Regulation of intracellular pH during oocyte growth and maturation in mammals

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Abstract

Regulation of intracellular pH (pH_i) is a fundamental homeostatic process essential for the survival and proliferation of virtually all cell types. The mammalian preimplantation embryo, for example, possesses Na^+/H^+ and $\text{HCO}_3^-/\text{Cl}^-$ exchangers that robustly regulate against acidosis and alkalosis respectively. Inhibition of these transporters prevents pH corrections and, perhaps unsurprisingly, leads to impaired embryogenesis. However, recent studies have revealed that the role and regulation of pH_i is somewhat more complex in the case of the developing and maturing oocyte. Small meiotically incompetent growing oocytes are apparently incapable of regulating their own pH_i , and instead rely upon the surrounding granulosa cells to correct ooplasmic pH, until such a time that the oocyte has developed the capacity to regulate its own pH_i . Later, during meiotic maturation, pH_i -regulating activities that were developed during growth are inactivated, apparently under the control of MAPK signalling, until the oocyte is successfully fertilized. Here, we will discuss pH homeostasis in early mammalian development, focussing on recent developments highlighting the unusual and unexpected scenario of pH regulation during oocyte growth and maturation.

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Introduction: growth and maturation of the mammalian oocyte

Mammalian oocytes grow within ovarian follicles, each comprising a single oocyte enclosed within a surrounding shell of granulosa cells. For every reproductive cycle, a group of primordial follicles, consisting of a small non-growing primordial oocyte and a single layer of granulosa cells, is selected to begin growing. The oocyte increases in size from $\sim 20\ \mu\text{m}$ in diameter to a species-dependent final size of 70–120 μm , with fully grown oocytes of the most common experimental model, the mouse, reaching about 75 μm by the end of the growth phase. Oocyte growth is an indispensable component of oogenesis, since in the mouse oocytes only become capable of resuming meiosis when they reach about 80% of their final size (Sorensen & Wassarman 1976, Wassarman *et al.* 1979). Oocyte growth is also absolutely dependent upon the presence of, and communication with, the surrounding granulosa cells (Eppig 1985, 1991, Buccione *et al.* 1990, Su *et al.* 2009). Granulosa cells are coupled to the enclosed oocyte via gap junctions that, at least in mouse, are composed principally of connexin 37 (Cx37) subunits (Simon *et al.* 1997, Kidder & Mhawi 2002). During growth, the

granulosa cells proliferate rapidly, such that the follicle expands, and eventually forms a fluid-filled antrum from which the fully grown oocyte, surrounded by cumulus granulosa cells, is ovulated.

Throughout the growth phase, the oocyte is arrested in first meiotic prophase with a prominent prophase nucleus, termed the germinal vesicle (GV). The LH surge serves as the trigger for ovulation, and is also the signal for the oocyte to resume meiosis. The GV breaks down and the oocyte completes the first meiotic division (MI), ending in an unequal cell division forming a tiny nonviable first polar body (Pb1) and a large oocyte. The oocyte immediately enters the second meiotic division (MII), but arrests in metaphase until successfully fertilized (termed the MII arrest). It is around this time that the oocyte is expelled from the follicle and begins its journey along the oviduct (fallopian tube), and granulosa cells secrete hyaluronic acid matrix causing them to be physically uncoupled from the oocyte, including loss of gap junctional communication (Eppig 1982). Successful fertilization in the ampulla of the oviduct initiates a cascade of events culminating in the resumption of meiosis, extrusion of the Pb2, and initiation of embryonic division. The cleaving embryo differentiates into a blastocyst some 5 days after ovulation (in mouse),

around which time the successful embryo attaches to the uterus marking the end of preimplantation (PI) development. Thus, having remained arrested from birth until it is recruited to grow at some point after puberty, the oocyte is transformed from a tiny primordial oocyte within the ovary to a partially differentiated embryo in a matter of a few weeks (see Fig. 1).

