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Differential regulation of cyclin B1 degradation between the first and second meiotic divisions of bovine oocytes

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Abstract

During mammalian oocyte maturation, two consecutive meiotic divisions are required to form a haploid gamete. For each meiotic division, oocytes must transfer from metaphase to anaphase, but maturation promoting factor (cyclin-dependent kinase 1/cyclin B1) activity would keep the oocytes at metaphase. Therefore, inactivation of maturation promoting factor is needed to finish the transition and complete both these divisions; this is provided through anaphase-promoting complex/cyclosome-dependent degradation of cyclin B1. The objective of this study was to examine meiotic divisions in bovine oocytes after expression of a full length cyclin B1 and a nondegradable N-terminal 87 amino acid deletion, coupled with the fluorochrome Venus, by microinjecting their complementary RNA (cRNA). Overexpression of full-length cyclin B1-Venus inhibited homologue disjunction and first polar body formation in maturing oocytes (control 70% vs. overexpression 16%; $P < 0.05$). However at the same levels of expression, it did not block second meiotic metaphase and cleavage of eggs after parthenogenetic activation (control: 82% pronuclei and 79% cleaved; overexpression: 91% pronuclei and 89% cleaved). The full length cyclin B1 and a nondegradable N-terminal 87 amino acid deletion caused metaphase arrest in both meiotic divisions, whereas degradation of securin was unaffected. Roscovitine, a potent cyclin-dependent kinase 1 (CDK1) inhibitor, overcame this metaphase arrest in maturing oocytes at 140 μM, but higher doses (200 μM) were needed to overcome arrest in eggs. In conclusion, because metaphase I (MI) blocked by nondegradable cyclin B1 was distinct from metaphase II (MII) in their different sensitivities to trigger CDK1 inactivation, we concluded that mechanisms of MI arrest differed from MII arrest.
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Keywords: Cyclin B1; Securin; MPF; Meiosis; Bovine; Oocytes

1. Introduction

Mammalian oocytes are arrested at the dictyate stage of the first meiotic prophase. Maturation-promoting factor (MPF; cyclin-dependent kinase 1/cyclin B1) activity drives oocytes into and through meiosis [1,2]. Its activity oscillates with entry and exit from meiosis I and meiosis II, and is regulated primarily by cyclin B1 synthesis and degradation in mammalian oocytes [3,4]. The initial increase in MPF activity is sufficient to drive germinal vesicle breakdown (GVBD), chromosome condensation, and microtubule polymerization in prophase I oocytes [5]. Then, MPF activity rises and reaches a plateau at the end of the first metaphase (MI). Anaphase-promoting complex/cyclosome (APC/C) activation at MI targets destruction of cyclin B1, but cyclin B1 is newly synthesized immediately after entry into meiosis II [6–8]. Oocytes

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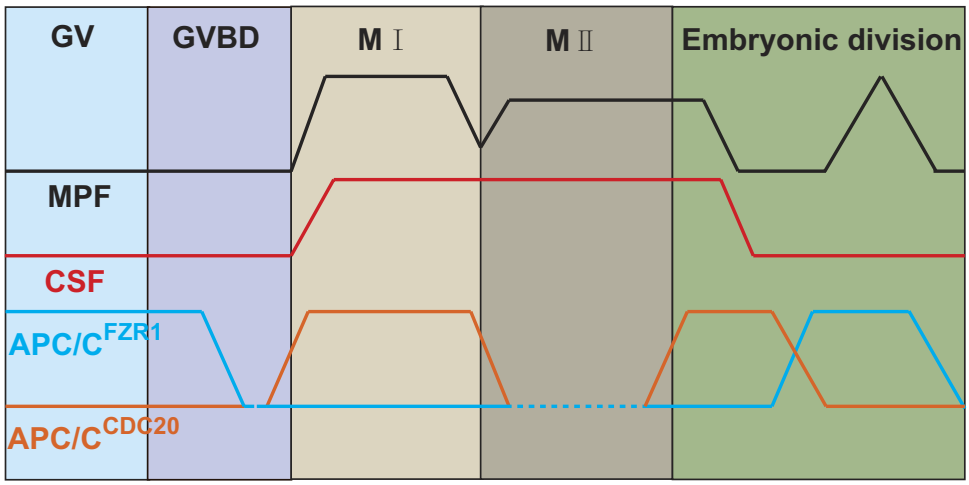

Fig. 1. Activity diagram of maturation promoting factor (MPF), cytostatic factor (CSF), and anaphase-promoting complex/cyclosome (APC/C) during oocyte maturation.

arrest at metaphase II (MII; and hereafter are termed "eggs") with high MPF activity because of cytostatic factor (CSF) activity, which inhibits APC/C [9,10]. An activity diagram of MPF, CSF, and APC/C during oocyte maturation is shown (Fig. 1).

Homologous chromosomes in meiosis I and sister chromatids in meiosis II are prevented from undergoing premature disjunction by cohesin complexes that hold the chromosomes together. At anaphase onset, the protease separase cleaves cohesion [11,12], resulting in a poleward movement of separated chromosomes, a process that also requires loss of cyclin-dependent kinase 1 (CDK1) activity [13,14]. Before anaphase, separase is inhibited through binding to a chaperone protein, securin, and at anaphase onset APC/C activity not only causes degradation of cyclin B1 but also securin, thereby activating separase.

Although cyclin B1 proteolysis is the dominant control for downregulation of CDK1 activity during cell division, additional mechanisms may contribute to CDK1 inactivation. These are likely important in oocytes, where not all of the cyclin B1 is degraded between meiotic divisions [15,16]. It has been demonstrated that MPF can bind free separase and in so doing nullify its kinase activity [17,18]. Interestingly, this interaction appears essential in mouse oocytes during meiosis I, because the first polar body was blocked when a functionally inhibitory antibody was injected into oocytes that prevented binding of MPF with separase [19]. The reason for the block to polar body extrusion was maintenance of some residual MPF activity, because it was rescued by incubation with the CDK inhibitor roscovitine.

The interaction of separase with MPF leads to mutual inhibition, not just inactivation of MPF. Inhibition of separase by MPF was most evident after cyclin B1 overexpression. Therefore, for mouse oocytes in either meiotic division, or for somatic cells, overexpression of nondegradable cyclin B1 inhibited chromosome disjunction [6,7,18,20–22].

Many human aneuploidies are derived from segregation errors during meiotic divisions [1,23]. Although mechanisms of chromosome separation in meiotic exit are important, this complex process has not yet been thoroughly studied in mammals other than the mouse. The objective of the present study was to determine the effects of expression of full length cyclin B1 and a nondegradable D-box mutant of cyclin B1 (D87 cyclin B1) on bovine immature oocytes and mature eggs.

2. Materials and methods

2.1. Reagents

All chemicals and media were from Sigma-Aldrich (Shanghai, China), unless otherwise indicated. Tissue culture medium-199 (M199) was from Gibco (Life Technologies Corporation, Beijing, China).

2.2. Oocyte collection and culture

Bovine ovaries were collected at an abattoir and transported to the laboratory within 6 h after collection. Ovaries were transported in saline solution (NaCl, 0.9% wt:vol) at 21 °C to 25 °C. Oocytes were obtained by aspiration of 2- to 8-mm follicles with a 12-gauge needle and a 10-mL syringe. The cumulus-oocyte com-

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