Investigating the roles of Naa15 and Naa10 proteins in NAT A/E complex formation and ribosome interaction

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Background and Aims

Multi-complex proteins are essential for nearly all cellular processes. Incorrect protein partnership leads to degradation and aggregation which can cause disease (Vamparys et al., 2016; Wiederstein et al., 2014). The N-terminal acetyltransferases (NATs) are important multi-complex proteins which undertake protein N-terminal acetylation at the ribosome, a common modification in yeast and human proteins. NAT mutations have been indicated in diseases including Ogden Syndrome, Lex microphthalmia syndrome and some cancers (Esmailpour et al., 2014; Gogoll et al., 2021; Koufaris and Kirmizis, 2020; Van Damme et al., 2012).

Two major NATs are NatA and Nat E, which form a complex on the ribosome. NatA is composed of a catalytic subunit (Naa10) and an auxiliary subunit (Naa15), whilst NatE only has a catalytic subunit (Naa50). Previous research has proposed that Naa10 interacts with Naa50, whilst Naa15 interacts with the ribosome (Alrasidi, 2018). Naa50 undertakes N-terminal acetylation, but without NatA it cannot interact with the ribosome. Still inconclusive is whether Naa15 interacts with both Naa10 and Naa50, or just Naa10.

My studentship aimed to determine whether both Naa10 and Naa15 are crucial for Naa50 binding to the ribosome, using ribosomal fractionation of *S. Cerevisiae* yeast expressing C-terminal Naa50 FLAG tags in wild type (M109), ΔNaa10 (M129) or ΔNaa15 (M122). Using immunoprecipitation, I then set out to determine how Naa50 interacts with their complex partners without Naa10 or Naa15.

Methods

Cell culture: *S. Cerevisiae* strains W303 (wild type), Naa50 $_{FLAG}$ (M109), ΔNaa15 $_{NAA50\ FLAG}$ (M122) and ΔNaa10 $_{NAA50\ FLAG}$ (M129) were grown under sterile conditions in YPD media to mid log phase then treated with cycloheximide to pause translation.

Ribosomal fractionation: To observe how Naa50 interacted with the ribosome, cycloheximide treated cell cultures were pelleted then lysed under physiological low salt conditions using glass beads. Extracts were ultracentrifuged through a sucrose cushion to produce a cytosolic fraction (S), a cushion fraction (C), and a pellet (P) fraction.

Co-immunoprecipitation: To see how Naa50 interacts with their binding partners, cell extracts were lysed under high physiological salt conditions to release proteins from the ribosome. Cytosolic fractions (S) were immunoprecipitated with anti-flag peptide to pull down Naa50 interacting proteins.

SDS-PAGE and Immunoblotting: Protein extracts were separated using SDS PAGE and transferred onto a nitrocellulose membrane. Naa50 FLAG tags were probed using anti-FLAG primary antibodies, whilst anti-US3 anti-zwf1 primary antibodies were used to indicate ribosomal and cytosolic proteins respectively.

Results: Naa50 ribosome interaction

To ascertain whether Naa50 still interacts with the ribosome in the absence of its partner proteins Naa15 or Naa10, low fractionation was used to separate out cytosolic proteins from ribosomal bound proteins.

Fractionation of M109 strain showed Naa50 was present in the cushion (C) and the ribosome (P) but not the cytosol (S), reflecting the location of the ribosomal marker US3 (figure 1), indicating that Naa50 bound to the ribosome as expected. In M122, the results show Naa50 in the cytosolic extract (S), some in the cushion (C) and none in the ribosomal fraction (P), mirroring the cytosolic marker Zwf1, and not the ribosomal marker US3, indicating that without Naa15, Naa50 can no longer interact with the ribosome and remains in the cytosol. M129 reflected the same results as M122 with Naa50 not present in the ribosome fraction. This illustrates that without Naa10, Naa50 cannot interact with the ribosome, and thus both Naa10 and Naa15 are needed for Naa50 ribosomal interaction.

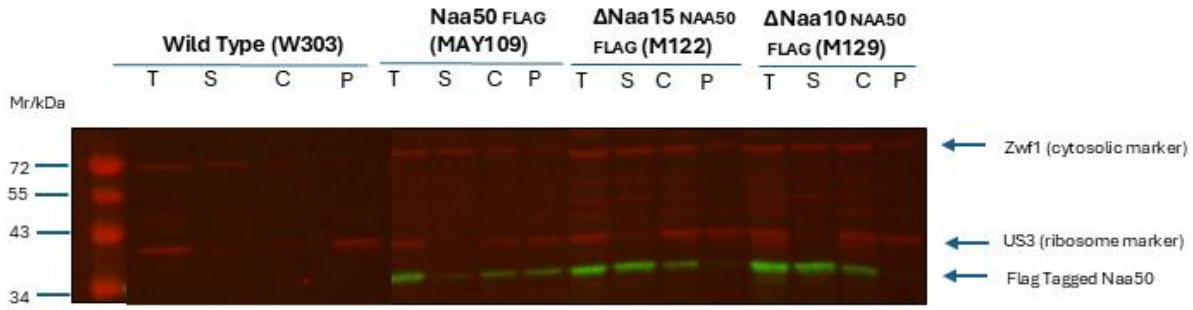

Figure 1 Naa10 and Naa15 are both required for Naa50 interaction with the ribosome. Wild type yeast strains (W303), FLAG tagged Naa50 yeast strains in wild type (M109), ΔNaa15 (M122) and ΔNaa10 (M129) were grown to mid log phase and treated with cycloheximide. Cells were lysed under physiological salt conditions before ultra centrifugation through a sucrose cushion to produce a cytosolic fraction (S) a cushion fraction (C) and a ribosomal pellet fraction (P). Fractions were analysed using SDS-PAGE and immunoblotting with anti-flag antibodies to detect Naa50 FLAG (green channel). Ribosomal markers US3 and cytosolic marker Zwf1 were also probed (red channel).