Given the changes in the physiological demands and external environment, it seems logical that the oocyte and early embryo should be equipped to adapt to its changing circumstances. Here, we review what we know about one major aspect of oocyte/embryo homeostasis – regulation of intracellular pH (pH_i). We approach the question in 'reverse' order. First, we will briefly review the relatively intuitive scenario of PI embryo development. Then we focus on recent work that has revealed the mechanisms and control of pH regulation in the growing and maturing oocyte to be far less obvious.

Cellular homeostasis: the pH regulation toolkit

Virtually all cells possess the ability to regulate against unwelcome perturbations in pH_i . Most cellular processes are acutely pH sensitive, and so it is unsurprising that impairment of pH_i regulation can critically compromise cell function and viability. Classic experiments in cultured somatic cells showed that growth and proliferation are impaired if pH_i regulation is compromised and pH_i is disturbed (Grinstein *et al.* 1989, Kapus *et al.* 1994), and pH_i dysregulation has been shown to jeopardize cell survival (Pouyssegur *et al.* 1984). The mammalian cell has therefore been equipped to maintain a normal pH_i in response to changing environments and metabolic acid generation. To correct alkalosis, most mammalian cells possess $\text{HCO}_3^-/\text{Cl}^-$ exchangers of the anion exchange (AE) family (AE – three isoforms with various splice variants), which export HCO_3^- in exchange for Cl^- , thereby raising pH_i (Alper

1991, Romero *et al.* 2004). The principal means for correcting acidosis are Na^+/H^+ exchangers (NHEs) of the NHE gene family, which export protons in exchange for Na^+ , thereby raising pH_i (Orlowski & Grinstein 2004). Out of the nine known NHE isoforms, five (NHE1–5) are known to reside at least partly in the plasmalemma, and may therefore regulate pH_i (Orlowski & Grinstein 2004, Romero *et al.* 2004). Dependent upon the cell type, these two main players may be backed by a supporting cast including Na^+ , $\text{HCO}_3^-/\text{Cl}^-$ exchangers (NBC) that relieve acidosis in some cell types by importing HCO_3^- (Romero *et al.* 2004), and V-type H^+ -ATPases (V-ATPases) that export protons across the plasmalemma in some cells (Merzendorfer *et al.* 1997, Nelson & Harvey 1999, Kawasaki-Nishi *et al.* 2003). The study of cellular pH_i regulation has been facilitated considerably by commercially available pH-sensitive fluorophores, such as BCECF and SNARF, which allow pH_i measurement during experimental manipulations in living cells. For example, real-time recording of the recovery of a cell from an induced acidosis or alkalosis can be performed, and identification of the transport systems responsible for the recovery can be achieved by inhibition of each system.

Role of pH in early embryo development

It is well established that pH_i regulation is of importance in early mammalian development. The PI mouse embryo has very active $\text{HCO}_3^-/\text{Cl}^-$ exchange for mitigating alkalosis (Baltz *et al.* 1991, Zhao *et al.* 1995, Zhao & Baltz 1996, Phillips & Baltz 1999), which is also seen in human (Dale *et al.* 1998, Phillips *et al.* 2000), hamster (Lane *et al.* 1999a), and, to a lesser extent, bovine embryos (Lane & Bavister 1999). The effectiveness of these exchangers is substantial; one-cell mouse embryos can efficiently progress to blastocyst in conditions of $\text{pH} \sim 8.2$ (normal embryo culture media $\text{pH} \sim 7.3$), provided $\text{HCO}_3^-/\text{Cl}^-$ exchange is functional (Zhao *et al.* 1995).

Figure 1 Regulation of intracellular pH changes in mammalian oogenesis and embryogenesis. Cartoon outlining the major milestones of oocyte development and early embryogenesis in mammals, indicating the relative level of pH regulation capacity displayed by the oocyte. Note that times indicated are for mouse, the most closely studied mammal in terms of pH_i regulation. Granulosa cells are indicated in green, and the germinal vesicle as a dotted line. Further details are in text and in Table 1.

