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Mouse meiosis expressed gene 1 is not phosphorylated in vivo

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Protein phosphorylation is a post-translational modification of proteins that plays critical roles in the regulation of many cellular processes including cell cycle, growth, apoptosis, and signal transduction pathways. Mouse meiosis expressed gene 1 (*Meig1*) was originally cloned in a search for genes essential for meiosis. However, studies from our laboratory and others demonstrated that the gene is critical for the final phase, spermiogenesis. MEIG1 forms a complex with parkin co-regulated gene 1 (PACRG) in the manchette, a unique structure only present in the elongating spermatids, and the complex is involved in transporting cargo proteins including sperm associated antigen 16 (SPAG16) for sperm flagella formation (Li et al., 2015). Our further studies demonstrated that as a monomer, MEIG1 binds to PACRG through a specific domain on the protein surface.

MEIG1 has only 88 amino acids with an estimated molecular mass of 10.8 kDa and a PI of 9.1. Due to its basic nature, MEIG1 migrates as a 15 kDa protein in SDS-PAGE gels. MEIG1 contains multiple potential serine and threonine phosphorylation sites. Post-translational modifications, including protein phosphorylation, play critical roles for proteins to conduct their biologic functions. It was reported that MEIG1 was phosphorylated and formed a dimer in male germ cells (Chen-Moses, Malkov, Shalom, Ever, & Don, 1997). The original goal of the study was to identify the phosphorylation sites of the MEIG1 protein in vivo and to further investigate the roles of these phosphorylated amino acids on MEIG1's function. Testes from adult wild type mice were removed and quickly frozen in an acetone-dry ice slurry, slowly warmed to room temperature, dried, weighed, and homogenized on ice in 8 M urea, 2% Triton X-100, and 20 mM dithiothreitol and phosphatase activity was inhibited with this procedure (Rembold et al., 2004). Protein concentration was measured with a 2D Quant kit (GE Healthcare Bio-Sciences, 80-6483-56). The samples were treated with a 2-D Clean-Up kit (GE Healthcare Bio-Sciences, 80-6484-51) and separated by two

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identical 4-20% criterion™ Tris-HCl protein gels (BIO-RAD, #3450104). After electrophoresis, one gel was transferred to a PVDF membrane for a Western blot analysis using the specific anti-MEIG1 antibody; the other gel was stained to evaluate protein phosphorylation status using the Pro-QTM Diamond phosphoprotein gel stain kit from Promega (Catalog number: P33300). The stain can visualize the proteins that are phosphorylated at a similar sensitivity as Western blot analysis using the ECL system. In Western blot analysis using the specific anti-MEIG1 antibody, a single band was detected at about 15 kDa with a PI of about 9, resembling the MEIG1 protein (Figure 1a). After staining with the Pro-Q Diamond phosphoprotein gel stain kit, multiple positive signals were observed. However, no signal was observed at 15 kDa and PI around 9 (Figure 1b), where the MEIG1 protein was detected in the Western blot analysis. The original conclusion for MEIG1 dipolymer/phosphorylation might be drawn by low specificity of the MEIG1 antibody used previously (Chen-Moses et al., 1997). A protein at about 31 kDa was detected in the testis of the global *Meig1* knockout when a higher concentration of our specific anti-MEIG1 antibody was used, indicating that the 31 kDa band represents a non-specific protein instead of the MEIG1 protein (Figure 1c). Our studies demonstrate that mouse MEIG1 does not undergo major phosphorylation in the mouse testis, and phosphorylation is not the major mechanism for MEIG1 to conduct biologic function in vivo.

