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Reactive Oxygen and Nitrogen Species During Meiotic Resumption From Diplotene Arrest in Mammalian Oocytes

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ABSTRACT

Mammalian ovary is metabolically active organ and generates by-products such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) on an extraordinary scale. Both follicular somatic cells as well as oocyte generate ROS and RNS synchronously and their effects are neutralized by intricate array of antioxidants. ROS such as hydrogen peroxide (H_2O_2) and RNS such as nitric oxide (NO) act as signaling molecules and modulate various aspects of oocyte physiology including meiotic cell cycle arrest and resumption. Generation of intraoocyte H_2O_2 can induce meiotic resumption from diplotene arrest probably by the activation of adenosine monophosphate (AMP)-activated protein kinase A (PRKA)—or Ca^{2+}-mediated pathway. However, reduced intraoocyte NO level may inactivate guanylyl cyclase-mediated pathway that results in the reduced production of cyclic 3′,5′-guanosine monophosphate (cGMP). The reduced level of cGMP results in the activation of cyclic 3′,5′-adenosine monophosphate (cAMP)-phosphodiesterase 3A (PDE3A), which hydrolyses cAMP. The reduced intraoocyte cAMP results in the activation of maturation promoting factor (MPF) that finally induces meiotic resumption. Thus, a transient increase of intraoocyte H_2O_2 level and decrease of NO level may signal meiotic resumption from diplotene arrest in mammalian oocytes. J. Cell. Biochem. 111: 521–528, 2010.

KEY WORDS: H_2O_2; NO; OOCYTE; cGMP; cAMP; MPF; MEIOTIC CELL CYCLE

In mammals, free radicals are generated as by-products of normal cellular metabolism and serve as key signal molecules in various physiological and pathological processes [Agarwal et al., 2005; Fujii et al., 2005]. Ovary is a metabolically active organ and generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) on an extraordinary scale during various physiological processes [Fujii et al., 2005]. The biphasic roles of ROS and RNS have been reported, that is, transient increase or decrease of intraoocyte ROS and RNS levels modulate oocyte physiology, while their sustained high levels result in oxidative and nitrosative stresses that lead to negative consequences such as cell cycle arrest and apoptosis [Agarwal et al., 2005; Fujii et al., 2005; Peyrot and Ducrocq, 2008]. Several studies have been conducted to find out roles of ROS and RNS in ovulated eggs arrested at metaphase-II [Goud et al., 2005, 2006, 2008a,b] but the possible mechanism of their action during meiotic resumption from diplotene arrest have not been reviewed so far. Hence, the purpose of this review is to summarize data, from several laboratories including ours, on the beneficial effects of ROS and RNS during meiotic resumption from diplotene arrest in mammalian oocytes and to propose their possible mechanism of actions.

GENERATION OF ROS

ROS are inevitably generated during physiological processes of oxygen consumption, the levels of which are enhanced under some pathological conditions [Fujii et al., 2005]. ROS are generated via various reactions within the body either by non-enzymatic reactions, for example, Fenton reaction in the presence of transient metal ion or by enzymatic system, for example, xanthine oxidase [Agarwal et al., 2005; Fujii et al., 2005; Harrison, 2002]. Biological reactions such as electron transfer and oxygenase reaction (utilize oxygen molecules as the substrate) are also involved in ROS production. Since the mitochondrial respiratory chain is the main oxygen consuming system of a cell, majority of ROS are generated from this system under physiological conditions. Three major types of ROS reported so far are superoxide ($O_2^{\cdot-}$), hydrogen peroxide

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(H_2O_2), and hydroxyl radical (.OH) [Agarwal et al., 2005; Combelles et al., 2009]. Superoxide radical is formed when the electrons leak from the electron transport chain and dismutation of superoxide ion results in the formation of H_2O_2 [Agarwal et al., 2005]. The H_2O_2 is also generated as a by-product of mitochondrial respiration and other metabolic processes [Winterbourn and Hampton, 2008]. The cellular concentrations of H_2O_2 in submicromolar range induces signaling pathway, while higher concentrations results in oxidative stress [Winterbourn and Hampton, 2008].

