NAD binding | Khan, A.N. and Lewis, P.N. (2006) Use of substrate analogs and mutagenesis to study substrate binding and catalysis in the Sir2 family of NAD-depepent protein deacetylases. J. Biol. Chem. 281, 11702-11711. |
| NA | NA | Background |
| S36A | 49.8±0.6 | 1.42±0.02 | 0.34±0.07 | Decrease in affinity for NAD and a role in NAD binding |
| R45A | 10.3±1.8 | 0.86±0.06 | 0.15±0.04 | Plays a role in catalytic steps beyond the initial NAM cleavage and/or in stabilizing the acetyl-lysine-ADPR intermediate. |
| N116A | NA | NA | Background | Asn116 hydrogen-bonds with a structurally conserved catalytic water molecule that is proposed to carry out nucleophilic attack to resolve the cyclic intermediate |
| H135A | 16.9±0.6 | 0.88±0.10 | 0.1±0.03 | NAD binding |
| K178A | 11.6±0.1 | 0.68±0.09 | 0.1±0.02 | Located in the small zinc-binding domain, away from the enzyme’s active site; Does not play a direct role in catalysis; The correct positioning of the small domain is essential for proper orientation of the two substrates in the active site.  |
| V228A | 12.5±0.6 | 1.91±0.11 | 0.89±0.21 | Acetyl-lysine binding |
| P230A | 9.5±1.2 | 2.23±0.45 | 0.1±0.03 | Acetyl-lysine binding |
| N248A | 17.9±1.6 | 0.78±0.07 | 0.25±0.06 | Results in inefficient cleavage of NAM, with decrease in overall catalytic activity, and decrease binding affinity for NAD rather than a defect in catalysis. |
| D263A | NA | NA | Background | Buried within the large domain away from theHst2 active site |
| H338A | 12.2±1.1 | 1.68±0.11 | 1.03±0.25 |  |
| I117F | * A nicotinamide inhibition and base-exchange site (D pocket) that is distinct from the so-called ‘‘C pocket’’ binding site for the nicotinamide group of NAD+ was identified
 | Sanders, B.D. Zhao, K. Slama, J.T. Marmorstein, R. (2007) Structural basis for NAM inhibition and base exchange in Sir2 enzymes. Mol. Cell 25, 463-472. |
| I117V |
| **Sir2Af1** | S24A | * Significantly reduce NAD-NAM exchange and deacetylation activities
 | Min, J. Landry, J. Sternglanz, R., Xu, R.M. (2001) Crystal structure of sir2 homolog-NAD-complex. Cell 105, 269-279. |
| Q98A |
| H116A |
| E161A |
| S24A | * Map to the B or the C NAD binding site.
* Enzymatic activity is severely affected by mutations
* Conserved residues at the C site are directly involved in catalysis.
 |
| Q98A |
| H116A |
| E161A |
| N99 |
| S24A | * This residue may be involved in the cleavage of the glycosidic linkage between NAM and ribose
 | Min, J., Landry, J., Sternglanz, R., and Xu, R. M. (2001) *Cell* **105,** 269–279. Chang, J.H. Kim, H.C. Hwang, K.Y. Lee, J.W. Jackson, S.P. Bell, S.D. Cho, Y. (2002) Structural basis for the NAD-dependent deacetyase mechanism of sir2. J Biol. Chem. 277, 34489-34498. |
| R33A |  |
| E45A |  |
| H80N | * Minor effect on enzyme activity and NAD binding
 |
| D101N | * Although the primary role of Asp101 in site C is to stabilize the local structure, we cannot completely exclude the possibility that this residue may act as a base in the deprotonation of 2’-OH or 3’-OH
 |
| F159A | * Significantly affects the deacetylation activity. Phe-159 may guide the substrate into the correct position in the active site during catalysis.
 |
| H116D | * His-116 formed a hydrogen bond to the O3’ of NAD
 |
| H116N | * Deprotonates the 2’-OH directly or indirectly through 3’-OH activation.
 |
| **Sir2Af2** | M70R | * Increase desuccinylation activity
 | Fischer, F, Gertz, M. Suenkel, B. Lakshminarasimhan, M. Schutkowski, M. Steegborn, C. (2012) Sirt5 deaceylation acetivities show differential sensitivities to nicotinamide inhibition. PLOS One 7, e45098. |
| **Sir2Tm** | D101N | * Asp101 is a key residue in the C pocket for NAM binding.
* Mutation in the C pocket enables the enzyme to catalyze NAAD-dependent deacetylation, be inhibited by nicotinic acid, and synthesize NAAD from NAD+ and nicotinic acid strongly supports the role of C pocket as the sole NAM binding site in sirtuins.
* Lead to not only a weaker binding of the substrate but also a significant loss in NAD+-dependent deacetylation activity.