PI mouse embryos also exhibit NHE activity for correcting acidosis (Gibb *et al.* 1997, Lane *et al.* 1998, Steeves *et al.* 2001, Harding *et al.* 2002). Oddly, NHE activity in mouse embryos varies widely among mouse strains, and in at least some strains it is not robust (Steeves *et al.* 2001). As is the case for $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity, NHE activity is present in the PI embryos of human (Phillips *et al.* 2000), hamster (Lane *et al.* 1998), and bovine (Lane & Bavister 1999), and inhibition impairs PI development (Lane *et al.* 1998). In addition, early embryos possess monocarboxylate transporters that cotransport H^+ and can influence pH_i of the embryo (Harding *et al.* 1999), although this is not pH regulation *per se*, but a manifestation of passive transport of H^+ as lactic acid across the plasma membrane. In short, the mammalian PI embryo is well equipped with pH_i regulation machinery that is essential for embryo development.

It is worth also noting that pH_i -regulatory exchangers perform a second role in embryos distinct from pH_i regulation, which is to promote the formation of the blastocoel (embryo cavitation). Formation and expansion of the blastocoel are the result of an osmotic gradient driven by Na^+ accumulation within the cavity, which can be prevented by NHE inhibitors (Manejwala *et al.* 1989). Immunolocalization of NHE3 (SLC9A3) on the inner surface of the blastocoel (Barr *et al.* 1998) and inhibition of blastocoel formation using a NHE3-selective inhibitor (Kawagishi *et al.* 2004) are very consistent with NHE3 playing the dominant role in blastocoel formation in mouse. In contrast, AE2 (SLC4A2)-mediated $\text{HCO}_3^-/\text{Cl}^-$ exchange is necessary for Cl^- efflux from the blastocoel (Zhao *et al.* 1997). The molecular identities (i.e. which isoforms) of the exchangers that regulate against pH changes during PI development are yet to be reported.

pH regulation in the growing oocyte

In stark contrast to embryos, a recent series of studies has revealed that ovarian oocytes that are growing and have yet to achieve their fully grown size lack the ability to regulate pH_i . These studies took advantage of the fact that, in mice, a wave of follicular development occurs shortly after birth such that oocyte size and follicular development are closely related to age during post-natal days 5–20. Thus, the ability of oocytes of different sizes to regulate their pH_i could be examined. Small oocytes (< ~50 μm in diameter) that were isolated from the ovary and freed of granulosa cells (denuded) assumed a much lower 'resting' pH when placed in a standard culture medium than their fully grown (70–75 μm diameter) counterparts. Moreover, small oocytes apparently lacked both $\text{HCO}_3^-/\text{Cl}^-$ and Na^+/H^+ exchange activities, but these became increasingly active and capable of regulating pH_i with increasing oocyte size. This activation of the transporters occurred mainly over

the range 60–65 μm , mirroring the increase in resting pH within the oocyte, implying that the lower pH_i of smaller oocytes was a result of inadequate pH_i regulation (Erdogan *et al.* 2005).

Crucially, however, these initial experiments were unable to determine whether pH_i of growing oocytes behaves similarly within the physiological environment of the ovarian follicle. Key to addressing this question were experiments in which the pH_i of denuded and follicle-enclosed oocytes could be reliably and directly compared. This was achieved by microinjecting a pH-sensitive fluorescent dye into both types of oocytes, allowing the pH_i of the follicle-enclosed oocyte to be measured without any signal from the follicle cells (see Fig. 2A, for example). As in the previous study, small denuded oocytes adopted a much lower pH (pH ~6.8), than fully grown denuded oocytes (pH ~7.2). Strikingly, however, follicle-enclosed oocytes adopt essentially the same resting pH_i regardless of their size (pH ~7.2). Furthermore, fully grown denuded oocytes could efficiently recover from an experimentally induced acidosis or alkalosis, whereas in small oocytes normal pH_i was only re-established when the oocyte was still enclosed within the follicle. Thus, granulosa cells provide the small growing oocyte with the ability to regulate against pH_i changes, thereby allowing it to maintain a steady pH_i during growth. This ability of the granulosa cells to 'assist' the oocyte in regulating its pH_i is dependent upon gap junctions, since gap junction inhibition prevents small follicle-enclosed oocytes from dealing with induced pH changes, and causes the resting pH_i to drop (FitzHarris & Baltz 2006, FitzHarris *et al.* 2007).