FOLLICULAR PRODUCTION OF ROS

There are two possible sources for generation of ROS inside the follicular microenvironment, that is, follicular somatic cells and the oocyte. Generation of H_2O_2 in submicromolar range has been reported in the ovary of rat during various phases of estrous cycle [Singh and Pandey, 1997]. The various biomarkers of oxidative stress like superoxide dismutase (SOD), Cu–Zn superoxide dismutase (Cu–Zn SOD), Mn superoxide dismutase (Mn SOD), glutathione peroxidase, and lipid peroxides have been demonstrated in the ovary of several mammalian species including human [Shiotani et al., 1991; Suzuki et al., 1999; Agarwal et al., 2005]. The Cu–Zn SOD and Mn SOD are important primary intracellular ROS scavenger enzymes [Yu, 1994; Matos et al., 2009] and their activities have been reported in preantral, antral, and dominant follicles [Laloraya et al., 1989, 1998; Suzuki et al., 1999; Fujii et al., 2005; Matos et al., 2009]. The higher levels of ROS have been reported in the follicular fluids of Swine and Cow [Basini et al., 2008; Rizzo et al., 2009].

The generation of ROS within the follicle indicates its beneficial role during final maturation just prior to ovulation in mammalian oocytes [Behrman et al., 2001]. The generation of ROS in human follicular fluid is required in order to have better chance to a women undergoing in vitro fertilization cycle to get pregnant [Oyawoye et al., 2003; Pasqualotto et al., 2004; Baka and Malamitsi-Puchner, 2006; Pasqualotto and Pasqualotto, 2007]. This is indirectly supported by the observations that luteinizing hormone (LH; known to induce meiotic resumption in prevulatory oocytes and ovulation in most of the mammalian species) depletes ascorbic acid (a natural antioxidant) level in preovulatory follicles [Guarnaccia et al., 2000]. Taken together, these findings suggest that the generation of ROS inside the follicular microenvironment could be associated with final maturation, that is, meiotic resumption from diplotene arrest in mammalian preovulatory oocytes.

ROS AND OOCYTE MEIOTIC RESUMPTION IN VITRO

It has been now accepted that ROS act as signaling molecule and regulate various aspects of cell functions including meiotic cell cycle progression/arrest and apoptotic cell death [Chaube et al., 2005, 2008, 2009; Martin-Romero et al., 2008; Tripathi et al., 2009, 2010]. The generation of ROS is associated with meiotic cell cycle progression [Martin-Romero et al., 2008; Chaube et al., 2009] and increased developmental potential of oocytes during maturation [Attaran et al., 2000; Oyawoye et al., 2003; Wiener-Megnazi et al., 2004; Morado et al., 2009]. The oxidizing agent tertiary-butyl-hydroperoxide did not inhibit resumption of meiosis in mouse oocytes cultured in vitro indirectly suggesting the involvement of ROS in meiotic resumption [Tarin et al., 1996]. In vitro observations suggest that lower concentrations of H_2O_2 induce resumption from diplotene arrest in rat oocytes [Chaube et al., 2005]. A transient increase of intracellular H_2O_2 level is associated with the resumption of meiosis from diplotene arrest in denuded rat oocyte cultured in vitro [Chaube et al., 2009; Tripathi et al., 2009], while further increase of ROS is associated with meiotic cell cycle arrest and apoptosis [Chaube et al., 2005, 2008, 2009; Tripathi et al., 2009]. Further, ROS scavengers and cell permeable antioxidants inhibit spontaneous meiotic resumption in both cumulus-enclosed oocyte and denuded oocytes cultured in vitro indirectly suggesting the involvement of ROS during meiotic resumption from diplotene arrest [Combelles et al., 2009; Takami et al., 1999; Pasqualotto and Pasqualotto, 2007; Tripathi et al., 2009].

The downstream pathway by which ROS induce meiotic resumption needs to be elucidated. However, few studies indicate that H_2O_2 and other oxidative stress inducers stimulate AMP-activated protein kinase (AMPK) [Choi et al., 2001; Chen et al., 2006; Chen and Downs, 2008]. The activated AMPK induces meiotic resumption in mouse diplotene arrested oocyte cultured in vitro [Cheon et al., 2000; Downs et al., 2002; Chen et al., 2006; LaRosa and Downs, 2006; Chen and Downs, 2008]. Another possibility exist that H_2O_2 stimulates the release of intracellular Ca^{2+} from mitochondria and induces meiotic resumption [Suzuki et al., 1997; Lee et al., 2000; Chaube et al., 2008]. Taken together, these studies suggest that transient increase in the level of intracellular ROS through AMPK and/or Ca^{2+}-mediated pathway induce meiotic resumption from diplotene arrest in mammalian oocytes.

