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Author(s):
Lobsiger, Joel

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Towards Structural Investigation of the Yeast SWI/SNF Chromatin Remodeling Complex and X-ray Crystal Structure of the Yeast ARP7/ARP9 Heterodimer

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presented by

JOEL LOBSIGER
M Sc in Molecular Life Sciences, University of Bern

born on 14. 10. 1982

citizen of Wohlen b. Bern

accepted on the recommendation of

Prof. Dr. Timothy J. Richmond, Examiner
Prof. Dr. Kaspar P. Locher, Co-examiner
Prof. Dr. Gebhard F.X. Schertler, Co-examiner

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III. Summary

In eukaryotes, genomic DNA is packed repetitively into a highly structured nucleoprotein complex called chromatin. The organization and remodeling of chromatin structure within the nuclear compartment play a crucial role in transcription regulation, replication, DNA-damage repair and recombination. SWI/SNF is one of the four well-known families of ATP-dependent chromatin remodeling factors with the ability to regulate gene transcription. The yeast SWI/SNF, which comprises 12 subunits, is required for the expression of several highly inducible genes as well as the transcription of numerous genes which are expressed during late mitosis. As the prototype for an ATP-dependent chromatin remodeling family, the high-resolution structure determination of the SWI/SNF complex or its subcomplexes would provide important insights into the mechanism of the entire remodeling factor family.

Little structural information is available on SWI/SNF chromatin remodeling complexes. In order to understand their general and specific functions at the molecular level, X-ray structural investigation is needed. Using current bio-molecular and biophysical methods such as multi-protein and polyprotein expression systems, chaperone co-expression, MBP fusions, and X-ray crystallography, this work seeks to find an expression and purification strategy for single subunits, subcomplexes and the whole complex suitable for structural and functional analysis.

Yeast SWI/SNF chromatin remodeling subunits and subcomplexes were probed for bacterial or insect cell expressions. As shown in Chap. 3, only a few subunits such as RTT102, SNF11, TAF14, ARP9 and SNF12 yielded significant amounts of stable and correctly-folded protein under various conditions. Those subunits were then successfully expressed in large-scale, purified to homogeneity and used for protein crystallization trials. To date, a successful crystallization condition has not been found for any single subunit.

Consequently, methods for enhanced protein expression and methods for stabilizing expressed proteins suitable for purification and initial structural investigation were needed. As described in Chap. 4, protein co-expression (co-infection, MultiBac, polyprotein, bicistronic expression), chaperon co-expression, fusion-partner expression (MBP), and construct-truncation methods were tested for enhancing protein expression level, correct
protein folding, and improved protein stability. Supplementing the expression reaction with chaperones did not lead to enhanced solubility of aggregated and insoluble SWI/SNF subunits. The addition of MBP as a fusion partner helped increase expression and solubility of some subunits. Co-expression of ARP7 and ARP9 yielded a soluble, non-aggregated heterodimer suitable for crystallization.

As described in Chap. 5 and based on the results of Chap. 3 and 4, several subcomplexes were successfully assembled in vitro. Some of them yielded significant amounts of stable protein complexes and were purified to homogeneity for crystallization trials. A successful crystallization condition has not yet been found for any of these subcomplexes.

In Chap. 6, the crystal structure of the S. cerevisiae heterodimeric module of nuclear actin-related protein ARP7 and ARP9 is presented. The crystal structure was solved to a resolution of 3.1 Å by using the single-anomalous dispersion (SAD) method based on a methylmercury derivative dataset. Automatic as well as manual model building and refinement resulted in a model with acceptable R_{cryst}/R_{free} and geometry as determined by PHENIX. Coordinates were deposited in the protein data bank (accession number: 3WEE). In addition to providing anchor points (cysteine and methionine amino acids) for model building, the anomalous difference map calculated from the S-SAD dataset helped the interpretation of additional density in the ARP7/ARP9 interface as an N-cyclohexyl-3-aminopropanesulfonic acid (CAPS) molecule, a sulfur-containing crystallization buffer component. Moreover, four free sulfur ions were detected from the anomalous difference map of the S-SAD dataset.

The crystal structure of the yeast ARP7/ARP9 heterodimer presented explains structurally how several insertions into the conserved actin-fold lead to dimerization and not to F-filament formation like in actin. Furthermore, it was shown that the ARP7/ARP9 module reveals its dimerization interface and is not altered when bound in a complex with the SWI/SNF SNF2 HSA domain and the regulatory protein RTT102. In vitro interaction studies of ARP7, ARP9, and the ARP7/ARP9 heterodimer showed that they have, like other actin-related proteins, binding affinity for core histones. The lack of nucleosome binding activity, however, suggests that the dimeric ARP7/ARP9 module is a histone recognition module involved in histone binding or H2A/H2B displacement rather than a binding module for direct nucleosome interaction.
IV. Zusammenfassung


Rekombinante Proteinexpression einzelner SWI/SNF-Untereinheiten und diverser Subkomplexe wurden in verschiedenen Expressionssystemen gestestet. Wie in Kapitel 3