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The polycomb group protein EED varies in its ability to access the nucleus in porcine oocytes and cleavage stage embryos☆

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ABSTRACT

Chromatin-modifying complexes serve essential functions during mammalian embryonic development. Polycomb group proteins EED, SUZ12, and EZH2 have been shown to mediate methylation of the lysine 27 residue of histone protein H3 (H3K27), an epigenetic mark that is linked with transcriptional repression. H3K27 trimethylation has been shown to be present on chromatin in mature porcine oocytes, pronuclear and 2-cell stage embryos, with H3K27 trimethylation decreasing at the 4-cell stage and not detectable in blastocyst stage embryos. The goals of this study were to determine the intracellular localization of the polycomb group protein EED in porcine oocytes and cleavage stage porcine embryos produced by *in vitro* fertilization and to determine the binding abilities of karyopherin α subtypes toward EED. Our results revealed that EED had a strong nuclear localization in 4-cell and blastocyst stage embryos and a strong perinuclear staining in GV-stage oocytes; EED was not detectable in the nuclei of pronuclear or 2-cell stage embryos. An *in vitro* binding assay was performed to assess the ability of EED to interact with a series of karyopherin α subtypes; results from this experiment revealed that EED can interact with several karyopherin α subtypes, but with varying degrees of affinity. Together these data indicate that EED displays a dynamic change in intracellular localization in progression from immature oocyte to cleavage stage embryo and that EED possess differing *in vitro* binding affinities toward individual karyopherin α subtypes, which may in part regulate the nuclear access of EED during this window of development.

1. Introduction

Gene expression is affected by epigenetic modifications such as DNA methylation, covalent histone modifications, and chromatin packaging. Posttranslational modification of histone proteins, including methylation, acetylation, phosphorylation, and ubiquitination are hypothesized to constitute a "histone code" (Jenuwein and Allis, 2001) that control gene transcription. This "histone code" hypothesis proposes that covalent modifications of histone proteins contribute to transcriptional regulation and that these modifications provide a mechanism for encoding information through successive generations of cell division (Jenuwein and Allis, 2001). Methylation of the lysine residue 27 of histone H3 (H3K27) is an epigenetic mark that is linked with transcriptional repression (Trojer and Reinberg, 2006); H3K27 can be mono-, di-, or trimethylated (Pasini et al., 2004).

Chromatin-modifying complexes, including the polycomb group proteins, serve essential functions during mammalian embryonic development (Li, 2002). The polycomb group proteins embryonic ectoderm development

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(EED), suppressor of zeste 12 (SUZ12), and enhancer of zeste 2 (EZH2) form the Polycomb-Repressive Complex 2 (PRC2) (Cao et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002; Czermin et al., 2002). PRC2 has been shown to mediate methylation of H3K27. Although EZH2 has been shown to be the catalytic subunit that mediated lysine methylation, EZH2 requires EED and SUZ12 for activity *in vivo* (Erhardt et al., 2003). EED knockout embryos die during early gestation in mice; EED functions during gastrulation and serves a role in maintaining X-inactivation in trophoblast cells (Faust et al., 1995, 1998; Wang et al., 2001). EZH2 knockout mice have a growth defect of the ectoderm and, like SUZ12 knockouts, exhibit peri-implantation lethality (O'Carroll et al., 2001; Donohoe et al., 1999). EZH2 has been shown to be the histone methyltransferase that is responsible for the methylation of H3K27 (Czermin et al., 2002).

For chromatin remodeling enzymes to interact with interphase chromatin, they must first be transported through the nuclear envelope to reach the nuclear chromatin. Several nuclear trafficking pathways exist in eukaryotic cells; one the best characterized transport pathways is nuclear import mediated by the karyopherin α/β heterodimer (Görlich and Kutay, 1999). In this system, karyopherin α functions to bind proteins that possess a classical nuclear localization signal. To date, seven karyopherin α subtypes have been characterized in mammals (Cabot and Prather, 2003; Tejomurtula et al., 2009; Kelley et al., 2010; Hu et al., 2010). All karyopherin α subtypes possess an importin β binding (IBB) domain and a series of armadillo (ARM) repeats; these ARM repeats are responsible for recognizing the nuclear localization signal within the presumptive karyopherin α cargo. Karyopherin α subtypes have been shown to be differentially expressed at discrete stages of embryo development. Studies have also shown that some nuclear localization signal-bearing proteins are preferentially imported by only specific karyopherin α subtypes (Talcott and Moore, 2000; Welch et al., 1999; Tejomurtula et al., 2009; Wang et al., 2012).