Although these experiments reveal that granulosa cells are required to assist the oocyte in pH regulation, and that oocyte–granulosa gap junction communication is essential for this function, the mechanism of this assistance is somewhat more difficult to establish conclusively. One possibility is that oocyte–granulosa gap junctions allow the granulosa cells to pass to the oocyte a factor(s) that activates pH_i -regulatory mechanisms endogenous to the oocyte. Alternatively, gap junctions may allow the granulosa cells' direct access to the ooplasm, thereby allowing granulosa cells to regulate the pH_i of the oocyte on its behalf. Although both possibilities are plausible, the available evidence is more consistent with a model in which pH-regulatory transporters in the granulosa cells 'directly' regulate the pH of the oocyte through the gap junctions. Using techniques in which intact shells of granulosa shells are prepared from follicles, it was found that granulosa cells themselves possess robust and substantial pH-regulatory mechanisms (FitzHarris & Baltz 2006). These include $\text{HCO}_3^-/\text{Cl}^-$ exchange for correcting alkalosis, Na^+/H^+ exchange for acidosis recovery provided by expression of two different NHE isoforms (NHE1 (SLC9A1) and NHE3), and a Na^+ -independent means of acidosis recovery which was identified as a V-ATPase, or proton pump

Figure 2 Follicle-enclosed oocytes recover from an induced acidosis unless Na^+/H^+ exchanger isoforms 1 and 3 are simultaneously inhibited. (A) Example of follicle-enclosed oocytes isolated from the ovaries of 10-day-old mice and microinjected with the pH-sensitive fluorophore SNARF-dextran (10 kDa). Note that the 10 kDa dextran prevents the indicator from diffusing through gap junctions, which permit the passage of molecules $< \sim 1$ kDa, thereby allowing oocyte pH to be measured directly without any contaminating fluorescence from the granulosa cells. (B) Summary of known locations of active NHEs within murine follicles. (C) The ability of the oocyte to recover from acidosis is assessed using the NH_4Cl -pulse assay. The 10 min pulse of NH_4Cl causes a net acidosis. Note that following re-addition of Na^+ , pH_i recovers normally in the presence of 10 μM cariporide, a concentration that inhibits all Na^+/H^+ exchange attributable to NHE1 (SLC9A1). However, recovery from acidosis is completely inhibited by 10 μM S3226, which inhibits both isoforms 1 and 3. Recovery was not inhibited by 1 μM S3226, which would be expected to inhibit NHE3 (SLC9A3) but not NHE1 (not shown; see Schwark *et al.* 1998, Masereel *et al.* 2003 regarding selective NHE inhibitors). Thus, inhibition of NHE isoforms 1 and 3 is necessary to prevent recovery.

(FitzHarris *et al.* 2007). In each case, the transporter activities measured in the granulosa cells closely resembled those found in the follicle-enclosed oocyte. Most compellingly, the V-ATPase activity was never evident in denuded oocytes, regardless of size, but is easily detected in granulosa cell-enclosed oocytes. Thus, the array of pH_i regulation mechanisms available to the small follicle-enclosed oocyte matches that of the follicle cells.

To further address the question, we have sought to exploit the different array of transporters in the two cell types. Granulosa cells express both NHE1 and NHE3, and simultaneous inhibition of both isoforms is necessary to suppress the ability of granulosa cells to recover from acidosis. Fully grown denuded oocytes, on the other hand, regulate against acidosis using Na^+/H^+ exchange provided exclusively by NHE1 (see Table 1; FitzHarris *et al.* 2007). We reasoned that if regulation against oocyte acidosis in small follicle-enclosed oocytes is performed by Na^+/H^+ exchange within the granulosa cells, then inhibition of both NHE1 and NHE3 should be necessary to prevent acidosis recovery in follicle-enclosed oocytes. Conversely, if granulosa cells activate Na^+/H^+ exchange native to the oocyte, then inhibition of NHE1 alone should be sufficient to prevent acid correction in small follicle-enclosed oocytes. The results of these experiments are shown in Fig. 2C. Oocytes within follicles exposed to high concentrations of cariporide,

