CPEB2 IS REQUIRED FOR TIGHT JUNCTION ASSEMBLY FOR ESTABLISHMENT OF PORCINE TROPHECTODERM EPITHELIUM

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Introduction

After fertilization, the embryo undergoes continuous mitotic cell division and loses distinct cell boundaries during the process of compaction. After compaction, the first epithelial cells are established in the outer portion of this structure, which contains a cavity formed by water influx brought about by an ion gradient, water channels, and tight junction (TJ) assembly. Recent study reported that apical localization of TJP1 (ZO-1) transcripts mediated by post-translational mechanisms for TJ assembly and mammary epithelial cell polarity. Cytoplasmic polyadenylation element binding protein (CPEB) is an RNA-binding protein that promotes elongation of poly(A) tails and regulates wild post-translational mechanisms. CPEB depletion in mammary epithelial cells is known to disrupt tight junction (TJ) assembly via mislocalization of TJP1, but the role of CPEB in the biological functions associated with TJs in trophectoderm epithelium has not yet been studied.

Materials and Methods

Cumulus-oocyte complexes were collected from porcine ovaries obtained from a local slaughterhouse and incubated in in vitro maturation medium for 44 h at 39 °C. Parthenogenetic activation was used an Electro Cell Manipulator 2001 (BTX, Inc., San Diego, CA, USA) on 280 mM mannitol medium supplemented with 0.01 mM CaCl2 and 0.05 mM MgCl2. Using a FemtoJet microinjector (Eppendorf, Hamburg, Germany) and a TE2000-U inverted microscope (Nikon Corporation, Tokyo, Japan), CPEB2 dsRNA (1 μg/μL) was then injected into the cytoplasm after 6 h of electrical stimulation. After injection, the embryos were placed in PZM-5 solution and cultured in an incubator for 144 h.

Results and Discussion

CPEB2 was detected in both the nuclei and apical cytoplasm at the 4- and 8-cell stages, localized to cell-cell contact after the initiation of the morula stage. Its depletion led to retarded blastocyst formation caused by impaired TJ assembly. Moreover, transcription of TJ-associated genes, including TJP1, CXADR, and OCLN, was not affected, but the corresponding proteins were not properly localized at the apical cell membrane in morulae, suggesting that CPEB2 confers mRNA stability and/or determines subcellular localization for translation. Remarkably reduced relative levels of TJP1 transcripts bearing the 3′-untranslated region were noted, indicating that CPEB2 mediates TJP1 mRNA stability. In conclusion, our findings demonstrate that because of its regulation of TJP1, CPEB2 is required for TJ assembly during porcine blastocyst development.