

Abstract

The aim of this work was to find and characterize genes that seem to be important for normal preimplantation development. We characterized three genes in more detail – centromeric protein F (CENPF; mitotin), ubiquitin C-terminal hydrolase-L1 (UCHL1) and nucleophosmin (NPM1; B23; numatrin; NO38). CENPF and nucleophosmin were shown to start their expression at late 8-cell stage, i.e. at major embryonic genome activation (EGA), and were hence supposed to be important during bovine preimplantation development. CENPF plays crucial role during cell division, especially by mediating the interaction of kinetochores and microtubules. Nucleophosmin is a multifunctional nucleolar phosphoprotein, whose most important roles are rRNA processing, chaperoning, ribosome biogenesis and centriole duplication control. Further, we characterized the role of UCHL1 during fertilization of bovine oocytes. UCHL1 is a deubiquitinating enzyme that controls cytoplasmic protein degradation, recycling of free ubiquitin from proteasome products and is involved in regulation of physiological apoptosis.

We studied the function of CENPF and nucleophosmin using RNA interference approach. Since UCHL1 protein is very stable, this method is not suitable for studying the UCHL1 function. We thus used two UCHL1 inhibitors that block its hydrolase activity. The embryos with silenced *CENPF* mRNA arrest at 8-cell stage and have lower morphological quality than the control embryos. The development up until the 8-cell stage is likely enabled by the storage of maternal protein. In somatic cells and post-EGA embryos the protein is degraded at the end of each cycle, but we detected no such degradation in the pre-EGA embryos. Similarly, we confirmed the importance of UCHL1 for normal course of bovine fertilization. In oocytes matured in UCHL1 inhibitor, high rate of polyspermy was found after fertilization. This was likely caused by defects in cortical granule relocalization and extrusion. On the other hand, the embryos with silenced nucleophosmin mRNA were able to develop until the blastocyst stage. Even though a large portion of maternal protein was degraded a sufficient amount was still stored during whole preimplantation embryogenesis and enabled the development of embryos without any detectable defects.

Further, we have focused on identifying of new potentially important genes, whose role during preimplantation development will be determined in the following studies. Among these genes, especially cullin 1 seems to play an important role during bovine preimplantation development. Its transcription from embryonic genome starts at late 8-cells stage. Moreover, we detected two transcript variants of cullin 1 expressed from different genes, one of which (cullin 1-like; XM_589507.3) was present from MII oocytes to early 8-cell stage and the second one (cullin 1; XM_876699) was present from late 8-cell stage to the blastocyst stage. These two genes are 83% homologous in sequence, both present on chromosome 4 but localized to two different regions.