Investigating the Direct Effect of Phosphorylation on the Oligomeric State and Activity of yPRMT1

Emily Wilson, Anthony Peidl, Joan M. Hevel
Department of Chemistry and Biochemistry, Utah State University

Introduction to Arginine Methylation and yPRMT1

Arginine Methylation is an essential post-translational modification that affects various biological processes. Arginine methylation is catalyzed in mammalian cells by protein arginine methyltransferases (PRMTs). The most abundant isomer, PRMT1, utilizes S-adenosylmethionine (SAM) as a methyl donor (Figure 1). Proper regulation of PRMT1 activity is imperative as studies link dysregulated arginine methylation to diseases [2].

One potential way to regulate PRMT1 activity is through post-translational modifications and oligomerization. A previous publication states phosphorylation of serine 9 directly regulates the oligomeric state and activity of yeast PRMT1 (yPRMT1) [1]. However, the methods and experimental design used are insufficient to come to the stated conclusions, and contradict the preliminary data collected by the Hevel lab [2, 3]. The specific aims of this project are:

1-Recombinantly express and purify wildtype (WT), S9A (control), S9E (phosphomimic) yPRMT1
2-Determine the native oligomeric state
3-Quantify methyltransferase activity

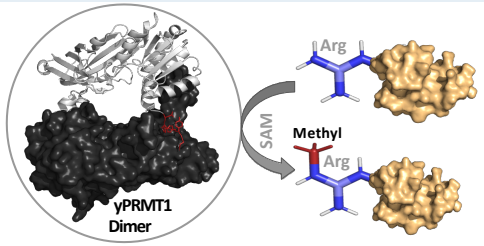

Figure 1. Arginine Methylation via yPRMT1. Previous research suggests that yPRMT1 must exist in at least a dimer, or higher order oligomers, to be active.

Using More Novel and Conclusive Methods *in Vitro*

The previous publication performed experiments *in vivo*. Many components exist within the cell that may result in increased activity, such as protein regulators. To eliminate these potential confounding variables, this study used *in vitro* techniques to directly probe the effect of phosphorylation. Given that, in the previous publication, all effects *in vivo* were mimicked using the E substitution, we recombinantly expressed and purified WT, S9A, and S9E yPRMT1 constructs. To characterize the native oligomeric state, we utilized Analytical Ultracentrifugation (AUC) in conjunction with Native-PAGE. To determine methyltransferase activity, we performed kinetics assays with radiolabeled SAM.

Recombinant Expression and Purification of yPRMT1

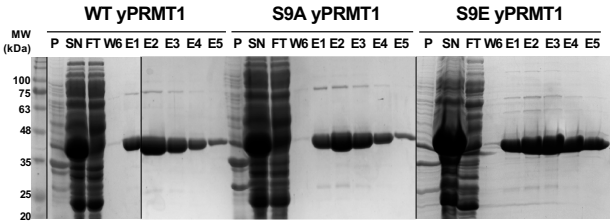

Figure 2. Expression in BL21 Codon+(RIPL) *E. Coli* and purification using nickel affinity chromatography yields large amounts of pure protein. Each sample was loaded on SDS PAGE gel with the following lanes: Pellet (P), Supernatant (SN), Flowthrough (FT), Wash 6 (W6), and Elutions 1-5 (E1-E5). Thick bands are seen near the molecular weight of yPRMT1, which is approximately 39,800 kDa for each construct.

Characterization of the Oligomeric State of yPRMT1 Constructs

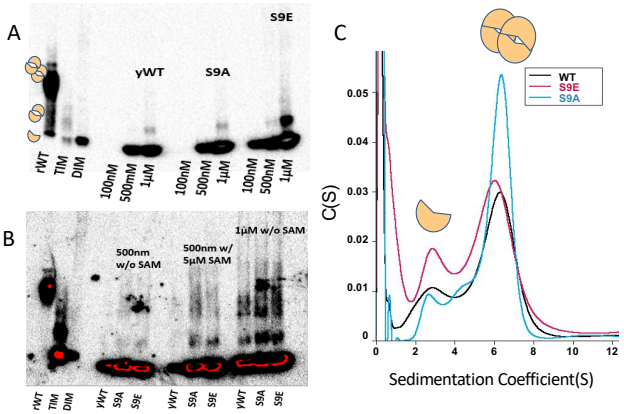

Figure 3. Native-PAGE and AUC experiments show variation in oligomeric state. A) yPRMT1 constructs compared to rat WT, TIM(), and DIM () standards. 1µM concentrations show dimerization for all constructs. B) yPRMT1 constructs with and without SAM. C) AUC using 3µM protein indicates constructs exist predominantly as a tetramer, with a smaller fraction of monomer.

Quantification of methyltransferase activity of yPRMT1 Constructs

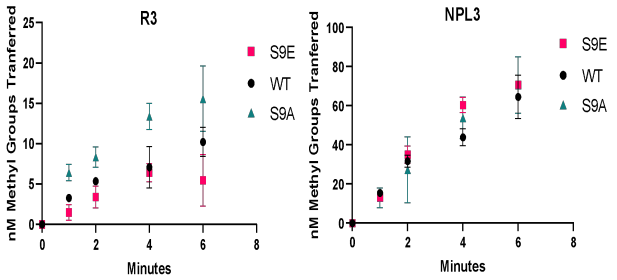

Figure 4. Methylation activity assays of each yPRMT1 construct. Methylation activity of protein with either R3 peptide substrate (left) or NPL3 protein substrate (right). S9E does not exhibit increased activity, as suggested by the previous publication. These data indicate that phosphorylation does not directly increase yPRMT1 activity.

Conclusions and Future Studies

Collectively, these results indicate that phosphorylation of Ser9 does not *directly* affect the oligomerization state and activity of yPRMT1. The *in vitro* methods of this study show different results from the previous publication. Further investigation will help provide insight as to how posttranslational modifications and oligomerization can regulate yPRMT1 and other methyltransferases.

Potential Future Studies:
- **Determine *in vivo* regulators of yPRMT1 activity.**
- **Determine how existing as a tetramer relates to function.**
- **Why both dimers and tetramers can methylate substrates.**

References

[1] Messier, Vincent, et al. (2013) *A Nutrient-Responsive Pathway That Determines M Phase Timing through Control of B-Cyclin MRNA Stability.* Cell.
[2] Morales, Y. (2016). *Characterization of the Substrate Interactions and Regulation of Protein Arginine Methyltransferase 1* [Dissertation]. Utah State University.
[3] Ortolano, A. N. (2019). *Characterizing Oligomeric State Changes of Protein Arginine Methyltransferase 1 as a Mechanism for Regulating Activity* [Master's thesis]. Utah State University.