The Intracellular pH-regulatory $\text{HCO}_3^-/\text{Cl}^-$ Exchanger in the Mouse Oocyte Is Inactivated during First Meiotic Metaphase and Reactivated after Egg Activation via the MAP Kinase Pathway

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The $\text{HCO}_3^-/\text{Cl}^-$ exchanger is quiescent in the unfertilized mouse egg but is highly active in regulating intracellular pH in the early embryo and required for normal development. We show here that the $\text{HCO}_3^-/\text{Cl}^-$ exchanger is active in first meiotic prophase (GV) oocyte but inactivated during meiotic metaphase before the MI to MII transition. Reactivation does not occur until the activated egg enters interphase. A quiescent $\text{HCO}_3^-/\text{Cl}^-$ exchanger is not simply a general feature of metaphase, because activity did not decrease during first mitotic metaphase. Inactivation of the $\text{HCO}_3^-/\text{Cl}^-$ exchanger during MI coincided with the activation of MAP kinase (MAPK), whereas its reactivation coincided with the loss of MAPK activity after egg activation. Maintaining high MAPK activity after egg activation prevented the normal reactivation of the $\text{HCO}_3^-/\text{Cl}^-$ exchanger. Inactivating MAPK in unfertilized MII eggs resulted in $\text{HCO}_3^-/\text{Cl}^-$ exchanger activation. Preventing MAPK activation during first meiotic metaphase prevented the inactivation of $\text{HCO}_3^-/\text{Cl}^-$ exchange. Conversely, activating MAPK in the GV oocyte resulted in inactivation of $\text{HCO}_3^-/\text{Cl}^-$ exchange. These results imply that the $\text{HCO}_3^-/\text{Cl}^-$ exchanger in mouse oocytes is negatively regulated by MAPK. Thus, suppression of pH-regulatory mechanisms during meiosis is a novel function of MAPK and cytostatic factor activity in the oocyte.

INTRODUCTION

Intracellular pH (pH_i) in mammalian cells is maintained within a narrow range by transport mechanisms, including the Na^+/H^+ antiporter, which exports H^+ and thus corrects low pH_i , and the $\text{HCO}_3^-/\text{Cl}^-$ exchanger, which exports HCO_3^- and thus corrects increases in pH_i (Roos and Boron,

1981; Alper, 1994; Orlowski and Grinstein, 1997). The $\text{HCO}_3^-/\text{Cl}^-$ exchanger is highly active in preimplantation mammalian embryos from the pronuclear egg through the blastocyst stages (Baltz *et al.*, 1991; Zhao and Baltz, 1996; Lane *et al.*, 1999a; Phillips *et al.*, 2000; Phillips and Baltz, 1999), and exchanger activity is required for mouse embryos to maintain pH_i and for normal embryo development (Zhao *et al.*, 1995).

In a number of marine invertebrates and amphibians, activation of pH_i -regulatory transporters is a fundamental event of egg activation (Johnson *et al.*, 1976; Webb and Nuccitelli, 1981; Dube *et al.*, 1985; Dube, 1988; Epel, 1988; Freeman and Ridgway, 1993; Dube and Eckberg, 1997). In the sea urchin, the Na^+/H^+ antiporter is quiescent in the egg but becomes highly active within minutes of fertilization (Johnson *et al.*, 1976; Epel, 1988), causing a permanent increase in pH_i that is required for subsequent embryo development. In mammals, however, no increase in pH_i follows fertilization (Kline and Zagray, 1995; Ben Yosef *et al.*, 1996;

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Abbreviations used: Ca^{2+}_i , intracellular calcium; CSF, cytostatic factor; GV, germinal vesicle (prominent nucleus of prophase I oocytes); GVBD, germinal vesicle breakdown (release from prophase I arrest); IVF, in vitro fertilization; MAPK, mitogen-activated protein kinase (ERK1,2); MBP, myelin basic protein; MI, first meiotic metaphase; MII, second meiotic metaphase; MPF, maturation- (or mitosis-) promoting factor; OA, okadaic acid; pH_i , intracellular pH

Phillips and Baltz, 1996; Dale *et al.*, 1998; Phillips *et al.*, 2000), and it had been assumed that activation of pH_i-regulatory mechanisms is not a feature of mammalian fertilization.

We recently showed, however, that the HCO₃⁻/Cl⁻ exchanger is quiescent in ovulated mouse eggs and only becomes activated several hours after fertilization (Phillips and Baltz, 1999). Similarly, both the HCO₃⁻/Cl⁻ exchanger and Na⁺/H⁺ antiporter are quiescent in hamster eggs and become activated after fertilization (Lane *et al.*, 1999a, 1999b). Fertilization-induced activation of the HCO₃⁻/Cl⁻ exchanger in mouse or Na⁺/H⁺ antiporter in hamster did not require protein synthesis or rely on protein trafficking, indicating that preexisting exchanger proteins in the membrane likely become activated by fertilization (Lane *et al.*, 1999b; Phillips and Baltz, 1999). However, the mechanism of activation after fertilization was unknown.

