SUMOylation is Required for Nuclear Organization in Mouse Oocytes

Tessa E. Steenwinkel[1,2]; Sovanny R. Taylor[3,4,5]; Gabe J. Hohensee[3,4]; Steven Boeynaems[3,4,6]; Stephanie A. Pangas[1,2,3,5,7]

1. Dept. of Pathology & Immunology, Baylor College of Medicine, Houston, TX, USA
2. Graduate Program in Development, Disease Models & Therapeutics, Baylor College of Medicine, Houston, TX, USA
3. Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA
4. Jan and Dan Duncan Neurological Research Institute, Houston, TX, USA
5. Cancer & Cell Biology Graduate Program, Baylor College of Medicine, Houston, TX, USA
6. Therapeutic Innovation Center, Baylor College of Medicine, Houston, TX, USA
7. Dept. of Molecular & Cellular Biology, Baylor College of Medicine, Houston, TX, USA

In developed countries, women are choosing to have children later in life. During a woman's reproductive years, the quality and quantity of oocytes decrease due to several factors, including chromatin organization, mitochondrial dysfunction, and loss of protein homeostasis. SUMOylation is a post translational modification involved in protein homeostasis, subcellular localization, and the formation of membraneless organelles by phase separation. SUMOylation becomes dysregulated during aging, leading to dysregulation of its target proteins. In the ovary, loss of the SUMOylation conjugation enzyme, *Ube2i* (*Ubc9*), from postnatal oocytes causes female infertility, with oocytes that arrest in metaphase I of meiosis I, but the mechanism by which loss of SUMOylation disrupts oocyte development is not fully characterized. Normally, as the oocyte reaches its terminal growth state, transcription is repressed and the oocyte uses stored RNA transcripts for later use in meiosis and embryonic development. *Ube2i-Zp3cre* oocytes show defects in these processes as transcription continues and some maternal RNAs decrease. We hypothesize the lack of SUMOylation disrupts the ability of *Ube2i-Zp3cre* oocytes to form phase separated condensates essential for transcriptional regulation and mRNA processing in the nucleus, thereby preventing proper development. Confocal imaging of fully grown, germinal vesicle (GV) stage*Ube2i-Zp3cre* oocytes show mislocalization of SUMO1 and SUMO2-3, including loss of SUMO1 and SUMO2-3 condensates in the nucleus. We further analyzed the number of SC-35 positive speckles and their volume, as these speckles are closely tied to active transcription, mRNA processing, and have stable association with nearby chromatin. We found that SC-35 positive nuclear speckles are greatly decreased in number and size in the fully grown GV*Ube2i-Zp3cre* oocytes as compared to similar stage wildtype oocytes. We hypothesize this may be related to speckle collapse due to a lack of SUMOylation and proper association with chromatin. Because nuclear speckles are sites of RNA processing and splicing, we analyzed splicing events in RNA-sequencing data comparing *Ube2i-Zp3cre* oocytes to controls. Informatic analysis showed almost 600 alternative splicing patterns, including genes associated with actin binding and the nuclear membrane essential for proper nuclear structure. This work shows that in the absence of SUMOylation nucleus becomes disordered leading to a loss of controlled transcription in GV oocytes. These studies were supported by NIH grant R01HD085994 and NSF GRFP.