* Since Asp101 directly forms a hydrogen bond with the amide group of the nicotinamide fragment which carries significant positive charge in the reactant complex, it is expected that the mutation would result in the weak binding of the substrate.
 | Avalos, J. L.; Bever, K. M.; Wolberger, C. (2005) Mechanism of Sirtuin Inhibition by Nicotinamide: Altering the NAD(+) Cosubstrate Specificity of a Sir2 Enzyme. Mol. Cell, 17, 855−868. |
| H116A | * NAD+ orientation is not dependent on contacts with His116
* The histidine is likely needed as a general base in later reaction steps
 | Hoff, K. G., Avalos, J. L., Sens, K., Wolberger, C. (2006) Insights into the Sirtuin mechanism from ternary complexes containing NAD+ and acetylated peptide, Structure 14, 1231-1240. |
| H116Y | * the acetyl lysine side chain and the side chain of Phe33 may aid in portioning NAD+ for catalysis
 |
| Q115 | * Another key factor in binding and positioning NAD+ into its productive conformation.
 | Liang, Z.; et al. Investigation of the Catalytic Mechanism of Sir2 Enzyme with QM/MM Approach: SN1 vs SN2? J. Phys. Chem. B 2010, 114, 11927−11933. |
| N165D | * A negatively charged residue at position 165 increases the Sir2Tm affinity for peptides that contain a positively charged side chain at peptide position -1, but not for peptides with an alanine at position -1
 | Cosgrove, M.S. Bever, K. Avalos, J.L. Muhammad, S. Zhang, X. Wolberger, C. (2006) The structural basis of sirtuin substrate affinity. Biochemistry 45, 7511-7521 |
| N165A | * A Sir2Tm with an alanine at position 165 is not able to discriminate among this group of substrates. Therefore, the identity of the residue at position 165 in Sir2Tm likely plays an important role in sirtuin substrate recognition.
 |
| F33A | * The role of Phe33 in protecting the O-alkylamidate intermediate from hydrolysis and the base exchange reaction with nicotinamide, which regenerates acetyl-lysine and NAD+,
 | Hawse, W.F. et al (2008) Structural insights into intermediate steps in the Sir2 deacetylation reaction, Structure 16, 1368-1377.) |
| D101N | * The D101N mutation would lead to the disruption of key hydrogen bonds in the nicotinamide binding pocket and the change of the binding conformation of NAD+
 | Hu, P. Wang, S. Zhang, Y. (2008) Highly dissociative and concerted mechanism for the nicotinamide cleavage reaction in Sir2Tm enzyme suggested by Ab initio QM/MM molecular dynamics simulations. J. Am. Chem. Soc. 130, 16721-16728 |
| **SIRT1** | F273L | Mutants showed dramatic decrease of sensitivities to compound 4 indicating that the side chains of the residues take part in the inhibitor interaction.Compound 4:   The compounds were predicted to bind to the C and D pockets of SIRT1 | Wu, J. Li, J. Xu, MH. Liu, D. (2014) Structure–activity relationship and interaction studies of new SIRT1inhibitors with the scaffold of 3-(furan-2-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole. Bioorg. & Med. Che. Lett 24, 3050-3056. |
| I347A |
| F414A |
| **SIRT2** | H187A | * Displayed ~3000-fold lower catalytic efficiency compared with wild type enzyme.
* Used as control to ensure any observed effects were due to the catalytic activity of enzyme.
 | Borra, M.T. O’Neill, F.J. Jackson, M.D. Marshall, B. Verdin, E. Foltz, K.R. Denu, J.M. (2002) Conserved enzymatic production and biological effect of O-acetyl-ADP-ribose by silent information regulator 2- like NAD-dependent deacetylases. J. Biol. Chem. 277, 12632-12641. |
| H171Y | * Abolishes its enzymatic activity
 | Frye, R.A. (1999). Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem. Biophys. Res. Commun. 260, 273–279. |
| Q167A | * Reduced the SIRT2 histone deacetylase activity, B site
 | Finnin, M.S. Donigian, J.R. Pavletich, N.P. (2001) Structure of the histone deacetylase SIRT2. Nature Structural Biology. 8, 621-625. |
| H187A |
| N168 | * Reduced the SIRT2 histone deacetylase activity, C site
 |
| D170 |
| **SIRT5** | T69D | * Loss activity (locating in the flexible cofactor binding loop)
 | Fischer, F, Gertz, M. Suenkel, B. Lakshminarasimhan, M. Schutkowski, M. Steegborn, C. (2012) Sirt5 deaceylation acetivities show differential sensitivities to nicotinamide inhibition. PLOS One 7, e45098. |
| R105L | * Retained WT deacetylation activity(WT:2.10.4nmol.mg-1.min-1, R105L:2.4±0.1nmol.mg-1.min-1). Partially gained SIRT3-like NAM sensitivity (IC50=18923uM)
 |
| R105M | * Loss the desuccinylase activity
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