a NHE1-specific inhibitor, recover from an induced acidosis as normal, whereas simultaneous inhibition of NHE1 and NHE3 using relatively high concentrations of S3226 prevents oocyte recovery in the follicle, implying that pH_i regulation in the granulosa cells has to be inhibited to prevent pH_i regulation in the follicle-enclosed oocyte. Although the possibility that granulosa cells provide a signal to the growing oocyte that activates Na^+/H^+ exchange other than NHE1 cannot be formally ruled out, this seems unlikely given that the fully grown oocyte utilizes only NHE1. Thus, we strongly favour a model in which the small growing oocyte, which is incapable of regulating its own pH, has instead 'outsourced' this function to the granulosa cells.

The growing oocyte thus provides the first clear example of a mammalian cell that is incapable of regulating its pH_i by conventional means until the oocyte reaches such a size that its own pH_i-regulatory transport is activated. The mechanism by which this transport activity is eventually activated in the oocyte remains to be established. Since a wave of new protein synthesis accompanies oocyte growth (Schultz & Wassarman 1977), it is easy to imagine that the activation of transport represents the first time when the transporters reside in the plasma membrane at sufficient abundance to affect cytosolic pH. Accordingly, the granulosa cells have highly developed pH regulation mechanisms,

Table 1 Summary of pH regulation during oogenesis and embryogenesis in mammals.

	Granulosa cells	Growing and fully grown GV oocyte	Unfertilized egg	Early preimplantation embryo	Late preimplantation embryo
Acidosis correction	Na ⁺ /H ⁺ exchange by NHE1, 3 V-type H ⁺ -ATPase activity (FitzHarris <i>et al.</i> 2007)	Na ⁺ /H ⁺ gradually activated during growth: NHE1 responsible (FitzHarris <i>et al.</i> 2007)	Little or no transport activities detected (G FitzHarris & JM Baltz, unpublished data; Lane & Bavister 1999, Lane <i>et al.</i> 1999a, Phillips & Baltz 1999, Phillips <i>et al.</i> 2002)	Na ⁺ /H ⁺ exchange active – possibly NHE1 and 3 (Lane <i>et al.</i> 1998, Phillips <i>et al.</i> 2000, Harding <i>et al.</i> 2002)	NHE3 participates in blastocoel expansion (Kawagishi <i>et al.</i> 2004)
	Possible NBC activity (FitzHarris <i>et al.</i> 2007)	<i>Transcripts for NHE1 and 3 detected (FitzHarris <i>et al.</i> 2007)</i>	<i>Transcripts for NHE1, 3 and 4 detected (G FitzHarris & JM Baltz, unpublished data; Harding <i>et al.</i> 2002)</i>	MCT1 active two-cell stage onwards (Gibb <i>et al.</i> 1997, Harding <i>et al.</i> 1999)	Na ⁺ /H ⁺ exchange active: possibly by NHE1, 3, 4 (Lane <i>et al.</i> 1998, Harding <i>et al.</i> 2002)
	<i>Transcripts for NHE1 + 3 detected (FitzHarris <i>et al.</i> 2007)</i>			<i>NHE1 and 3 transcripts detected (Barr <i>et al.</i> 1998)</i>	<i>NHE1 and NHE3 protein at apical and basolateral membrane respectively</i>
				<i>MCT1, 2, 3 transcripts detected two-cell onwards (Harding <i>et al.</i> 1999)</i>	<i>NHE1 transcripts, but not NHE3 (Barr <i>et al.</i> 1998)</i>
Alkalosis correction	HCO ₃ ⁻ /Cl ⁻ exchange active, isoform unknown (FitzHarris & Baltz 2006)	HCO ₃ ⁻ /Cl ⁻ exchange gradually activated during growth, isoform unknown (Erdogan <i>et al.</i> 2005, FitzHarris & Baltz 2006)	No transport activities detected (Phillips & Baltz 1999; Lane <i>et al.</i> 1999b)	HCO ₃ ⁻ /Cl ⁻ exchange active, isoform unknown (Baltz <i>et al.</i> 1991, Zhao <i>et al.</i> 1995, Phillips <i>et al.</i> 2000)	pH-regulatory HCO ₃ ⁻ /Cl ⁻ exchange active, isoforms unknown (Zhao <i>et al.</i> 1995)
			<i>AE2 transcripts detected (Phillips & Baltz 1999)</i>	<i>AE2 protein in one-cell embryos (JM Baltz & SL Alper, unpublished data) and four- to eight- cell embryos (Zhao <i>et al.</i> 1995)</i>	<i>AE2 immunolocalizes to basolateral membrane and involved in Cl⁻ efflux during blastocoel formation (Zhao <i>et al.</i> 1997)</i>
				Transcripts and protein for AE2 one cell onwards, and AE3 two cell onwards (Zhao <i>et al.</i> 1995)	Transcripts and protein for AE2 and AE3 (Zhao <i>et al.</i> 1995)

