Nop56/58:Fibrillarin complex in Box C/D RNPs

Malachi A. Blundon

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1: Introduction

Ribosomal RNAs (rRNAs) constitute approximately 80% of the RNA in rapidly dividing cells[1]. These RNAs form the core of the ribosome unit. There are four types of eukaryotic rRNAs currently identified. Three of the four rRNAs are made by chemically modifying and cleaving a single large precursor rRNA. The fourth is synthesized from a separate group of genes transcribed by RNA polymerase III and does not require any modification[2]. Chemical modifications occur in the 13,000 nucleotide-long precursor rRNA before the rRNAs are cleaved out and assembled into ribosomes. It is speculated these modifications aid in the folding, assembly of the final rRNAs, and function of ribosomes. Each modification is made at a specific position of the precursor rRNA. These positions are specific by "guide RNAs" that position themselves through base-pairing to the precursor rRNA[2]. This allows an RNA-modifying enzyme to locate the appropriate position for modification. Other "guide RNAs" promote cleavage of the precursor rRNAs into the mature rRNAs[2]. It is proposed this cleavage is promoted by causing conformational changes in the precursor rRNA that expose these specific sites to nucleases. All of these guide RNAs are members of a large class of RNAs called small nucleolar RNAs (snoRNAs).

The two different types of rRNA modifications are directed by two different families of snoRNPs. These families of snoRNAs are referred to as antisense C/D box and H/ACA box snoRNAs[1]. These families are named based on the presence of conserved sequence motifs in the snoRNA. In general the C/D box members guide methylation and H/ACA members guide pseudouridylation[1].

Ribosomal RNAs of all organisms contain site specific 2'-O-methylation (Figure 1). All C/D RNPs share four core proteins: Nop56, Nop58, fibrillarin, and 15.5 kDa. Fibrillarin contains the methyltransferase catalytic site of the complex[3]. The 15.5 kDa protein initiate C/D RNP assembly by binding to the kink-turn motif formed by the conserved box C and D sequences[3]. Once the 15.5 kDa protein has bound to the box C and D sequences the Nop56/58:fibrillarin complex assembles with the RNA (Figure 2). This step assembly is believed to be dependent on a direct contact between the box C/D motif and Nop56/58, as well as protein-protein interactions between Nop56/58 and fibrillarin[3]. This suggests that Nop56/58 is important for positioning the catalytic subunit on the target RNA via physical contact between the guide RNA and fibrillarin (Figure 2). The target RNA is recruited to the complex through its 10-21 Watson-Crick base-pair which compliments with the guide RNA that is unwound upon modification and prior to ribosome assembly[3]. Box C/D RNAs contain a bipartite arrangement of two box C/D motifs designated as C/D and C'/D' (Figure 2). These antisense regions guide the target methylation at two distinct sites. This arrangement presents a symmetrical molecular scaffold allowing the bipartite RNA to dock into the RNP complex, placing a methyltransferase near the less conserved box C'/D' motif (Figure 2). A second structural feature of box C/D RNAs is the variation in spacing between the two box C/D motifs. This spacing is defined between 12-18 bps (Figure 2), and has been suggested there is a certain flexibility in the distance between the two box C/D motifs as a deletion in the spacer sequence of yeast U24 is tolerated *in vivo*[4].

