Experimental report

13/02/2017

Proposal: 8-03-871 **Council:** 4/2016

Title: Investigation of the molecular mechanism of Rtt109 activation by Vps75

Research area: Biology

This proposal is a new proposal

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Samples: Protein in 150 mM NaCl, 50 mM citrate ($C_6H_8O_7$), 1 mM DTT ($C_4H_{10}O_2S_2$)

Instrument	Requested days	Allocated days	From	To
D22	2	1	09/06/2016	10/06/2016
			12/09/2016	14/09/2016
			15/11/2016	17/11/2016

Abstract:

The Regulator of Ty1 Transposition protein 106 (Rtt109) is a fungal histone acetyltransferase required for histone H3 K9, K27 and K56 acetylation. These acetylation sites have been linked to processing and folding of nascent H3 and play an integral role in replication- and repair-coupled nucleosome assembly. An intriguing feature of Rtt109 is its activation by two structurally unrelated histone chaperones, Asf1 and Vps75, which likely stimulate Rtt109 activity via different mechanisms.
Using an approach combining nuclear magnetic resonance (NMR) spectroscopy, small-angle scattering (SAS) and crystallography we aim to gain an insight into the structural basis for Rtt109 activation and specifity of H3 acetylation. We find that Rtt109 can adopt different structures in solution and that the equilibrium between the two is shifted upon Vps75 association. In addition, we were able to reconstitute a complex containing Rtt109 and both Asf1 and Vps75 and to map the interaction interfaces of this 150 kDa complex. We now intend to further explore the conformational changes of individual subunits in the complex by contrast-variation SANS.

Experimental report for proposal 8-03-871, BAG8-34 on D-22.

Investigation of the molecular mechanism of Rtt109 activation by Vps75

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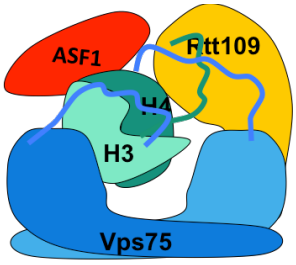
Biological background and the aim of the study

The *Regulator of Ty1 Transposition protein 106* (Rtt109) is a fungal histone acetyltransferase required for histone H3 K9, K27 and K56 acetylation. These acetylation sites have been linked to processing and folding of nascent H3 and play an integral role in replication- and repair-coupled nucleosome assembly. An intriguing feature of Rtt109 is its activation by two structurally unrelated histone chaperones, Asf1 and Vps75 [1]. The two chaperones likely stimulate Rtt109 activity via different mechanisms. This suggestion is based on the fact that Vps75 forms a tight complex with Rtt109, while Asf1 interacts only transiently. Rtt109 - Asf1 association has been proposed to be responsible for K56 acetylation, while the Rtt109-Vps75 interaction is required for K9 and K27 acetylation [2,3]. Although crystal structures of isolated Rtt109 and Rtt109 in complex with Vps75 exist [3,4], these raise questions regarding the stoichiometry of the complexes as both 2:1 and 2:2 Vps75-Rtt109 complexes were observed, and give a limited understanding of the activation of Rtt109 upon chaperone association. As such, there is a clear demand for a full characterization of Rtt109 complexes with Vps75, Asf1 and the substrate.
Previous to the SANS experiments, we found that Rtt109 can adopt two different conformations in solution and that the equilibrium between the two is shifted upon Vps75 association. In addition, we found that the Rtt109-Vps75 complex stoichiometry is dependent on buffer conditions. Using an optimized protocol, we were able to reconstitute a 150 kDa complex containing Rtt109:Vps75:(H3-H4):Asf1 in the ratio 1:2:1:1 and to map the interaction interfaces of this complex by chemical shift perturbations.
We aimed to understand the structural basis for Rtt109 activation and specificity of H3 acetylation. In order to investigate this, we needed to further explore the conformational changes of individual subunits in the complex.

Measured samples and results

In order to characterize a complex containing Rtt109 and both Asf1 and Vps75, we used contrast-variation SANS to obtain information on the shape of each of the proteins within the context of the whole complex (see Table 1 and Fig.1) and the relative position of subunit pairs.

Table 1. The samples measured on D22 during the allocated time. All samples are complexes reconstituted with one copy of Rtt109, two copies of Vps75, one copy of Asf1 and one H3-H4 dimer.



Schematic representation of the complex containing Rtt109 (50 kDa), Vps75 (60 kDa dimer), H3 (15 kDa), H4 (10 kDa) and Asf1 (20 kDa) .