Italics indicate expression data, whereas standard type refers to functional experiments. Note that the majority of work has been carried out on mouse and hamster, but the table also includes data from cow and human. Omission of an exchanger type does not indicate its absence at that developmental stage, rather the absence of available data. NHE1 also known as SLC9A1; NHE3 also known as SLC9A3; AE2 also known as SLC4A2; AE3 also known as SLC4A3.

which, via gap junctions, regulate the ooplasm against pH change until the oocyte is capable of performing this function alone. Although it has previously been demonstrated that protons or proton equivalents can diffuse through gap junctions in cardiomyocytes (Swietach *et al.* 2003, Zaniboni *et al.* 2003), the mammalian follicle provides the first demonstration of gap junctions allowing one cell to be entirely responsible for regulating the pH_i of its neighbour in a physiologically relevant setting. A clean and conclusive experiment demonstrating the developmental importance of pH_i cooperativity in oocyte growth is yet to be presented and, indeed, difficult to conceive. Nonetheless, given the well-established effect of pH_i perturbations on cellular function, it is hard to imagine that oocyte growth and development would be unaffected at ~ 0.5 pH units less than normal. It is noteworthy that since all other cell types including early embryos (Lane *et al.* 1999a, 1999b, Steeves *et al.* 2001, Harding *et al.* 2002) possess these mechanisms, a developmental switch must occur that inactivates pH regulation specifically within the female germ cell, the timing and mechanism of which are as yet unknown.

That granulosa cells provide physiological support for the growing oocyte is not without precedent. It has long been known that metabolic cooperativity between oocyte and granulosa cells underpins oocyte energy production. Although oocytes utilize glucose poorly, granulosa cells readily metabolize glucose and supply the oocyte with substrates that it can use (Biggers *et al.* 1967). The granulosa cells also facilitate uptake of some amino acids and nucleotides, which are ineffectively taken up by denuded oocytes, an effect dependent upon gap junctions (Cross & Brinster 1974, Heller & Schultz 1980, Colonna & Mangia 1983, Haghighat & Van Winkle 1990, Su *et al.* 2009). Recent work has shown that in the case of some amino acids, granulosa cells can respond to the increased demands of the oocyte by upregulating transporters in order to provide the oocyte with more substrate (Eppig *et al.* 2005). pH_i regulation distinguishes itself from these mechanisms insofar as granulosa cells provide rapid homeostatic support to the oocyte in the form of an acute (seconds to minutes) response to pH_i change using pre-existing transporters. It remains to be seen whether granulosa cells also take up the slack on behalf of the oocyte in terms of other aspects of ionic homeostasis, although a similar phenomenon has been noted for $\text{Na}^+/\text{Ca}^{2+}$ exchange in immature *Xenopus laevis* oocytes (Supplisson *et al.* 1991).

pH regulation mechanisms during oocyte maturation and fertilization

As outlined above, by the time it is fully grown, the GV stage mouse oocyte has developed robust mechanisms for regulating its own pH_i (Erdogan *et al.* 2005, FitzHarris

& Baltz 2006, FitzHarris *et al.* 2007). Similarly, the PI embryo from the pronuclear one-cell stage onwards exhibits robust ability to regulate its own pH_i (see above). Thus, given that developmental stages occurring both before and after are each capable of regulating pH_i , it was surprising that the ovulated MII oocyte exhibits little or no ability to regulate its pH_i .