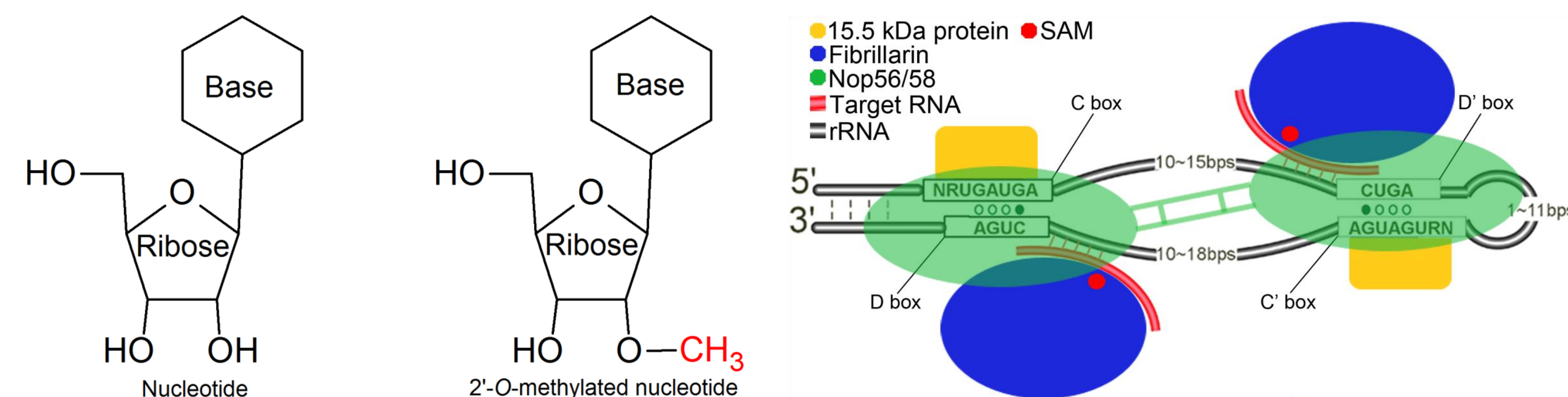


Figure 1: Methylation of a RNA base.

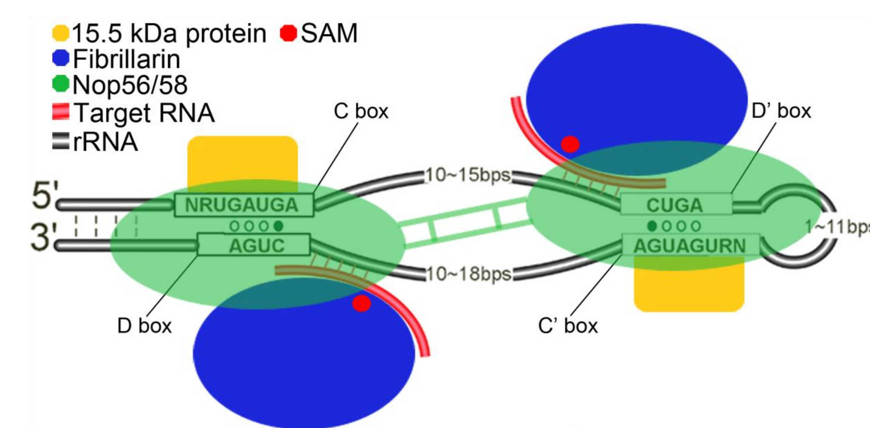


Figure 2: A schematic diagram of the Nop56/58 RNP complex with an unmodified box C/D RNA.

2: Overall Structure

As shown in Figure 3, Nop56/58 contains three distinct domains: the N-terminal, the coiled-coil, and the C-terminal domains. The coiled-coil domain allows the entire protein to loop back such that the N-terminal and the C-terminal domains are adjacent to each other in space. The Fibrillarin protein interacts with Nop56/68 via the least conserved N-terminal domain at helices α1 and α2. Nop56/58 has about 40 amino acids residues that are enriched in lysine and negatively charged residues (KKE/D tail). However, this KKE/D tail is not required for Nop56/68 function *in vivo* as suggested by prokaryotic homologues[5].

