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SEEING THE INVISIBLE – STUDY OF TRANSIENTLY FORMED PROTEIN CONFORMATION FOUND IN DOMAIN 1.1 OF BACTERIAL TRANSCRIPTION FACTOR

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Biomolecules undergo a variety of motions at various timescales. Motions at microsecond to millisecond timescales are often associated with transitions between ground states and higher energy states. Methods of structural biology allow detailed characterization of ground state structure and dynamics. However, the studies of higher energy state conformations are more difficult, because of their low occupancies and short lifetimes. Due to this fact, studies of excited states are often omitted. But nuclear magnetic resonance provides methods to investigate motions associated with these transitions and structures of excited states.

In here, we investigate such conformational exchange, between well-defined ground state and transiently formed excited state which has been detected in domain 1.1 of primary sigma transcription factor from *Bacillus subtillis*. Sigma factor is essential for initiating the process of transcription, a fundamental cellular process. With the use of relaxation dispersion experiments we obtained structural information about orientations of bond vectors and secondary structure propensities within the excited state. Our results suggest that the excited state (populated only about 3% in the solution at 25 °C) has significantly lower propensity to form a stable secondary structure in the regions of helix I and helix III compared to the ground state.

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A UBIQUITOUS DISORDERED PROTEIN INTERACTION MODULE ORCHESTRATES TRANSCRIPTION ELONGATION

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During eukaryotic transcription elongation, RNA polymerase II (RNAP2) is regulated by a chorus of factors. Here, we identified a common binary interaction module consisting of TFIIS N-terminal domains (TNDs) and natively unstructured TND-interacting motifs (TIMs). This module was conserved among the elongation machinery and linked complexes including transcription factor TFIIS, Mediator, super elongation complex, elongin, IWS1, SPT6, PP1-PNUTS phosphatase, H3K36me3 readers, and other factors. Using nuclear magnetic resonance, live-cell microscopy, and mass spectrometry, we revealed the structural basis for these interactions and found that TND-TIM

sequences were necessary and sufficient to induce strong and specific colocalization in the crowded nuclear environment. Disruption of a single TIM in IWS1 induced robust changes in gene expression and RNAP2 elongation dynamics, which underscores the functional importance of TND-TIM surfaces for transcription elongation.The references should be listed immediately at the end of text without a heading.

1.Cermakova et. al, *Science*, **374** (2021), 1113-1121.

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STRUCTURES OF PHAGE 812 NECK SUGGEST A MECHANISM FOR GENOME RETENTION AND RELEASE IN *HERELLEVIRIDAE*

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Herelleviridae phage 812 infects a wide range of *S. aureus* strains and is considered a promising candidate for phage therapy against bacterial infections, including those resistant to antibiotics. The virion is formed by an icosahedral capsid enclosing a double-stranded DNA genome, and a double layered contractile tail terminated by a baseplate. The neck region joins the capsid to the tail and functions in capsid assembly, genome packaging, its retention, and release. The neck consists of four protein complexes forming the DNA channel: portal, adaptor, stopper, and tail terminator. We determined the structural features of the neck in virion, genome-releasing intermediate and empty particle using cryo-electron microscopy, as well as the structure of a closed conformation of the stopper complex by X-ray crystallography. The portal provides a dodecameric DNA binding site anchoring the genomic end inside the capsid. This high-avidity binding site recognizes B-form DNA shape with locally narrowed minor groove. The DNA binding may be important during the initial stages of genome packaging to prevent DNA escape from the capsid caused by packaging terminase slippage. In the late stages of packaging, high genome pressure inside the phage head hinders DNA escape by inducing a compression of the portal crown which obstructs the opening of portal tunnel valve loops. The stopper hexamer blocks genome escape in the packaging intermediate particle prior to tail attachment. The stopper gating function involves a shifting of a zinc-ribbon loop and a strand-to-helix conformational switching of the gating valve loop. After the attachment of the tail, the stopper adopts an open conformation and primes the DNA for release by allowing its descent toward the tail. The study of structural changes in phage 812 neck proteins throughout stages of phage life cycle thus expands our understanding of the functions fulfilled by the head-to-tail joining region of the viral particle.

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HOW DO SorC PROTEINS RECOGNIZE THEIR DNA OPERATORS?

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The SorC family is a large family of bacterial transcription regulators involved in the control of carbohydrate metabolism and quorum-sensing [1, 2]. SorC protomers constitute an N-terminal helix-turn-helix DNA-binding domain (DBD) and a C-terminal effector-binding domain (EBD) that perceives a change in the environment by binding a phosphor-sugar molecule which affects oligomerization state and subsequently the affinity to the DNA operator.