GENERATION OF RNS

The nitric oxide (NO) is another important signaling molecule that can modulate oocyte physiology. It is generated either by enzymatic or non-enzymatic pathway in various cell types including mammalian oocytes. The NO is generated by a group of enzymes such as neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), and inducible nitric oxide synthase (iNOS) in mammalian ovaries [Agarwal et al., 2005; Fujii et al., 2005]. These enzymes convert L-arginine to L-citrulline and generate NO [Bush et al., 1992]. This is further supported by the observations that competitive inhibition of L-arginine uptake by L-lysine or L-ornithine reduces NO generation [Inoue et al., 1993]. In non-enzymatic pathway, NO is generated from nitrite at low-pH under reducing conditions involving hydrogen peroxide and D- or L-arginine [Palmer and Moncada, 1989; Nagase et al., 1997]. However, NO generated through non-enzymetic pathway may play a role to similar biological events as NO generated enzymatically [Aktan, 2004].

FOLLICULAR PRODUCTION OF RNS

During follicular growth and development, oocytes are arrested at diplotene stage of meiotic cell cycle for a long time inside follicular

microenvironment in mammalian ovary. The diplotene arrest in follicular oocyte might be due to synthesis and secretion of RNS such as NO through NOS-mediated pathway either from follicular cells or from oocyte itself or both. This notion is strengthened by the observations that all three NOS isoforms nNOS, eNOS, and iNOS are localized at various stages of folliculogenesis in rat and porcine ovaries [Van Voorhis et al., 1995; Zackrisson et al., 1996; Kim et al., 2005]. The levels of eNOS and iNOS increase in theca and granulosa cells during follicular development in mouse ovary [Chmelikova et al., 2009]. Similarly, eNOS mRNA has been detected in rat ovary and porcine granulosa cells cultured in vitro [Van Voorhis et al., 1995; Takesue et al., 2003]. Taken together, these findings suggest the involvement of NOS isoforms (nNOS, eNOS, and iNOS) during follicular growth and development. The NO generated through NOS-mediated pathway from follicular somatic cells might be associated with the maintenance of meiotic arrest at diplotene stage in preovulatory oocytes for a long time in follicle (Fig. 1).

Diplotene arrested follicular oocytes are also capable of generating NO through NOS-mediated pathway. This notion is supported by the observation that eNOS level increases in preovulatory oocytes of porcine during the acquisition of meiotic competency [Hattori et al., 2001; Takesue et al., 2003; Mitchell et al., 2004; Kim et al., 2005]. Similarly, an increased level of eNOS has also been reported in rat oocytes [Jablonka-Shariff and Olson, 1997]. Further, an increased iNOS expression has been observed in immature oocytes of mouse [Jablonka-Shariff and Olson, 1998; Mitchell et al., 2004] during final stages of follicular development. These studies suggest that oocyte also generate NO through NOS-mediated pathway that might be associated with the maintenance of meiotic arrest at diplotene stage inside the follicular microenvironment (Fig. 2).

The high level of NO generated either by follicular somatic cells or by oocyte itself play an important role in the maintenance of meiotic arrest at diplotene stage suggesting the involvement of reduced NO level in resumption of meiosis from diplotene arrest. This possibility is further supported by Zhang et al. [2009] that a decreased level of NO through iNOS-mediated pathway might be involved during LH/human chorionic gonadotropin (hCG)-induced meiotic resumption from diplotene arrest in mammalian oocytes [Zhang et al., 2009]. This hypothesis is strengthened by the observations that hCG injection reduced iNOS expression in granulosa cells and thereby NO levels in follicular fluid and induced meiotic resumption from diplotene arrest in rat oocytes [Nakamura et al., 2002; Yamagata et al., 2002]. In contrast, NO donor-inhibited proliferation of cumulus cells and mRNA expression of LH receptor in porcine COCs cultured in vitro [Hattori et al., 2000]. Similarly, NO inhibits LH-induced disruption of gap junctions, cumulus cells expansion, mitogen-activated protein kinase (MAPK) activity, resumption of meiosis from diplotene arrest and increases cyclic 3′,5′-guanosine monophosphate (cGMP) production in rat [Sela-Abramovich et al., 2008].

