Experimental report for ESRF Expt. MX-1593, proposer Clemens Grimm, University of Wuerzburg, Beamline ID23-1

Overview

Under conditions of nutrient limitation, eukaryotic cells show a significantly altered protein expression profile. A significant part of this response to environmental changes is facilitated on the post-transcriptional level (Spriggs et al., 2010). During amino acid starvation, a particular mRNA species called 5'TOP mRNAs is selectively depleted from the cytoplasm and enriched in so called stress granules. Thiss suggests the existence of a specific regulatory circle acting exclusively on 5'TOP mRNA translation (Avni et al. 1997; Iadevaia et al. 2008). In 2011, the proteins TIA1-1 and TIAR were identified as specific 5´TOP mRNA binders (Damgaard et al, 2011), therefore representing the front-end of this regulatory circle. The hallmark of all 5'TOP mRNAs is a 5' cytosine followed by an uninterrupted stretch of 4 to 15 pyrimidine bases (Hamilton et al. 2006). We were able to map TIA-1/TIAR binding to the oligopyrimidine tract of 5´TOP mRNAs on the nucleic acid side and to the RRM2 and RRM3 domains of TIA-1/TIAR on the protein side.

To shed light on the atomic details of the 5´TOP mRNA recognition by TIA-1/TIAR, we have crystallized a complex between a protein fragment covering the three RRM domains of *Chaetomium thermophilum* TIA and an 11 nucleotide RNA oligomer representing the oligopyrimidine tract of a 5´TOP mRNA. We could collect an initial native dataset from these crystals with 2.5 Å resolution.

Experimental method

As we were so far unable to solve the native data by molecular replacement with single RRM structures as search models, we had prepared various heavy atom derivatives by soaking with diverse platinum, gold, mercury and gadolinium salts. With these derivatized crystals we performed a series of three-wavelength MAD experiments.

Evaluation and results

Around 20 heavy-atom soaked crystals featured good enough diffraction so that a set of three datasets (inflection point, peak, high energy remote) was collected on each of those crystals. The best derivatives were observed for platinum-soaked crystals. In the lowest resolution shell an overall D_{ano}/sigma(D_{ano}) of 2 was observed (Table 1). Using those datasets, a heavy atom search and the generation of experimental phases was attempted. However, so far no interpretable experimental density could be generated. We are currently working on the reproduction of the co-crystals to extend the derivative search.

Meanwhile, another crystal form measured in this session was identified as the fortuitously purified and crystallized bacterial protein ArnA, a key enzyme for Polymyxin resistance. We were able to solve the structure of those crystals by replacement with the model of substrate-bound ArnA. In comparison, the apo structure features significant differences to the previously solved substrate-bound form. This includes the formation of a central binding pocket (Fig. 1). This work is currently under review with *Acta Crystallographica section D.*

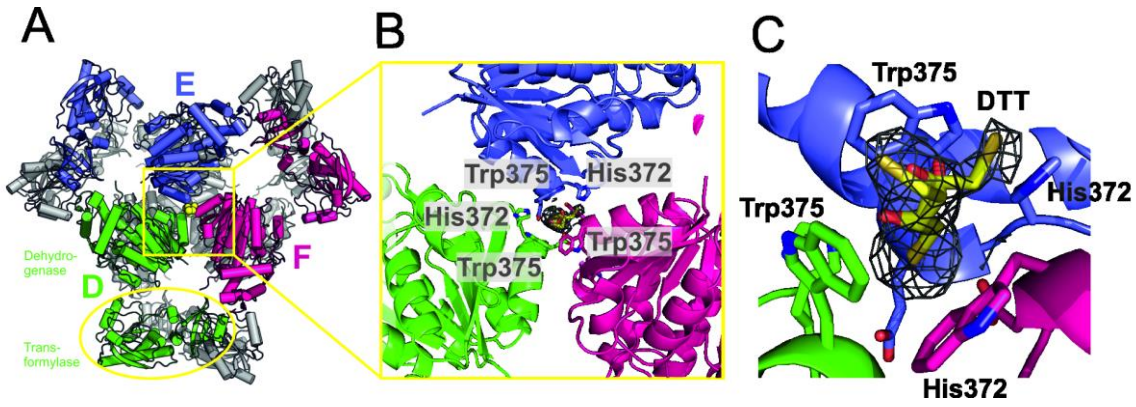

Fig 1. Crystal structure of apo ArnA, its six protomers are organized in a dimer of trimer fashion. The first trimer is depicted in colour, the second in grey. A, overall view. B and C, increasing zoom into the observed central binding pocket bound with a DTT molecule modelled in two alternative conformation. A $2F_{obs}$-F_{calc} simulated annealing omit map is shown as a mesh contoured on 0.8σ level.

	Number of Reflections								
Resol. Shell	Obsvd.	Unique	Possib.	Completeness	R_{sym}	I/Sigma	Anom. Corr.	Sig_{Ano}	N_{ano}
9.99	2690	373	390	95.6%	3.6%	43.90	75	2.000	171
7.15	4772	582	583	99.8%	4.6%	32.19	53	1.444	379
5.86	6291	720	722	99.7%	10.6%	16.86	28	1.086	529
5.08	7478	835	835	100.0%	15.0%	12.73	8	0.911	637
4.55	8543	934	936	99.8%	15.7%	12.16	10	0.879	737
4.16	9122	1024	1024	100.0%	26.6%	7.73	1	0.785	823
3.85	10188	1085	1085	100.0%	46.8%	4.75	1	0.755	888
3.60	10637	1182	1182	100.0%	66.1%	3.40	1	0.697	983
3.40	11382	1244	1245	99.9%	112.7%	2.08	2	0.662	1038
overall	**71106**	**7980**	**8002**	**99.7%**	**16.4%**	**11.14**	**12**	**0.870**	**6185**

Table 1: Data collection statistics of a Pt-derivatized TIA/TOP-mRNA co-crystal. Resolution shells with anomalous correlation significant at the 0.1% level are marked in red.

References

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(3) Avni, D. et al. (1997*) Nucleic Acids Res*. **25**:995-1001

(4) Iadevaia, V. et al. (2008) *RNA* **14**:1730-1736

(5) Hamilton, T. L. (2006) *Biochem. Soc. Trans*. **34**: 12-16