Zygote

cambridge.org/zyg

Finding of bands of higher molecular weight than expected in three proteins in bovine preimplantation embryos

Veronika Kinterova[1,2], Veronika Petruskova[1,3], Jiri Kanka[1] and Tereza Toralova[1]

[1]Laboratory of Developmental Biology, Institute of Animal Physiology and Genetics, Academy of Science of Czech Republic, v.v.i., Libechov, Czech Republic, [2]Department of Veterinary Sciences, Czech University of Life Sciences in Prague, Prague, Czech Republic and [3]Faculty of Science, Charles University in Prague, Prague, Czech Republic

Short Communication

Cite this article: K. Veronika *et al.* (2019) Finding of bands of higher molecular weight than expected in three proteins in bovine preimplantation embryos. *Zygote* **27**: 187–189. doi: 10.1017/S0967199419000133

Received: 16 August 2018
Revised: 30 November 2018
Accepted: 25 February 2019

Keywords:
IκBα; Molecular weight; p27; Preimplantation embryo; Skp1

Address for correspondence:
Veronika Kinterova. Institute of Animal Physiology and Genetics, Academy of Science of Czech Republic, v. v. i., Rumburska 89, 277 21 Libechov, Czech Republic.
E-mail: kinterova@iapg.cas.cz

Summary

We report here the existence of bands of higher molecular weight after western blot analysis in three proteins – Skp1, p27 and IκBα in bovine preimplantation embryos. This finding is specific to preimplantation embryos (from the 2-cell stage to the blastocyst stage) and not differentiated fibroblast cells in which these bands were of expected molecular weight. We suggest that these bands of higher molecular weight represent a complex of proteins that are characteristic of preimplantation embryos.

Introduction

Mammalian preimplantation development, as well as early embryonic development, in non-mammalian species is a very specific period of life. The cell cycle is much shorter, G1 and G2 phases are almost missing and the embryo lives only from maternal stores. Until the event called embryonic genome activation (EGA) or maternal-to-zygotic transition (MZT) the embryo is transcriptionally silent and only maternally derived mRNAs and proteins are present. As both these types of molecules have usually short lifetimes, their processing has to be altered so that they could be stored from oocyte maturation through fertilization to EGA and in some proteins even further. It has been shown that some maternal proteins are stored after EGA (Svarcova *et al.*, 2007; Ohsugi *et al.*, 2008; Toralova *et al.*, 2012). This prolonged stability may be ensured by some posttranslational modification or complex formation. Such complexes of maternal proteins are formed to persist to preimplantation development and are then involved in driving embryogenesis (reviewed in Lei *et al.*, 2013). This represents primarily proteins like zona pellucida proteins or the SCMC (subcortical maternal complex). However it is possible that maternal proteins form complexes or are modified in order to be preserved for preimplantation development. Such masking or modification was found in oocytes in protein CENPE (Duesbery *et al.*, 1997). Moreover, the SCMC has a molecular weight much larger than is expected by the total mass of all participating proteins (669–2000 kDa vs. expected 325 kDa) (Li *et al.*, 2008).

Material and methods

In vitro *fertilization and embryo culture*

Bovine cumulus–oocytes complexes were obtained from abattoir-derived ovaries. The cattle had been slaughtered (Slaughterhouse Rosovice) for publicly edible meat. Those ovaries were discarded without any utilization. Hence, an ethics statement in our paper was not required. The isolated oocytes were subjected to *in vitro* maturation and subsequent fertilization (Toralova *et al.*, 2012). The embryos were collected after an appropriate time of cultivation (Benesova *et al.*, 2016).

Western blotting

Unless otherwise indicated, chemicals were purchased from Sigma. Unlike the anti-p27 and anti-IκBα antibodies (in which was necessary to use 20 embryos per line), anti-Skp1 antibody gave a really intensive signal and therefore we used just six embryos per line. Embryos were lysed in 15 µl of Blue Loading Buffer (772, Cell Signaling Technology, Danvers, MA, USA) with dithiothreitol, boiled for 5 min and subjected to 12% SDS-PAGE. Proteins were

