The bacterial transcriptional regulator RutR is controlled by lysine acetylation.

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In eukaryotes histone acetylation is known to regulate gene expression. It is less well understood how lysine acetylation modulates and regulates gene expression in prokaryotes. Bacteria live in rapidly changing environments such as nutrient availability. This requires to precisely sense the metabolic state and translate this in altered protein functionalities. Bacteria use one-component systems of the TetR family to adjust gene expression programs to dynamic changes in the environment, such as nutrient availability . The TetR-family member RutR (pyrimidine utilization repressor) is a transcriptional repressor for the *rutA-G* operon involved in pyrimidine degradation. Additionally, RutR indirectly activates transcription of the *carAB* operon involved in pyrimidine biosynthesis [1-3]. Belonging to the TetR-family RutR is structurally composed of an N-terminal DNA-binding domain (DBD) encompassing a helix-turn-helix (HTH) motif (α2-α3) for binding to the major groove of the DNA and a C-terminal ligand-binding domain (LBD; α4-α9) [4,5]. Binding of uracil to this LBD is suggested to stabilize a conformation that is incompatible with DNA binding [1]. Recently, RutR was shown to be lysine acetylated at five distinct sites in the DBD and LBD [6-9]. However, how lysine acetylation affects RutR function is not known. We discovered additional acetylation sites in the unfolded RutR N-terminal tail preceding the DBD. Applying genetic code expansion using a synthetically evolved acetyl-lysyl-tRNA-synthetase (AcKRS3)/$tRNA_{CUA}$ (*Mb*PylT)-pair from *Methanosarcina barkeri*, we produced site-specifically lysine-acetylated RutR proteins in yield and purity suitable for biophysical studies including X-ray crystallography [10-13]. We solved a crystal structure of K52-acetylated RutR showing that K52-acetylation switches-off RutR DNA-binding exerting a steric and electrostatic mechanism. The interactions of acetylated RutR-variants with *carAB* and *rutAB* dsDNA operator sequences were analysed thermodynamically by isothermal titration calorimetry (ITC) and by electrophoretic mobility shift assays. For the first time, we applied the genetic code expansion concept in *E. coli in vivo* showing that RutR-acetylation modulates its transcriptional regulator activity. Finally, we show that RutR acetylation is catalysed enzymatically by *E. coli* lysine acetyltransferases and non-enzymatically by acetyl-phosphate. The sirtuin deacetylase CobB can revert RutR acetylation at the functionally important sites. As a model, we conclude that prokaryotes apply lysine acetylation of transcriptional regulators as sensors of the cellular metabolic state directly adjusting gene expression programs allowing to rapidly adjust to changing environmental conditions.

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[1.] Nguyen Le Minh, P., de Cima, S., Bervoets, I., Maes, D., Rubio, V., Charlier, D.: Ligand binding specificity of RutR, a member of the TetR family of transcription regulators in Escherichia coli. FEBS Open Bio **5**, 76-84 (2015). doi:10.1016/j.fob.2015.01.002

[2.] Shimada, T., Hirao, K., Kori, A., Yamamoto, K., Ishihama, A.: RutR is the uracil/thymine-sensing master regulator of a set of genes for synthesis and degradation of pyrimidines. Mol Microbiol **66**(3), 744-757 (2007). doi:10.1111/j.1365-2958.2007.05954.x

[3.] Nguyen Ple, M., Bervoets, I., Maes, D., Charlier, D.: The protein-DNA contacts in RutR*carAB operator complexes. Nucleic Acids Res **38**(18), 6286-6300 (2010). doi:10.1093/nar/gkq385

[4.] Palm, G.J., Buchholz, I., Werten, S., Girbardt, B., Berndt, L., Delcea, M., Hinrichs, W.: Thermodynamics, cooperativity and stability of the tetracycline repressor (TetR) upon tetracycline binding. Biochim Biophys Acta Proteins Proteom **1868**(6), 140404 (2020). doi:10.1016/j.bbapap.2020.140404
[5.] Orth, P., Schnappinger, D., Hillen, W., Saenger, W., Hinrichs, W.: Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system. Nat Struct Biol **7**(3), 215-219 (2000). doi:10.1038/73324
[6.] Kremer, M., Kuhlmann, N., Lechner, M., Baldus, L., Lammers, M.: Comment on 'YcgC represents a new protein deacetylase family in prokaryotes'. Elife **7** (2018). doi:10.7554/eLife.37798
[7.] Tu, S., Guo, S.J., Chen, C.S., Liu, C.X., Jiang, H.W., Ge, F., Deng, J.Y., Zhou, Y.M., Czajkowsky, D.M., Li, Y., Qi, B.R., Ahn, Y.H., Cole, P.A., Zhu, H., Tao, S.C.: YcgC represents a new protein deacetylase family in prokaryotes. Elife **4** (2015). doi:10.7554/eLife.05322
[8.] Venkat, S., Chen, H., Stahman, A., Hudson, D., McGuire, P., Gan, Q., Fan, C.: Characterizing Lysine Acetylation of Isocitrate Dehydrogenase in Escherichia coli. J Mol Biol **430**(13), 1901-1911 (2018). doi:10.1016/j.jmb.2018.04.031
[9.] Venkat, S., Gregory, C., Gan, Q., Fan, C.: Biochemical Characterization of the Lysine Acetylation of Tyrosyl-tRNA Synthetase in Escherichia coli. Chembiochem **18**(19), 1928-1934 (2017). doi:10.1002/cbic.201700343
[10.] Lammers, M.: Expression and Purification of Site-Specifically Lysine-Acetylated and Natively-Folded Proteins for Biophysical Investigations. Methods Mol Biol **1728**, 169-190 (2018). doi:10.1007/978-1-4939-7574-7_11
[11.] Graf, L.G., Vogt, R., Blasl, A.T., Qin, C., Schulze, S., Zuhlke, D., Sievers, S., Lammers, M.: Assays to Study Enzymatic and Non-Enzymatic Protein Lysine Acetylation In Vitro. Curr Protoc **1**(11), e277 (2021). doi:10.1002/cpz1.277
[12.] Lammers, M., Neumann, H., Chin, J.W., James, L.C.: Acetylation regulates cyclophilin A catalysis, immunosuppression and HIV isomerization. Nat Chem Biol **6**(5), 331-337 (2010). doi:10.1038/nchembio.342
[13.] Neumann, H., Peak-Chew, S.Y., Chin, J.W.: Genetically encoding N(epsilon)-acetyllysine in recombinant proteins. Nat Chem Biol **4**(4), 232-234 (2008). doi:10.1038/nchembio.73