Co-Immunoprecipitation

To see how Naa50 interacts with their binding partners in strains which lack one of the partners, co-immunoprecipitation was undertaken. The bound proteins were then eluted in order to observe which proteins were co-immunoprecipitated. It was hypothesised that the Naa50 would co-immunoprecipitate with both Naa10 and Naa15 in M109. In M107, Naa50 and Naa15 would be pulled down. In M122, it was predicted that Naa10 would no longer be pulled down as without Naa15 it could not interact with Naa50. In M129, it was unclear whether Naa50 and Naa10 would interact, indicating that Naa15 is not required for this interaction, or if Naa50 would not interact with Naa10 without the presence of Naa15.

The negative control (figure 2, left) shows the unbound fraction and peptide elution of buffer and flag peptide without yeast. IgG chain bands and other cross reacting peptides are shown, which can serve as a marker for non-relevant protein bands for all strains. It is observed that only M107 shows a FLAG tagged protein; FLAG tagged Naa10 in the supernatant, peptide elution and SDS elution. Naa50 was very close in size to the IgG light chain so it was difficult to distinguish between the bands. Additionally. M122 was not cultured at this point. Due to these factors, the experiment wasn't taken further.

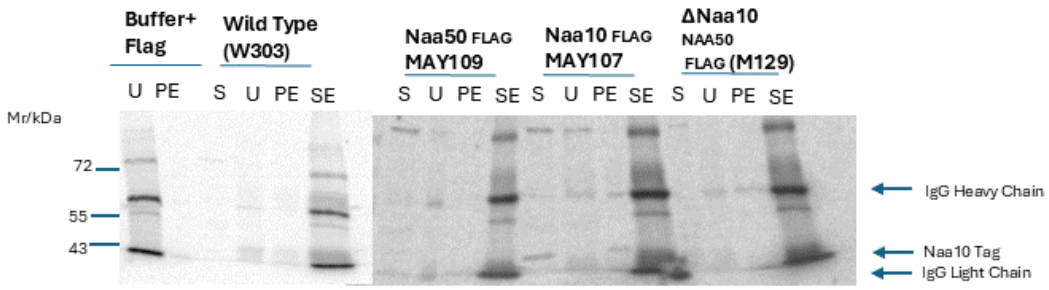

Figure 2 Investigation into how Naa50 interacts with their binding partners in strains which lack one of the partners. Wild type yeast strains (W303), FLAG tagged Naa50 wild type yeast strains (M109), FLAG tagged Naa10 wild type yeast strains, ΔNaa15 (M122) and ΔNaa10 (M129) were grown to late log phase. Cell extracts were lysed using glass beads under high salt conditions. Cytosolic fractions (S) were then immunoprecipitated with anti-flag antibodies to pull down proteins which interact with the Naa50 and Naa10. An unbound extract was taken (U), before the bound fraction was eluted under native conditions to produce a peptide elution (PE), and then the remaining proteins were denatured and eluted using SDS (SE) and the fractions were analysed using SDS-PAGE and immunoblotting.

In conclusion, the results gathered from the experimental work suggests that both Naa10 and Naa15 are essential for NatA/E ribosomal interaction. In order to determine whether both Naa10 and Naa15 bind to the Naa50 protein, co-immunoprecipitation will be repeated using a 12.5% polyacrylamide gel to distinguish the light chain from the Naa50 tag in the peptide elution, and silver staining will be used to observe co-immunoprecipitated proteins.

Impact

The work undertaken has aided our understanding of multi-subunit complex interaction, and what occurs when these complexes fail to form. The NATs, in particular, are important protein complexes in humans, and linked to diseases including Ogden syndrome. By understanding the impact of such protein deficiencies, it may be possible to find disease cures in the form of gene therapy for restoration of protein function (Makwana et al., 2024).

The results gathered have contributed to the Biochemical Society strategy; the findings have advanced molecular bioscience, and allowed me to develop skills and the chance to learn from scientists across different areas of expertise.

Skills

This opportunity has enabled me to refine my skills in cell culture, immunoblotting, fractionation and immunoprecipitation. I have honed my communication skills through collaboration with other scientists. I developed troubleshooting skills; I had to investigate why my cultures weren't growing and develop new strategies. This studentship has motivated me pursue a PhD.

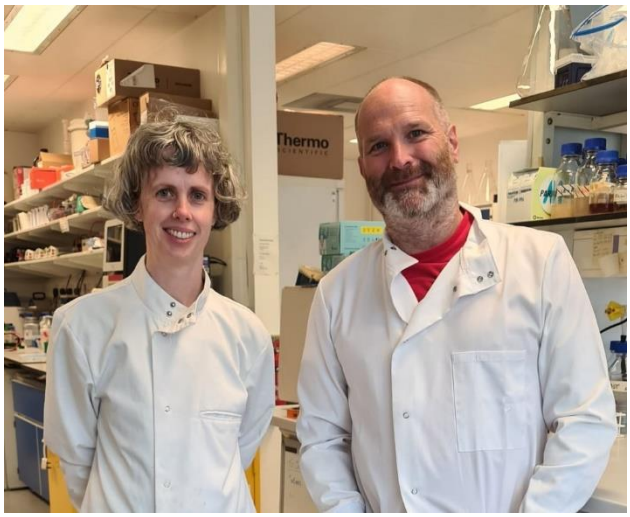

Figure 3 Amy Ashton (left) with Dr Martin Pool in the lab

Acknowledgements

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