Activation of Na⁺/H⁺ antiporter activity at fertilization in the sea urchin is controlled through intracellular calcium (Ca²⁺_i)-dependent signaling via protein kinase C (Swann and Whitaker, 1985; Epel, 1988). In mouse and hamster, chelation of Ca²⁺_i similarly inhibited the activation of pH_i-regulatory mechanisms after fertilization (Lane *et al.*, 1999b; Phillips and Baltz, 1999). We thus proposed that the increase in Ca²⁺_i and repetitive Ca²⁺_i transients that follows fertilization in mammals (Jones *et al.*, 1995) might be involved in activating pH_i-regulatory systems in mammalian eggs (Phillips and Baltz, 1999). We show here, however, that activation of the HCO₃⁻/Cl⁻ exchanger in mouse eggs after fertilization is independent of Ca²⁺_i transients. Instead, its activity is closely correlated with the meiotic cell cycle.

In most vertebrates, the fully grown oocyte is arrested in meiotic prophase, exhibiting a prominent nucleus or germinal vesicle (GV). On ovulation, meiosis resumes and the oocyte exits prophase I, as marked by the disappearance of the GV or GV breakdown (GVBD). Prophase I arrest appears to be maintained in mammalian eggs at least in part by an unknown inhibitory signal from the surrounding follicle cells, because most mammalian GV eggs spontaneously resume meiosis upon removal from the follicle. This is in contrast to many other animals, where resumption of meiosis requires a positive hormonal signal. In both cases, however, release from arrest appears to require a decrease in intracellular cAMP, whereas artificially maintaining elevated cAMP will prevent GVBD. After GVBD, the oocyte proceeds through first meiotic metaphase (MI), which ends with an unequal cytokinesis to emit the first polar body that removes one-half of the maternal chromosomes. The oocyte then reenters metaphase and becomes arrested in second meiotic metaphase (MII). On fertilization or egg activation, metaphase arrest is released, and the oocyte undergoes the second unequal cleavage to emit the second polar body and attain haploidy. The male and female genetic material form two separate pronuclei, which combine by the end of the first mitotic cell cycle to form the embryonic genome.

Two key players in regulating meiosis are maturation promoting factor (MPF), which induces metaphase, and cytotostatic factor (CSF), which maintains arrest of ovulated oocytes (in MII in mammals) until after fertilization. MPF is a complex of cyclin B and cdk1 kinase and is the common trigger of both meiotic and mitotic metaphases. In contrast, CSF, which stabilizes MPF and maintains metaphase arrest, is specific to meiosis. The MOS/MEK/MAPK pathway has

been shown to at least partly underlie CSF activity in oocytes (Colledge *et al.*, 1994; Hashimoto *et al.*, 1994; Gross *et al.*, 2000). Because, as we report here, HCO₃⁻/Cl⁻ exchanger activity is regulated by the meiotic cell cycle, we have investigated whether the signaling pathways known to control meiotic maturation in oocytes also regulate HCO₃⁻/Cl⁻ exchanger activity. Our results indicate, for the first time, that a pH_i-regulatory mechanism in a mammalian oocyte is controlled by the developmental status of the oocyte and is cell cycle dependent during meiosis.

MATERIALS AND METHODS

Chemicals and Solutions

Cycloheximide, demecolcine, hyaluronidase, pregnant mare's serum gonadotropin (PMSG), human chorionic gonadotropin (hCG), EGTA, dibutyryl cyclic AMP (dbcAMP), okadaic acid (OA), and nigericin were obtained from Sigma (St. Louis, MO). 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), valinomycin, 4-bromo-A23187, and the acetoxymethyl esters of SNARF-1 and Fura-2 were obtained from Molecular Probes (Eugene, OR). U0126 was obtained from Calbiochem (La Jolla, CA). All stock solutions were prepared in DMSO, except for cycloheximide and dbcAMP (water), and demecolcine and nigericin (ethanol), and stored at -20°C.

All media were based on KSOM embryo culture medium (Lawitts and Biggers, 1993; Phillips and Baltz, 1999), equilibrated with 5% CO₂/air except where noted. For pH_i measurements, 9 of the 10 mM Na lactate was replaced with NaCl, and BSA was omitted. All solutions contained 110 mM Cl⁻, except for Cl⁻-free media, where all Cl⁻ salts were replaced with corresponding gluconate salts as described previously (Phillips and Baltz, 1999).