CAMBRIDGE UNIVERSITY PRESS

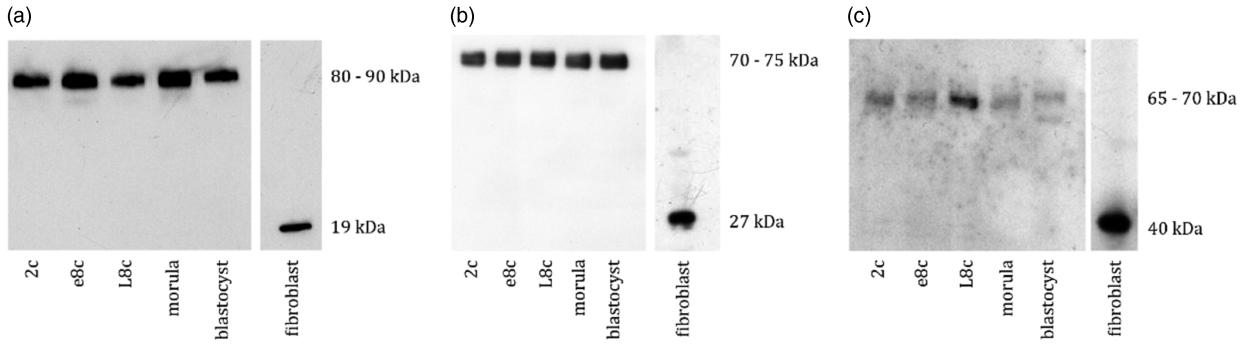

Figure 1. Western blot analysis of bovine preimplantation embryos and bovine fibroblasts. Representative images of western blots of embryos from 2-cell to blastocyst stage: (a) Skp1: 80–90 kDa vs. 19 kDa (six embryos per line). (b) p27: 70–75 kDa vs. 27 kDa (20 embryos per line). (c) IκBα: 65–70 kDa vs. 40 kDa (20 embryos per line). The expected band size responds to the band size in bovine fibroblasts. All experiments were repeated at least three times. 2c, 2-cell stage; e8c, early 8-cell stage, L8c, late 8-cell stage embryos.

transferred from gels to an Immobilon P membrane (Millipore Biosciences, Billerica, MA, USA) using a semidry blotting system (Whatman Biometra GmbH, Hoettingen, Germany) for 28 min at 5 mA/cm^2. Blocking of the membrane was performed in 5% BSA in TBS-Tween buffer (TBS-T, 20 mM Tris, pH 7.4, 137 mM NaCl and 0.5% Tween 20) for IκBα, and in 5% non-fat milk in TBS-T for Skp1 and p27, for 1 h and incubated overnight with following antibodies: IκBα - IκBα Antibody (Cell Signaling Technology 9242, Leiden, The Netherlands) 1:1000 in 5% BSA/TBS-T, p27 - Anti-p27 KIP 1 antibody (Abcam ab32034, Cambridge, UK) 1:1000 in 5% non-fat milk/TBS-T or Skp1–SKP1A monoclonal antibody, clone 1H8 (Abnova M01, Heidelberg, Germany) 1:1000 in 5% non-fat milk/TBS-T. After washing in TBS-T, the membranes were incubated with HRP-conjugated donkey anti-rabbit or donkey anti-mouse IgG antibody (both 1:7500; Jackson Immuno Research, Suffolk, UK) in 5% non-fat milk/TBS-T or in 5 % BSA/TBS-T for 1 h at room temperature. Proteins were visualized with Luminata Crescendo Western HRP (Merck Millipore, Darmstadt, Germany) or ECL (Amersham, GE Healthcare Life Science, UK). Precision Plus Protein™ Dual Color Standards (161-0374, Bio-Rad spol s.r.o., Czech Republic) were used for molecular weight estimation.

Results and discussion

Both embryos and fibroblasts were processed in the same way according to the protocol used in Toralova *et al.* (2012). All experiments were performed at least three times. When performing the western blot analysis, we found the existence of bands of higher molecular weight than expected, in all stages of bovine preimplantation development from the 2-cell stage until the blastocyst stage (representative blot in Fig. 1).