In the mouse and bovine embryo, it has been shown that global patterns of H3K27 methylation undergo dramatic changes during cleavage development (Erhardt et al., 2003; Ross et al., 2008). In bovine, the highest intensity of trimethylated H3K27 is present in oocytes. There is a gradual reduction of H3K27 trimethylation in the nuclei until the eight-cell stage; H3K27 trimethylation is then increased in morula and blastocyst stage bovine embryos (Ross et al., 2008). Global patterns of H3K27 methylation undergo reorganization during cleavage development in the porcine embryo as well. H3K27 trimethylation is present on chromatin in mature porcine oocytes, pronuclear and 2-cell stage embryos, is dramatically decreased at the 4-cell stage, and not detectable in blastocyst stage embryos (Park et al., 2009). Another study of H3K27 trimethylation in porcine embryos show H3K27 trimethylation decreases from the pronuclear to the 4-cell stage development, but is significantly increased in hatched blastocyst (Gao et al., 2010). In addition, it has been shown that the transcript levels of EED, SUZ12 and EZH2 are present in differing amounts at discrete stages of cleavage development (Park et al., 2009).

While it is known that EZH2 requires the additional PRC2 subunits to exert its methyltransferase activity *in vivo* (Erhardt et al., 2003), it is unclear how PRC2, or its individual subunits, are trafficked to the nucleus to gain access to chromatin. We hypothesized that the global change in H3K27 trimethylation previously reported in porcine embryos was due to restricted nuclear access of PRC2 proteins at discrete stages of porcine cleavage stage development. Therefore, the goals of this study were to determine the intracellular localization of endogenous EED in germinal vesicle (GV) stage porcine oocytes and cleavage stage porcine embryos produced *in vitro* and to determine which karyopherin α subtypes EED could interact with to mediate its nuclear import.

2. Materials and methods

2.1. Oocyte collection

All chemicals were obtained from Sigma Chemical Company (St. Louis, MO) unless stated otherwise. Porcine ovaries from prepubertal gilts were collected from a local slaughterhouse and transported to the laboratory in an insulated container. Cumulus-oocyte complexes (COCs) were aspirated from antral follicles measuring 3–6 mm in diameter using a disposable 10 ml syringe and an 18-gauge needle. Follicular fluid was pooled and allowed to gravity settle in a 37 °C incubator. COCs were resuspended in HEPES-buffered medium containing 0.01% polyvinyl alcohol (PVA) (Abeydeera et al., 1998). This suspension was examined under a dissecting microscope and COCs with multiple layers of intact cumulus cells were chosen for the experiments. For germinal vesicle (GV)-stage oocytes used in immunocytochemical staining studies, COCs were vortexed in 0.1% hyaluronidase in HEPES-buffered medium for 6 minutes to remove the cumulus cells. Denuded GV-stage oocytes were then rinsed in HEPES-buffered medium containing 3 mg/ml BSA.

2.2. In vitro maturation

Groups of 50–75 COCs were placed in culture dishes containing 500 μl of tissue culture medium 199 (TCM-199; Gibco BRL, Grand Island, NY) containing 0.14% PVA, 10 ng/ml epidermal growth factor, 0.57 mM cysteine, 0.5 IU/ml porcine FSH, and 0.5 IU/ml ovine LH under mineral oil. COCs were matured for 42–44 hours at 39 °C and 5% CO_2 in air, 100% humidity (Abeydeera et al., 1998). After maturation, cumulus cells were removed from COCs by vortexing in 0.1% hyaluronidase in HEPES-buffered medium containing 0.01% PVA for 4 minutes.

2.3. In vitro fertilization and embryo culture

Following three washes in HEPES-buffered medium and three washes in modified Tris-buffered medium (mTBM), 30–35 mature denuded oocytes were placed in 100 μl mTBM at 39 °C and 5% CO_2 and fertilized according to an established protocol (Abeydeera and Day, 1997), using fresh, extended boar semen at a concentration of 5×10^5 spermatozoa/ml. Briefly, boar semen was extended