The reduced intraoocyte NO level probably through cGMP signaling pathway may induce meiotic resumption from diplotene arrest in follicular oocytes. This reduced NO level in follicular fluid during ovarian stimulation protocol provides some beneficial effect to oocyte quality in human [Vignini et al., 2008]. Further, removal of diplotene arrested oocytes from preovulatory follicle probably deprive the supply of NO from follicular microenvironment to the oocyte resulting into decrease of intraoocyte NO level that might be associated with spontaneous resumption of meiosis under in vitro culture conditions [Chaube et al., 2009; Tripathi et al., 2009]. Taken together, these findings suggest that the reduced iNOS activity in response to LH/hCG surge or removal of oocyte from its follicular microenvironment result in the decrease of intraoocyte NO level that might be associated with meiotic resumption from diplotene arrest in mammalian oocytes. In contrast, in some mammalian species, increased NOS isoforms activities and NO level have been reported to induce meiotic resumption in diplotene arrested oocytes [Jablonka-Shariff and Olson, 1998; Hattori et al., 2000; Bu et al., 2003; Tao et al., 2005; Viana et al., 2007; Chmelikova et al., 2010]. However, the molecular mechanism(s) by which increased NO level induces meiotic resumption from diplotene arrest remains unclear.

RNS AND OOCYTE MEIOTIC RESUMPTION IN VITRO

Several studies have been conducted to find out the possible role of NO during exit from diplotene arrest in mammalian oocytes but the results are equivocal. Few studies suggest that high level of NO derived from both eNOS and iNOS induces meiotic resumption from diplotene arrest in porcine [Tao et al., 2004, 2005; Chmelikova et al., 2010], mouse [Jablonka-Shariff and Olson, 1998; Huo et al., 2005], murine [Jablonka-Shariff and Olson, 2000], and rat oocytes [Jablonka-Shariff et al., 1999]. On the other hand, few studies indicates that lower concentration (0.01 μM) of NO donor such as sodium nitroprusside (SNP) induces meiotic resumption, while higher concentrations (100 and 500 μM) inhibit meiotic resumption from diplotene arrest in bovine oocytes cultured in vitro [Bilodeau-Goeseels, 2007]. These finding are further supported by observations that NO donor inhibits meiotic resumption in mouse, rat, and bovine cumulus-enclosed oocytes [Nakamura et al., 2002; Bilodeau-Goeseels, 2007; Abbasi et al., 2009] by activating guanylyl cyclase (GC)–cGMP pathway [Bu et al., 2003]. Further, dual actions of NO (stimulation or inhibition; depending on its concentration) have been reported during meiotic resumption from diplotene arrest in mouse oocytes cultured in vitro [Bu et al., 2003]. More studies are required to elucidate the molecular mechanism(s) by which NO regulates meiotic cell cycle at diplotene stage in mammalian preovulatory oocytes.

The NO derived from iNOS-mediated pathway seems to play a role in the maintenance of meiotic arrest at diplotene stage. This possibility is supported by recent observations from our laboratory that an increase of iNOS immunoreactivity and higher level of intraoocyte NO are associated with maintenance of meiotic arrest at diplotene stage. On the other hand, reduced activity of iNOS and decreased intraoocyte NO level are associated with resumption of meiosis from diplotene arrest in rat oocytes [Tripathi et al., 2009]. This possibility is further strengthened by the observations that iNOS inhibitor such as aminoguanidine (AG) induces meiotic resumption from diplotene arrest in rat cumulus-enclosed oocytes cultured in vitro [Nakamura et al., 2002]. These studies support the hypothesis that reduced level of NO through iNOS-mediated pathway may play