MI mouse oocytes lack both $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity and the ability to recover from experimentally induced alkalosis (Phillips & Baltz 1999). A similar lack of $\text{HCO}_3^-/\text{Cl}^-$ exchange activity has been shown in MII hamster oocytes (Lane *et al.* 1999a). The lack of $\text{HCO}_3^-/\text{Cl}^-$ exchange activity in mature unfertilized eggs may not be a property of all mammals, however, as human MII eggs are able to recover from alkalosis, and recovery is inhibited by DIDS (Dale *et al.* 1998, Phillips *et al.* 2000).

Similar to $\text{HCO}_3^-/\text{Cl}^-$ exchange, there is no detectable NHE activity in the MII oocytes of hamster (Lane *et al.* 1999b) or mouse (G FitzHarris, C Zhou & JM Baltz, unpublished data). Unlike for $\text{HCO}_3^-/\text{Cl}^-$ exchange, human MII oocytes also lack NHE activity as well, and only exhibit this activity in embryos after fertilization, similar to mouse and hamster (Phillips *et al.* 2000). Therefore, somewhat unexpectedly, the robust pH_i -regulatory capacity, which is developed during oocyte growth and evident in the fully-grown GV oocyte, is downregulated during meiotic maturation, and then reactivated following fertilization.

The most in-depth investigations of this phenomenon have been carried out on the $\text{HCO}_3^-/\text{Cl}^-$ exchanger in the mouse. $\text{HCO}_3^-/\text{Cl}^-$ exchange activity and the ability to regulate pH_i against alkalosis begin to decrease after release from first meiotic prophase arrest, and decrease slowly during MI until exchanger activity becomes almost undetectable by the MI/II transition (Phillips *et al.* 2002) and is undetectable in the arrested MII egg. However, after egg activation either by fertilization or experimentally induced parthenogenetic activation, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity begins to reappear about 2–3 h post-egg activation and reaches a maximal level several hours later, when the zygotic pronuclei appear (Phillips & Baltz 1999, Phillips *et al.* 2002, Cetinkaya & Erdogan 2008). A similar time course of activation of NHE activity after egg activation has been seen in the hamster egg (Lane *et al.* 1999b). Thus, pH_i regulation is 'turned off' during meiotic maturation and is quiescent in the ovulated MII egg. It is then reactivated following fertilization, remaining present throughout subsequent PI embryogenesis.

Since pH_i regulation varies so dramatically during meiotic maturation, subsequent studies have focussed on the question of whether the same mechanisms that control meiotic progression and release from meiotic arrests may also participate in silencing the pH_i regulation machinery. GV arrest is mediated by high cAMP maintained by an activated G-protein-coupled receptor (Mehlmann *et al.* 2004). Thus, GV oocytes have

high cAMP levels, which then decrease to allow meiotic resumption. However, raising cAMP levels in oocytes after meiotic resumption did not reactivate $\text{HCO}_3^-/\text{Cl}^-$ exchange in MII eggs, indicating that high cAMP was not sufficient to maintain pH_i -regulatory activity (Phillips *et al.* 2002). Similarly, the intracellular Ca^{2+} oscillations that follow fertilization to trigger the release of the MII arrest are not required for reactivation of $\text{HCO}_3^-/\text{Cl}^-$ exchange (Phillips *et al.* 2002). Reactivation is also independent of protein synthesis (Phillips & Baltz 1999).