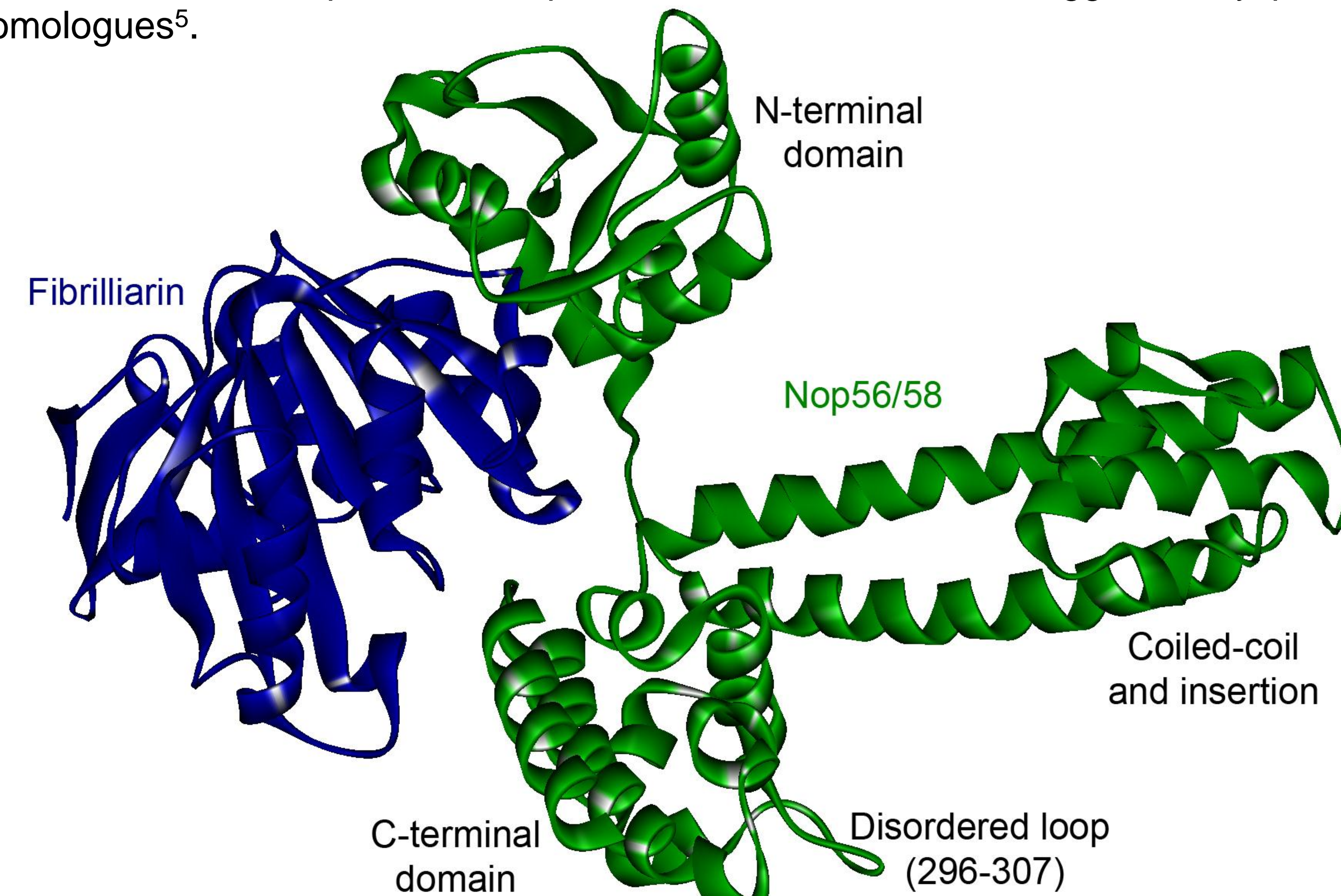


Figure 3: Overall structure of the Nop56/58:Fibrillarin complex.

3: Role for Nop56/58 in methylation cofactor binding by fibrillarin

As depicted in Figure 4, the methylation cofactor, *S*-adenosyl-L-methionine (SAM) was discovered and located at the predicted cofactor binding site. This suggests Nop56/58 plays a role in binding the methylation cofactor. In the presence of Nop56/58, SAM binding to fibrillarin is accompanied by a 7 Å downward shift of the conserved motif I loop (conserved sequence AASGT) away from the inhibitory position. This suggests that the conformational change in favor of the cofactor binding is facilitated by Nop56/58 binding.