We have studied the mechanism of how these proteins recognize their DNA and determined crystal structures of DBDs of two representatives, *bs*CggR and *bs*DeoR from *Bacillus subtilis* in complex with their DNA operators [3]. In previous studies, EBDs of these two proteins was also determined in their free form and in complex with their effector molecules [4, 5]. Our next question was how these proteins bind DNA in their full-length forms and what structural mechanism underlies the affinity change. To unveil this, we have combined methods of X-ray crystallography and cryogenic electron microscopy (cryo-EM), thus completing the partial structural information about separated EBDs and DBDs [3-5]. To gain an overall picture of *bs*CggR and *bs*DeoR in complex with their cognate DNA

duplexes, we determined 4.5 Å cryo-EM structure and 3.6 Å resolution crystal structure of *bs*CggR and *bs*DeoR DNA complexes, respectively. These models represent the first comprehensive insight into the DNA recognition by the SorC protein family.

1. Fillinger, S., Boschi-Muller, S., Azza, S., Dervyn, E., Branlant, G. and Aymerich, S. (2000) Two glyceraldehyde-3-phosphate dehydrogenases with opposite physiological roles in a nonphotosynthetic bacterium. *J Biol Chem*, **275**, 14031-14037.
2. Taga, M.E., Semmelhack, J.L. and Bassler, B.L. (2001) The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in Salmonella typhimurium. *Mol Microbiol*, **42**, 777-793.
3. Šoltysová, M.S., I.; Fábry, M.; Brynda, J.; Škerlová, J.; Řezáčová, P. (2021) Structural insight into DNA recognition by bacterial transcriptional regulators of the SorC/DeoR family. *Acta Crystallographica D Structural Biology*, **77**, 1411-1424.
4. Rezácová, P., Kozísek, M., Moy, S.F., Sieglová, I., Joachimiak, A., Machius, M. and Otwinowski, Z. (2008) Crystal structures of the effector-binding domain of repressor Central glycolytic gene Regulator from Bacillus subtilis reveal ligand-induced structural changes upon binding of several glycolytic intermediates. *Mol Microbiol*, **69**, 895-910.
5. Škerlová, J., Fábry, M., Hubálek, M., Otwinowski, Z. and Rezáčová, P. (2014) Structure of the effector-binding domain of deoxyribonucleoside regulator DeoR from Bacillus subtilis. *Febs J*, **281**, 4280-4292.

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STRUCTURE-FUNCTIONAL INSIGHTS INTO THE DANTROLENE BINDING SITE OF THE HUMAN CARDIAC RYANODINE RECEPTOR: TOWARDS A DEEPER UNDERSTANDING OF HEART ARRHYTHMIAS

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Ryanodine receptors (RyRs) are the largest known ion channels. Their main physiological role is to release calcium from the sarcoplasmic/endoplasmic reticulum into the cytosol of muscle cells. Three isoforms of this channel (1-3) have been identified in humans; isoforms 1 and 2 (RyR1 and 2) are predominantly expressed in skeletal and cardiac muscle, respectively. Mutations identified in the genes of the human RyR1 and 2 isoforms are responsible for several severe dystrophies and cardiac arrhythmias, which often result in death at a young age [1].

Dantrolene is a postsynaptic muscle relaxant that decreases excitation-contraction coupling in muscle cells. It is currently used as an effective treatment for malignant hyperthermia, muscle spasticity, neuroleptic malignant syndrome and it is also used during resuscitation [2]. It has been shown that dantrolene binds to the skeletal (RyR1) and cardiac (RyR2) muscle ryanodine receptors that are responsible for Ca^{2+} release from the sarcoplasmic reticulum. Dantrolene binding sites are found in both RyR1 and RyR2 isoforms [3]. Although the structure of the RyR1 and RyR2 N-terminal domain has been solved, little is known about the molecular basis of its interaction with dantrolene. In the present work we have prepared several mutants of the hRyR2 N-terminal domain (hRyR2 NTD) in the dantrolene binding site (residues 600–620) by site-directed mutagenesis and investigated the effect of these mutations on the folding and thermal stability of this fragment. Our results show that these mutations have an impact on the thermal stability of the hRyR2 NTD. We also measured the binding affinities between dantrolene and the wild-type and mutant hRyR2 NTD and used molecular docking to model dantrolene binding in hRyR2. This work helps to clarify the role of the dantrolene binding site in hRyR2 and to characterize dantrolene binding on the molecular level.

1. V. Bauerová-Hlinková., D. Hajdúchová, J.A. Bauer. *Molecules* **25,** (2020), 2-29.
2. S. Kobayashi, M. Yano, T. Suetomi, M. Ono, H. Tateishi, M. Mochizuki, X. Xu, H. Uchinoumi, S. Okuda, T. Yamamoto, N. Koseki, H. Kyushiki, N. Ikemoto, M. Matsuzaki. *Journal of the American College of Cardiology* **53**, (2009), p: 1993-2005.
3. R. Wang, X. Zhong, X. Meng, A. Koop, X. Tian, P.P. Jones, B.R. Fruen, T. Wagenknecht, Z. Liu, S.R.W. Chen. *Journal of Biological Chemistry* **286**, (2011), p: 12202-12212.

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