These bands were not possible to be dissolved using dithiothreitol or high temperature (5 min boiling). Simultaneously, analysis of bovine fibroblast cells was performed and the bands emerged at the expected molecular weight. This shows that the bands of higher molecular weight might be specific and typical for preimplantation embryos. However, we cannot exclude that this phenomenon does not exist in another cells, especially non-differentiated, rapidly dividing cells. The described proteins play distinct roles in cell functioning, however all of these have connection to E3-ubiquitin ligase SCF complex (Skp1–Cullin1–F-box protein complex). Skp1 is an invariant member of this complex and is involved in its activation/deactivation control (Bai *et al.*, 1996; Zheng *et al.*, 2002). Besides participation in the ubiquitin-proteasome pathway, it is necessary for correct chromosome segregation and euploidy maintenance in mice (Piva *et al.*, 2002). It is supposed to play an important role during mammalian preimplantation development (Benesova *et al.*, 2016). Incorrect Skp1 expression is involved in development of malignancy (Piva *et al.*, 2002). P27 is a cell cycle regulator especially involved in G1 arrest and in regulation of transcription. Its decreased expression is involved in tumorigenesis and poor prognosis of disease progression (Slingerland & Pagano, 2000). IκBα is involved in NF-κB inhibition by masking its nuclear localization signal and dissociation of NFκB from DNA. Both p27 and IκBα are substrates of the SCF complex, in which Skp1 is incorporated. However, it does not seem that the higher band incidence is related in these three proteins, as the bands are in different heights. It is known that the proteins in early embryos often have multiple isoforms (Tay *et al.*, 2006), however the large difference in molecular weights does not support this explanation. Moreover, the band height is not a multiple of the expected value, so it is not consequence of polymerization.

The reason for the existence of bands of higher molecular weight remains to be elucidated. However, the finding of them in these three proteins speaks for its importance and common occurrence in preimplantation embryos. We suppose that these bands are complex of proteins that arise to preserve them for further stages of preimplantation and may be (as the higher molecular weight bands are present in all preimplantation stages, including blastocysts) to some period of postimplantation development. We assume that similar results can be found also in many other proteins in preimplantation embryos.

Acknowledgements. We would like to thank J. Tyleckova, J. Cervenka, R. Sucha, M. Zizkova and J. Novak for their helpful comments.

Financial support. The work was supported by grant GACR 13-24730P and CIGA 20172013.

Ethical standards. Not applicable.

Statement of interest. None.

References

Bai C, Sen P, Hofmann K, Ma L, Goebl M, Harper JW and Elledge SJ (1996) SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* **86**, 263–74.

Benesova V, Kinterova V, Kanka J and Toralova T (2016) Characterization of SCF complex during bovine preimplantation development. *PLoS One* **11**, e0147096.

Duesbery NS, Choi T, Brown KD, Wood KW, Resau J, Fukasawa K, Cleveland DW and Vande Woude GF (1997) CENP-E is an essential

https://doi.org/10.1017/S0967199419000133 Published online by Cambridge University Press

kinetochore motor in maturing oocytes and is masked during Mos-dependent, cell cycle arrest at metaphase II. *Proc Natl Acad Sci USA* **94**, 9165–70.

Lei L, Xukun L and Jurrien D (2013) The maternal to zygotic transition in mammals. *Mol Aspects Med* **34**, 919–38.

Li L, Baibakov B and Dean J (2008) A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Dev Cell* **15**, 416–425.

Ohsugi M, Zheng P, Baibakov B, Li L and Dean J (2008) Maternally derived FILIA-MATER complex localizes asymmetrically in cleavage-stage mouse embryos. *Development* **135**, 259–69.

Piva R, Liu J, Chiarle R, Podda A, Pagano M and Inghirami G (2002) *In vivo* interference with Skp1 function leads to genetic instability and neoplastic transformation. *Mol Cell Biol* **22**, 8375–87.

Slingerland J and Pagano M (2000) Regulation of the cdk inhibitor p27 and its deregulation in cancer. *J Cell Physiol* **183**, 10–7.

Svarcova O, Laurincik J, Avery B, Mlyncek M, Niemann H and Maddox-Hyttel P (2007) Nucleolar development and allocation of key nucleolar proteins require *de novo* transcription in bovine embryos. *Mol Reprod Dev* **74**, 1428–35.

Tay TL, Lin Q, Seow TK, Tan KH, Hew CL and Gong Z (2006) Proteomic analysis of protein profiles during early development of the zebrafish, *Danio rerio*. *Proteomics* **6**, 3176–88.

Toralova T, Benesova V, Vodickova Kepkova K, Vodicka P, Susor A and Kanka J (2012) Bovine preimplantation embryos with silenced nucleophosmin mRNA are able to develop until the blastocyst stage. *Reprod Camb Engl* **144**, 349–59.

Zheng JY, Yang XM, Harrell JM, Ryzhikov S, Shim EH, Lykke-Andersen K, Wei N, Sun H, Kobayashi R and Zhang H (2002) CAND1 binds to unneddylated CUL1 and regulates the formation of SCF ubiquitin E3 ligase complex. *Mol Cell* **10**, 1519–26.

https://doi.org/10.1017/S0967199419000133 Published online by Cambridge University Press