Sample	Buffer D_2O%/H_2O%
All 1H	0/100
All 1H (#4)	100/0
2H Rtt109 Asf1	42/58
2H Asf1 Vps75	42/58
70% 2H Asf1 Rtt109 (#1)	100/0
70% 2H Asf1 Vps75 (#2)	100/0
2H Rtt109 Vps75 (#5)	0/100
2H Rtt109 Vps75	42/58
2H Rtt109 Vps75 (#6)	100/0
2H Rtt109 (#3)	42/58
2H Vps75	42/58
samples measured during additional time	
2H Rtt109 Vps75Δ225	48/52
2H Vps75Δ225	48/52
2H Rtt109 1H Vps75Δ225	48/52
70% 2H Vps75	100/0
70% 2H Vps75 Rtt109	100/0
all 1H (repeat)	0/100

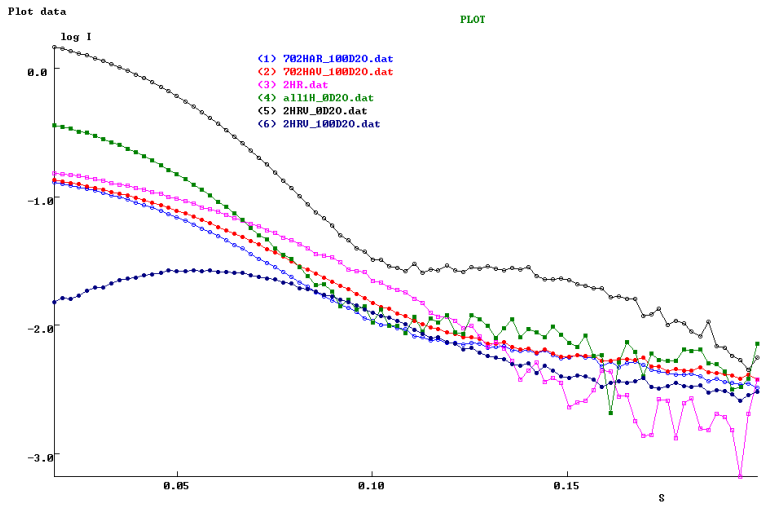


Fig 1. The samples measured on D22 during the allocated time. The numbers for the plotted curves (1-6) are added in the table 1.

The measurements were carried out at 25 °C in order to obtain data that could be combined with NMR restraints, which were employed to generate models of the complex using HADDOCK. The SANS data we collected on the various combinations of the subunits within the complex were used to score and cluster the HADDOCK-generated models.
The number of the measured SANS samples is sufficient to effectively discriminate between the models that had a similar NMR-restraints violation energy and allows us to select a final ensemble of the structures. However, Vps75 and H3 have long unstructured tails that are not present in the crystal structures we use for generating the complex models. To check how much the tails presence or absence influences model structures scoring, we collected SANS data on the full complex assembled with a truncated Vps75 (Vps75Δ225) lacking 226-264 C-terminal residues (Table 1). The sample concentration was kept the same to make the fitting (Chi^2 values) comparable. The existing crystal structure of Rtt109-Vps75 (PDB ID 3q66) and our HADDOCK models fitted significantly better to the curves for the complex with Vps75Δ225 (Table 2).

Rtt109-Vps75 structure (PDB ID 3q66)	Chi^2	Rtt109-Vps75 structure (HADDOCK)	Chi^2
vs. RV FL	22.3	vs. RV FL	6.8
vs. RVΔ225	3.3	vs. RVΔ225	0.59

Table 2. Fits for the Rtt109-Vps75 structures to the respective SANS curves. “RV FL” stands for a curve obtained with the full-length 2H Rtt109, Vps75 within the full complex, “RVΔ225” for 2H Rtt109, Vps75Δ225 within the full complex.

At the moment, we use the curves from Vps75Δ225 samples to score Rtt109-Vps75 models generated via molecular dynamics (MD). Afterwards, we use the 10 best scoring models together with Asf1-H3H4 structure (PDB ID 2hue) for HADDOCK ensemble docking. This approach allowed us to sample much more substrate positions.
Our current strategy is to first obtain a consensus structure of the core of the complex and after that model the tails on, using restraints provided by NMR and previously recorded SANS data for the full-length complex.

References

[1] Tsubota T, Berndsen CE, Erkmann JA, Smith CL, Yang L, Freitas MA, Denu JM, Kaufman PD. Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. Mol Cell. 2007; 25:703–712.
[2] Fillingham J, Recht J, Silva AC, Suter B, Emili A, Stagljar I, Krogan NJ, Allis CD, Keogh MC, Greenblatt JF. Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. Mol Cell Biol. 2008; 28:4342–4353.
[3] Tang Y, Holbert MA, Delgoshaie N, Wurtele H, Guillemette B, Meeth K, Yuan H, Drogaris P, Lee EH, Durette C, et al. Structure of the Rtt109-AcCoA/Vps75 complex and implications for chaperone-mediated histone acetylation. Structure. 2011; 19:221–231.