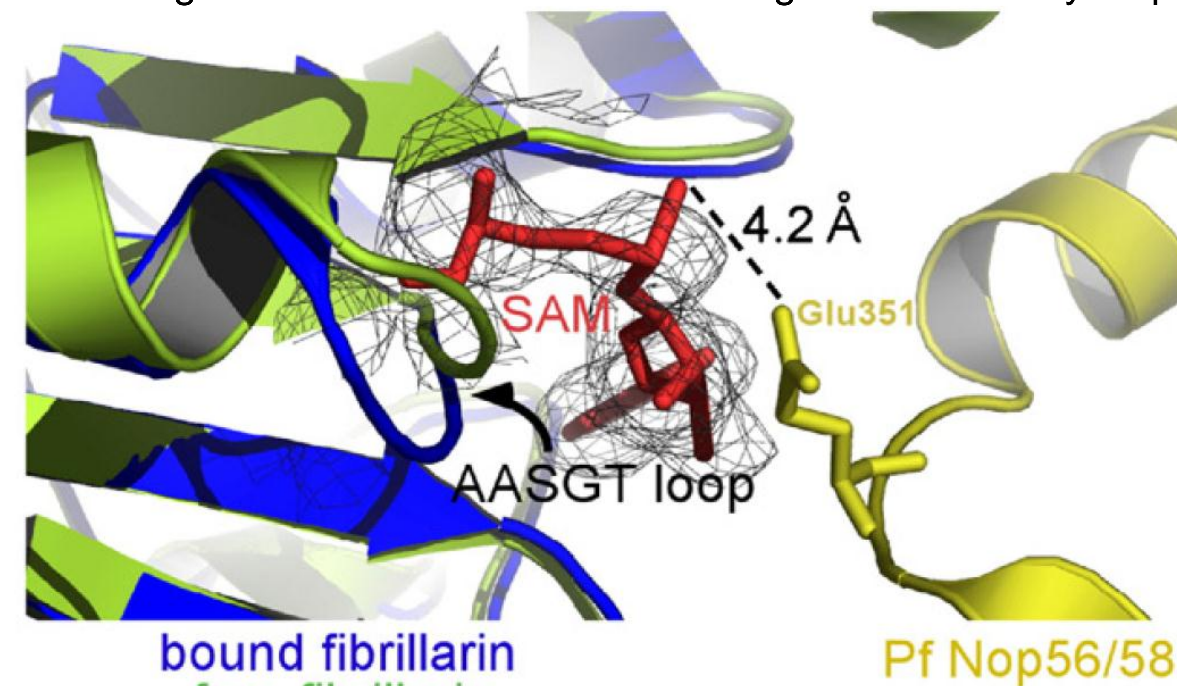


Figure 4: A schematic of SAM located in the methylation cofactor binding site. As shown here, Glu351 of Nop56/58 mediates the cofactor binding suggested by a conformational change (Orugani et al., 2007).

4: Dimerization of Nop56/58:fibrillarin complex through coiled-coil domain

The coiled-coil domain is located in the Nop56/58 protein. This coiled-coil domain mediates dimerization via a four-helix bundle that imposes a specific conformation and positioning of the N-terminal domain. It has been demonstrated that the coiled-coil domain is not highly conserved. In fact, large deletions or insertions of random amino acids do not disrupt the dimerization N-terminal domain at all. This low conservation and integrity of the coiled-coil domain suggests that it is not essential for Nop56/58's function.

