Nuclear Associations and *In Silico* Modeling Implicate Chromatin and Epigenetic Regulatory Mechanisms for Testis-Specific Actin Related Proteins in Spermiogenesis.

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Nuclear remodeling during spermatogenesis is critical for fertility with implications for germ-line epigenetic programing. However, the mechanistic regulation of nuclear dynamics and chromatin remodeling during germ-line morphogenesis to produce sperm is not well understood. Remodeling includes drastic chromatin rearrangements for unique transcriptional programing, meiotic recombination, and DNA repackaging in spermiogenesis. The latter includes transitions of unique histone variants and PTMs impacting transcription and chromatin organization. Here we explore the nuclear and molecular associations for testis-specific actin related proteins (ARPs).

ARP variants are known to contribute to somatic chromatin master regulatory complexes conserved in eukaryotes. We and others have shown that the testis specific ARPs ACTL7A and ACTL7B are required for fertility impacting sperm structure through cytosolic and cytoskeletal contributions in animals and humans. Here we report *in vivo*, *in vitro*, and *in silico* evidence for localization to the nucleus. Step-specific spacio-temporal IHC localization, subcellular fractionation westerns, and immuno-EM all provide evidence for temporally regulated nuclear localization. A putative nuclear localization signal was identified through *in situ* overexpression of specific ACTL7B domains. We further identified ACTL7B associations with nuclear proteins including nuclear importins, histones, and the chromatin remodeling complex associated proteins. These included RUVBL1/2 which are known components of nuclear chromatin remodelers that also classically contain somatic ARP variants. Using AI facilitated *in silico* modeling approaches, we developed and validated a model of relative ARP binding affinity with two distinct ARP containing domains in these complexes, and identified site-specific associations for ACTL7A and ACTL7B in the DNA associating HSA domain of these large multimeric nucleosomal regulatory complexes. We propose that through subunit swapping in otherwise conserved chromatin regulatory complexes, novel testis-specific complexes are formed. Transcriptomic profiling of *Actl7b* KO testis revealed a general up-regulation of transcripts with a notable change in testis-specific transcription factors. Together these data suggest a role in spermatid-specific chromatin remodeling through epigenetic mechanisms. Nucleosomes are classically regulated through recognition of histone PTMs including acetylation. We had previously noted an increase in acetylation of tubulin and mislocalization of the tubulin deacetylase HDAC6 in both *Actl7a* and *Actl7b* KO spermatids. We show here that global acetyl-lysine nuclear localization observed in WT spermatids is also absent. Additionally, in the absence of ACTL7A or ACTL7B there is a loss of intranuclear associated HDAC1 and HDAC3, which are known regulators of epigenetic associated histone acetylation changes that in turn regulate gene expression. From a holistic perspective of the data presented, herein we propose mechanistic and structural models for testis-specific ARPs in germ-line nuclear chromatin dynamics. Further investigation into the ARP-driven mechanisms regulating specific chromatin states in the

germline has exciting prospects in developing our understanding of heritability, specific disease states, and inter-generational epigenetics.