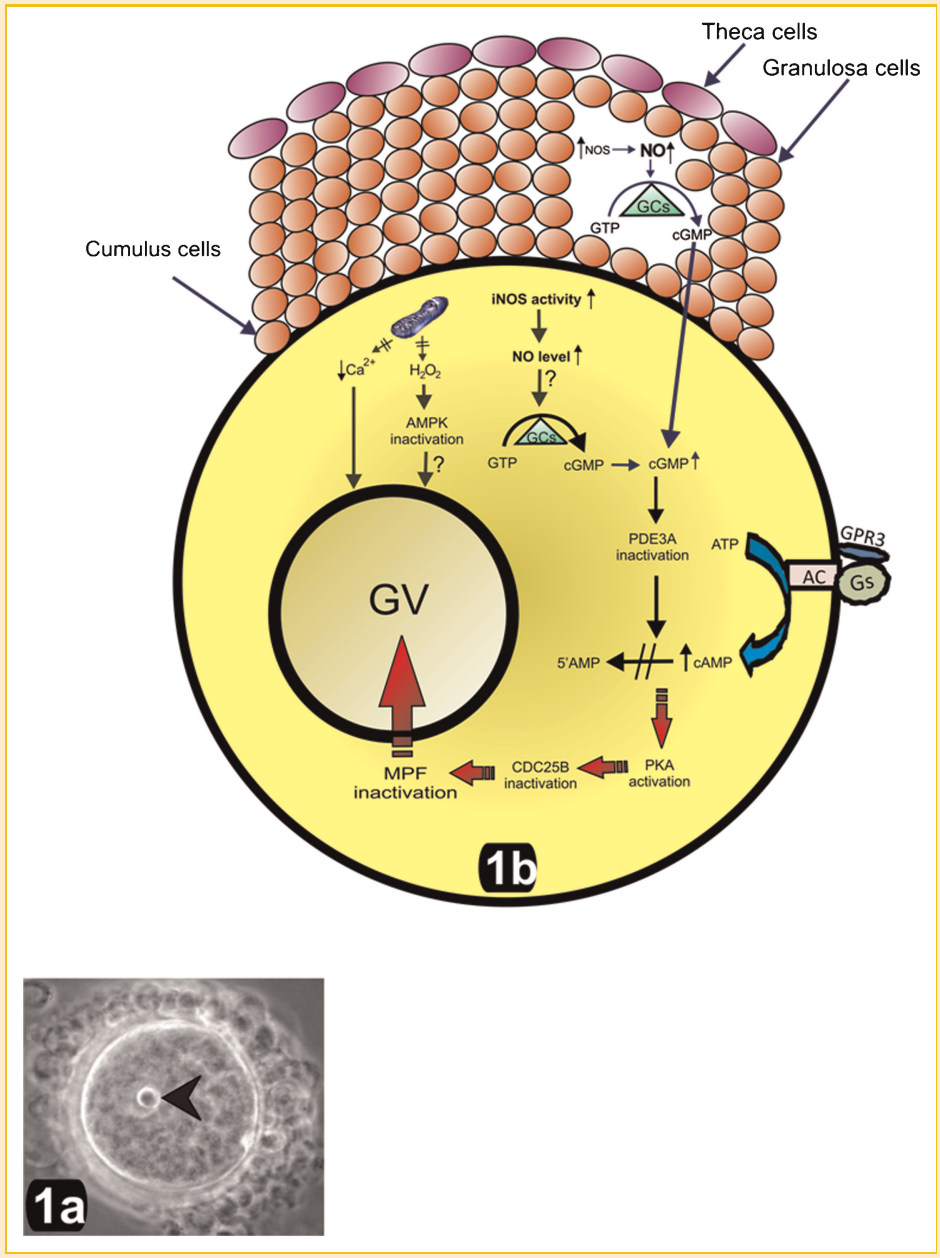

Fig. 1. a: A diplotene arrested rat cumulus-enclosed oocyte showing germinal vesicle and nucleolus (▶); original magnification, 400×. b: A proposed model for maintenance of meiotic arrest at diplotene stage in follicular oocyte. The NO produced through nitric oxide synthases in cumulus-granulosa cells stimulate generation of cGMP through GCs pathway. The cGMP from encircling somatic cells is transferred though gap junctions to the oocyte. An increased level of intraoocyte cGMP level may inactivate PDE3A in the oocyte. The NO is also produced by oocyte itself through iNOS-mediated pathway and possibly inhibits PDE3A through cGMP pathway. The inhibition of oocyte PDE3A prevents cAMP hydrolysis and increase intraoocyte cAMP level. The increased cAMP level may activate PKA which in turn inactivate CDC25B phosphatase and thereby MPF. The inactive MPF does not induce meiotic resumption and diplotene arrest is maintained. The reduced production of H_2O_2 and Ca^{2+} release from mitochondria may also maintain meiotic arrest at diplotene arrest. Symbol used: ▼, reduced; ▲ increased; ?, unestablished. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