MI arrest is maintained by cytosolic factor (CSF), a cytoplasmic signal that is due, at least in part, to the MOS/MEK/MAPK pathway, (Colledge *et al.* 1994, Hashimoto *et al.* 1994). The temporal variation of MAPK activity during meiotic maturation inversely parallels the activity of pH_i -regulatory mechanisms described above. Manipulations that induce ectopically high MAPK activity in GV oocytes or maintain high levels in activated eggs (Moos *et al.* 1995) reveal that high MAPK activity is not compatible with the activity of $\text{HCO}_3^-/\text{Cl}^-$ exchange in mouse oocytes (Phillips *et al.* 2002). Conversely, inhibiting MAPK activity using the specific MEK inhibitor U0126 in MI oocytes activated the normally quiescent $\text{HCO}_3^-/\text{Cl}^-$ exchanger (Phillips *et al.* 2002). Unpublished experiments in which a constitutively activated form of MEK (Moos *et al.* 1996) was expressed in parthenogenetically activated eggs to more specifically maintain high MAPK activity after egg activation has shown that maintaining high MAPK activity prevented reappearance of $\text{HCO}_3^-/\text{Cl}^-$ exchange, confirming a role for MEK and MAPK (C Zhou & JM Baltz, unpublished data). Thus, the MAPK cascade that is necessary for CSF activity appears to have a novel role in controlling pH_i -regulatory mechanisms during meiosis.

Several questions remain to be answered. For example, what is the mechanism by which MAPK signalling inactivates the transporters during oocyte maturation? Although, in other cells, NHE1 is known to be regulated by MAPK via its effector P90rsk, MAPK activity normally activates Na^+/H^+ exchange, rather than inactivating it (Orlowski & Grinstein 2004), and little is known about the effect of MAPK signalling on $\text{HCO}_3^-/\text{Cl}^-$ exchangers. Alternatively, perhaps MAPK triggers a process of transporter internalization and/or degradation during oocyte maturation, which is reversed following fertilization. Thus, the mechanism by which CSF inactivates transport in eggs requires further study. In addition, the physiological benefit of inactivating pH regulation in the unfertilized egg is obscure. One plausible possibility relates to the repetitive pulsatile increases in $[\text{Ca}^{2+}]_i$, which occur after fertilization to trigger development. The re-activation of pH_i regulation (when MAPK activity decreases) corresponds well with the cessation of Ca^{2+} oscillations (Jones *et al.* 1995, Marangos *et al.* 2003). Some pH-regulatory transporters are sensitive to Ca^{2+} or its downstream

pathways, and thus inactivation of pH_i -regulatory transporters may prevent Ca^{2+} -triggered pH_i fluctuations following fertilization. Another possibility relates to cell volume regulation. In many cells, functionally coupled Na^+/H^+ and $\text{HCO}_3^-/\text{Cl}^-$ exchangers act to protect cells against unwanted shrinkage by importing NaCl to increase intracellular osmotic pressure (Jiang *et al.* 1997, Hoffman *et al.* 2009). Early embryos possess a novel mechanism for regulating their cell volume that relies on accumulation of glycine rather than inorganic ions (Steeves *et al.* 2003). This mechanism becomes activated during meiotic maturation (Tartia *et al.* 2009), i.e. during the period when Na^+/H^+ and $\text{HCO}_3^-/\text{Cl}^-$ exchanger is inactivated. Thus, it is possible that the inactivation of pH_i -regulatory mechanisms during meiosis occurs to allow the switch to glycine-based control in embryos. These possibilities remain to be investigated.

Conclusion

In summary (Fig. 1 and Table 1), the small growing oocyte lacks the ability to regulate pH_i , and therefore has to rely upon the surrounding granulosa cells. Paradoxically, the fully grown oocyte apparently has the machinery necessary to regulate its own pH, but specifically inactivates this at the same time as it uncouples itself from the granulosa cells that were previously providing support (gap junction inactivation and cumulus expansion). Only after fertilization are these transporters re-activated. A summary of the transport mechanisms that have been identified so far as active in mammalian oocytes and embryos is presented in Table 1. The challenge now is to understand the precise mechanisms that control the inactivation and reactivation of pH_i -regulatory mechanisms, and to ascertain how the changing pH of the external environment (follicle, oviduct, and uterus) and the changes in the manner in which the oocyte/embryo handles acid and alkali together impact pH_i to affect the metabolism of the oocyte and embryo throughout PI development. Improved *in vitro* oocyte growth and maturation remain an important hurdle in the clinic, and understanding the interaction between the oocyte and its environment during these developmental milestones will likely be key in the development of improved culture systems.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research/work reported.

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