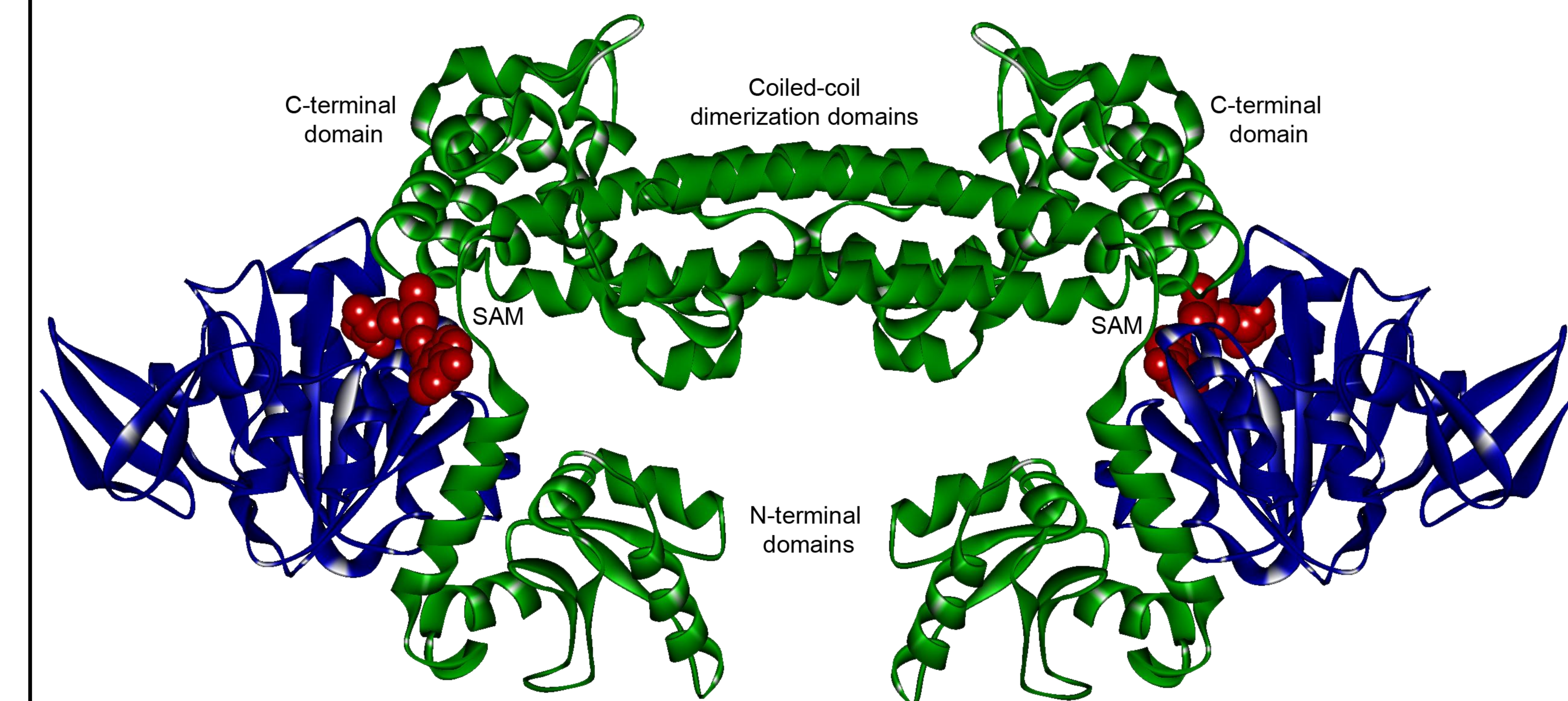


Figure 5: The coiled-coil domain of the Nop56/58 interacts with another CC domain in order to form a dimer with another Nop56/58:fibrillarin complex.

5: Nop56/58 can exist in two different conformations

Nop56/58 protein can exist in two different conformations, designated Af and Pf respectively. Each conformation was named by the conformation exhibited in specific archaeal bacteria, *P furiosus* and *A. fulgidus.* It has been determined that the N-terminal domain of Nop56/58 can exist in either a horizontal or vertical state. This ultimately shifts the physical position of the fibrillarin protein. Interestingly enough, the physical positioning from either of these conformations do not disrupt the methylation process of the complex.