an important role during exit from diplotene arrest in mammalian preovulatory oocytes.

The downstream pathway by which reduced NO level induces meiotic resumption has not been clearly elucidated. However, few studies indicate that iNOS-derived NO increases cGMP level and thereby maintain meiotic arrest at diplotene stage, while decrease in NO level and thereby reduced cGMP level may induce meiotic resumption [Tornell et al., 1990; Nakamura et al., 2002; Huo et al., 2005; Bilodeau-Goeseels, 2007; Wang et al., 2008; Zhang et al., 2009]. The reduced cGMP level activates cyclic 3′,5′ adenosine monophosphate (cAMP)-phosphodiesterase 3A (PDE3A) and reduces intraoocyte cAMP level [Tornell et al., 1990]. The reduction of intraoocyte cAMP inactivates protein kinase A (PKA) since high cAMP level and PKA activation play a critical role in the

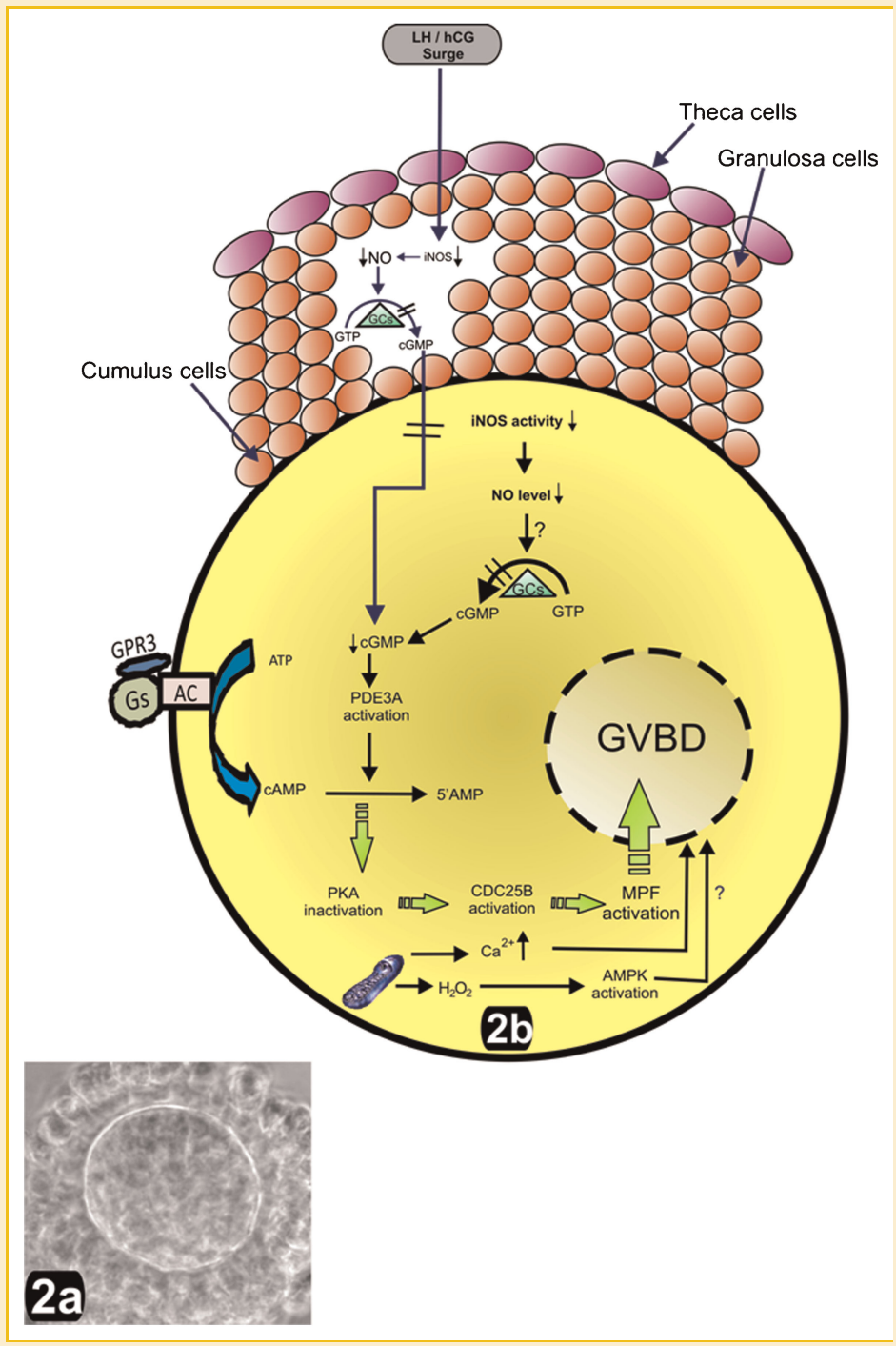

Fig. 2. a: Resumption of meiosis from diplotene arrested cumulus-enclosed oocyte as shown by germinal vesicle break down (GVBD) and disappearance of nucleolus; original magnification, 400×. b: A proposed model of LH/hCG-induced meiotic resumption from diplotene arrest in in preovulatory oocyte. LH/hCG reduces iNOS activity and induces disruption of gap junctions between cumulus-granulosa cells and oocyte. The interruption of communication between cumulus-granulosa cells and oocyte may block the transfer of cGMP produced through NO–GCs pathway. The reduced iNOS activity and thereby decreased intraoocyte NO level further decreases oocyte cGMP level. The net reduction in cGMP level may activate PDE3A that reduces cAMP level generated by oocyte itself through GPR3/AC pathway. The decrease in the cAMP level results in the inactivation of PKA activity, which in turn stimulates CDC25B phosphatase in the oocyte. The activated CDC25B phosphatase induces MPF activity that finally induces resumption of meiosis. Generation of tonic level of ROS and Ca^{2+} release from mitochondria may also be associated with the induction of meiotic resumption from diplotene arrest. Symbols used: ▼, reduced; ▲ increased; ?, unestablished. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