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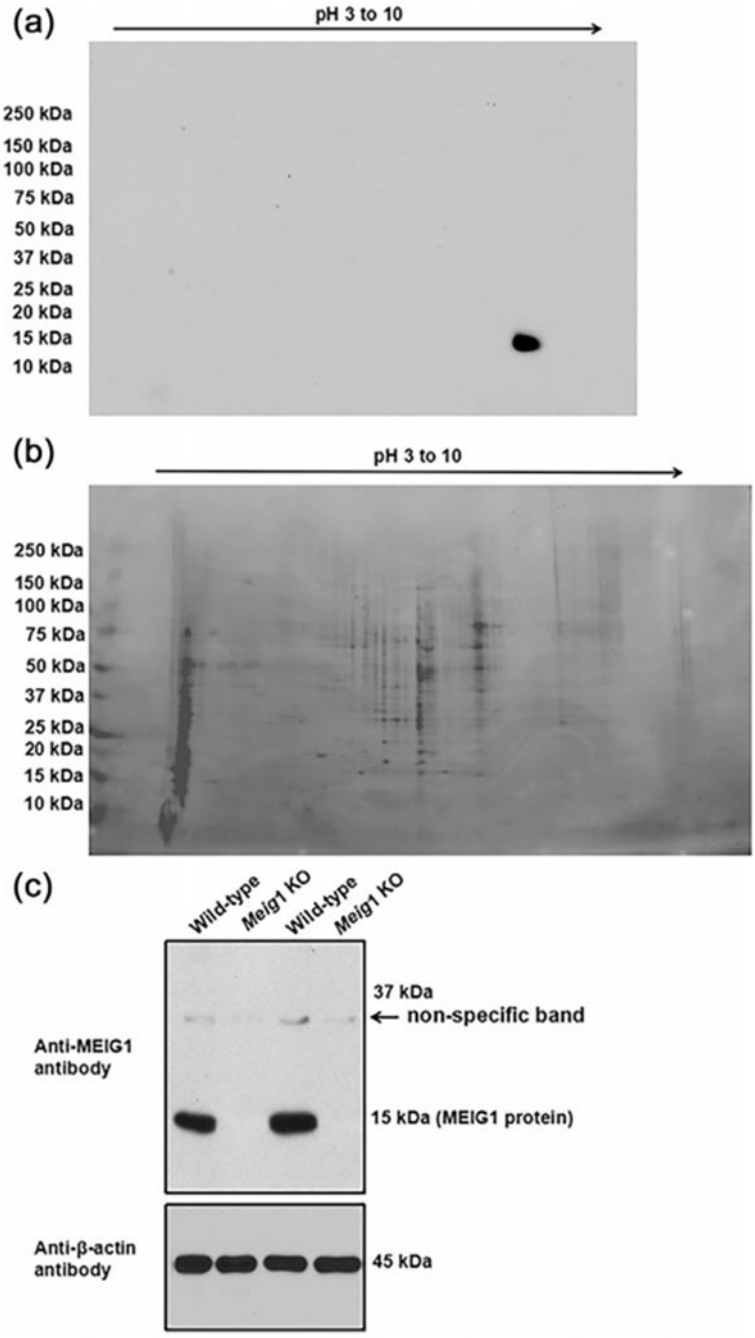

FIGURE 1.

Examination of phosphorylation status of mouse MEIG1 in the testis. Testicular protein was extracted from 3-month-old C57 mice, and was loaded onto two 2D PAGE gels with 75 ug of total protein for each gel. After 2D electrophoresis, one gel was used for 2D Western blot analysis using a specific anti-MEIG1 antibody generated in our laboratory (a. 1:8000 dilution), and the other gel was used to visualize the phosphorylated proteins using the Pro-Q Diamond phosphoprotein gel stain kit (b). Notice that the only one band visualized by Western blotting using the specific anti-MEIG1 antibody was not visualized in the gel

stained to visualize phosphorylated protein, indicating that MEIG1 does not undergo major phosphorylation in vivo. When the anti-MEIG1 antibody was used with a 1:2000 dilution, an about 31 kDa protein was also detected in the mouse testicular extracts, which likely represents a non-specific band as it was present in the MEIG1-deficient mice (c)