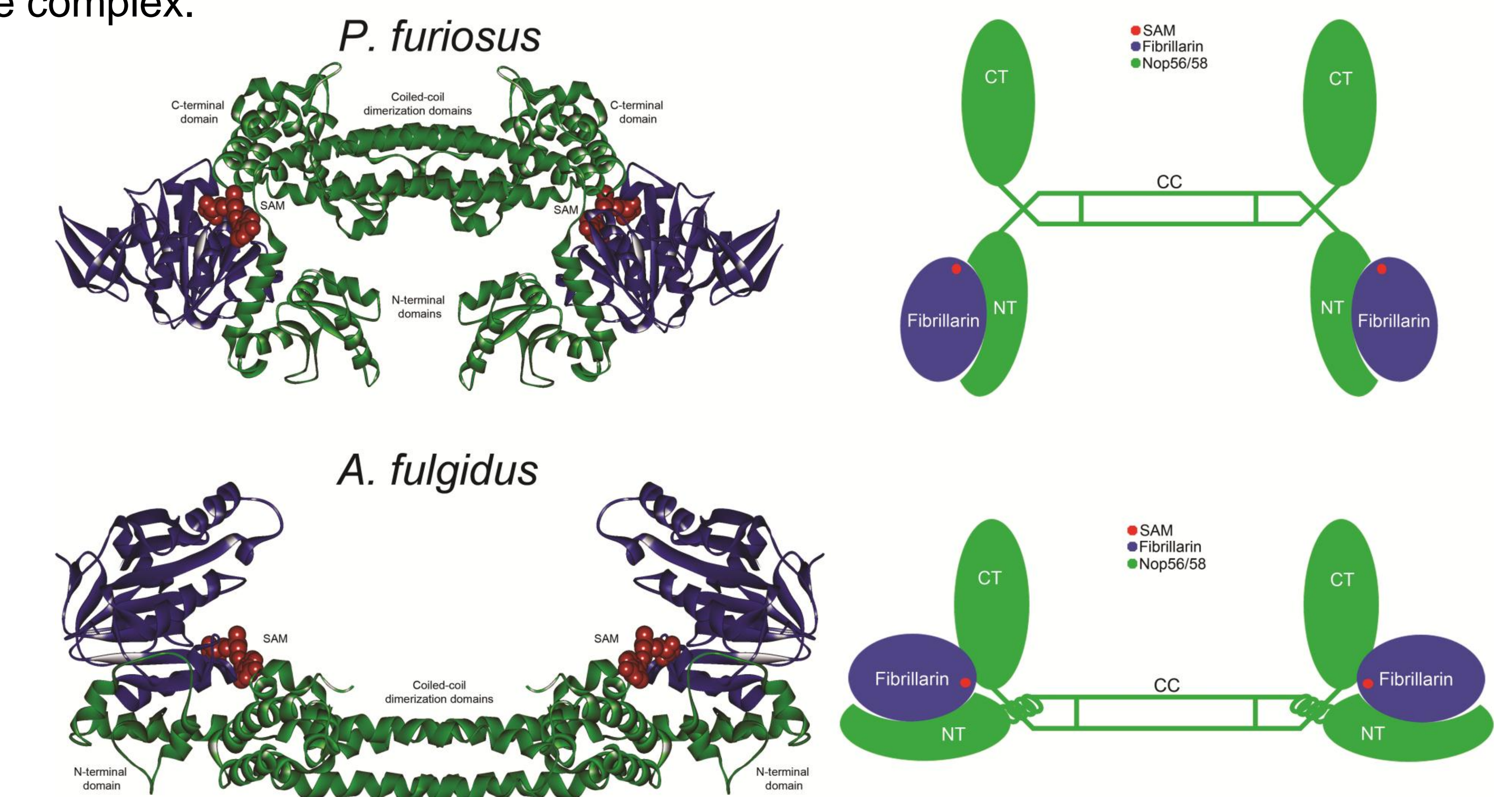
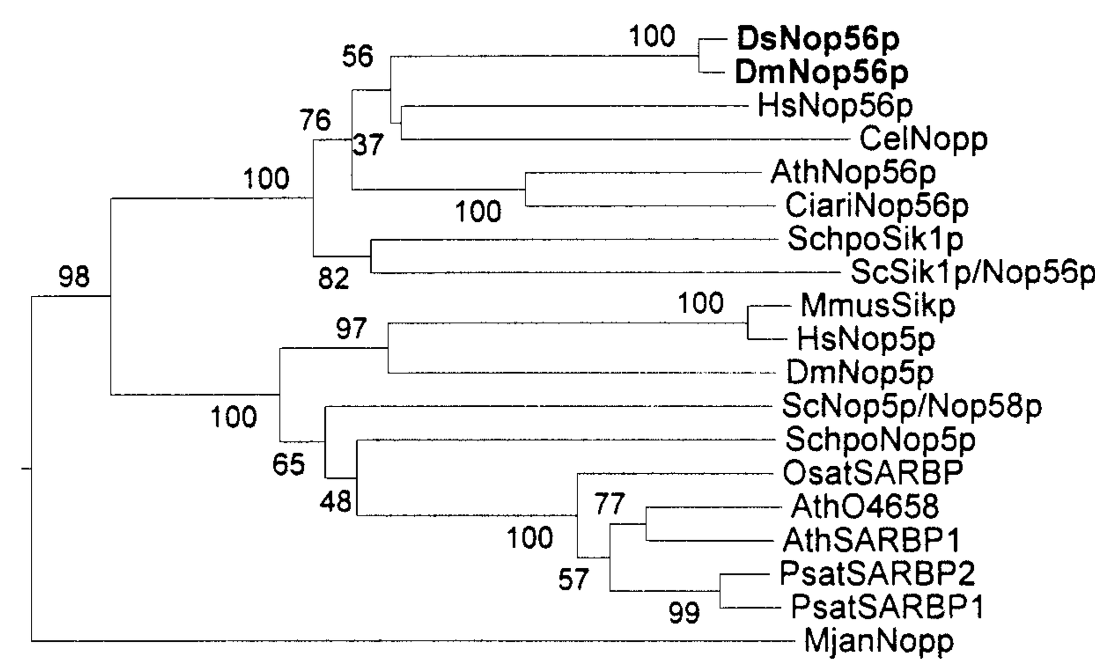


Figure 6: A schematic and protein presentation of the two conformations the Nop56/58:fibrillarin complex can exist in. Both conformations allow the correct methylation of the unmodified rRNA.

6: Nop56/58 is conserved throughout phylogeny

Below is a amino acid sequence analysis of the Nop56 protein. Conserved amino acids are boxed in black. Sequence was compared between yeast, fruit fly, human, round nematode, etc. From this sequence, a phylogenic tree was constructed using NCBI's COBALT sequence alignment tool. This phylogenic analysis was supported by Garcia-Plenells et al., 2000.



7: References

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6. Garcia-Plenells et al. (2000). Dnop56, a Drosophila gene homologous to the yeast nucleolar NOP56 gene. *Genetica , 109*, 275-282.