maintenance of meiotic arrest [Han and Conti, 2006]. The inactive PKA in the oocyte activates CDC25B phosphatases [Tornell et al., 1990; Eppig et al., 2004; Liang et al., 2007; Solc et al., 2008]. Active CDC25B dephosphorylates cyclin-dependent kinase1 (CDK1), a catalytic subunit of maturation promoting factor; MPF) that leads to MPF activation and meiosis is resumed from diplotene arrest [Duckworth et al., 2002; Han and Conti, 2006]. Hence, the reduced intraoocyte NO level possibly through cGMP/PDE3A/cAMP/PKA/CDC25B/MPF pathway may induce meiotic resumption from diplotene arrest in mammalian oocytes.

SUMMARY

Both oocyte and somatic cells encircling oocyte inside follicular microenvironment generate ROS and RNS to regulate meiotic cell cycle in preovulatory oocytes. The molecular mechanism(s) by which ROS and RNS regulate meiotic resumption in preovulatory oocytes have not yet been well defined. Based on the available data, we propose that a window of stimulation apparently exist whereby generation of tonic level of ROS either by AMPK pathway or by Ca^{2+}-mediated pathway induces meiotic resumption, while higher level results in meiotic cell cycle arrest at diplotene stage. Similarly, higher level of RNS maintains meiotic arrest, while decrease in its level stimulate cGMP/PDE3A/cAMP/PKA/CDC25B/MPF pathway to induce meiotic resumption from diplotene-arrest. Hence, possibility exist that a transient increase of intraoocyte H_2O_2 level and decrease of NO level are associated with meiotic resumption from diplotene arrest in preovulatory oocytes. However, further studies are required to elucidate the physiological roles of ROS and RNS in the regulation of meiotic cell cycle at diplotene stage in mammalian oocytes.

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