Oocyte and Egg Collection and Egg Activation

HEPES-buffered medium (HEPES-KSOM, pH 7.4; Lawitts and Biggers, 1993) was used for oocyte and egg collection. Female CF1 mice (6–8 week old; Charles River, Montreal, Quebec, Canada) were superovulated with 5 IU PMSG given intraperitoneally (IP). GV oocytes were collected from minced ovaries 45–48 h post-PMSG. dbcAMP (300 μM) was present during collection and culture to maintain GV arrest (Cho *et al.*, 1974) unless otherwise specified. For unfertilized eggs, ovulation was induced with 5 IU hCG IP 48 h post-PMSG, and eggs were collected 13.5–16 h post-hCG as previously described (Phillips and Baltz, 1999). Eggs and oocytes were cultured in KSOM droplets under mineral oil in 5%CO₂/air at 37°C (Lawitts and Biggers, 1993).

For activation with Sr²⁺, eggs were incubated for 2 h in KSOM with CaCl₂ omitted and 10 mM SrCl₂ added (Fraser, 1987; Bos-Mikich *et al.*, 1995). For activation with cycloheximide, eggs were incubated for 2 h with 50 μg/ml cycloheximide (Siracusa *et al.*, 1978; Moos *et al.*, 1996a). After activation, eggs were washed and transferred to culture in KSOM.

Intracellular pH and Ca²⁺ Measurements

pH_i and Ca²⁺_i measurements using a quantitative fluorescence imaging microscopy system (Inovision, Durham, NC) have been described previously (Baltz *et al.*, 1991; Zhao *et al.*, 1995; Baltz and Phillips, 1999). Briefly, pH_i was determined in oocytes loaded intracellularly with the pH-sensitive fluorophore, SNARF-1 (Zhao *et al.*, 1995; Baltz and Phillips, 1999). Calibration of ratio with pH_i was done by the nigericin/high K⁺ method with valinomycin added to collapse the K⁺ gradient (Thomas *et al.*, 1979; Baltz and Phillips, 1999). Ca²⁺_i was determined with Fura-2 (Baltz and Phillips, 1999; Phillips and Baltz, 1999). Ca²⁺_i was estimated from the intensity ratio as previously described (Gryniewicz *et al.*, 1985; Baltz and Phillips, 1999). Each replicate consisted of simultaneous measure-

ments made upon groups of 5–20 eggs or oocytes. pH_i was averaged for the group at each time point (Baltz and Phillips, 1999; Phillips and Baltz, 1999). All measurements were made with oocytes or eggs in a temperature- and atmosphere-controlled chamber (37°C , 5% CO_2/air).

Cl^- Removal Assay for $\text{HCO}_3^-/\text{Cl}^-$ Exchange Activity

$\text{HCO}_3^-/\text{Cl}^-$ exchanger activity was quantified by the Cl^- removal method (Zhao and Baltz, 1996; Baltz and Phillips, 1999; Phillips *et al.*, 2000; Phillips and Baltz, 1999). On exposure of cells to Cl^- -free solution, the $\text{HCO}_3^-/\text{Cl}^-$ exchanger will run in reverse, resulting in intracellular alkalization due to HCO_3^- influx coupled to Cl^- efflux (Nord *et al.*, 1988). Increased pH_i upon Cl^- removal thus indicates $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity, and the initial rate of alkalization provides a quantitative measure of activity (Nord *et al.*, 1988). Here, SNARF-1-loaded eggs or oocytes were placed in the chamber for 10 min, after which the solution was changed to Cl^- -free, low-lactate KSOM for 20 min. The initial rate of intracellular alkalization upon Cl^- removal was determined using linear regression (SigmaPlot 5; SPSS, Chicago, IL), and exchanger activity is reported as the change in pH_i per minute (pH U/min). This assay for $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity has been extensively described and validated in mouse oocytes and embryos (Zhao *et al.*, 1995; Zhao and Baltz, 1996; Baltz and Phillips, 1999; Phillips and Baltz, 1999; Phillips *et al.*, 2000). The change in pH_i observed after external Cl^- removal was confirmed to be diagnostic of $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity by its pharmacological profile and HCO_3^- requirement (Baltz *et al.*, 1991). In addition, the lack of $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in ovulated eggs indicated by this assay has been confirmed by demonstrating their inability to recover from ammonium-induced alkalosis (Phillips and Baltz, 1999), ruling out the alternative possibility that unfertilized eggs instead have an abnormally low internal Cl^- concentration that would prevent $\text{HCO}_3^-/\text{Cl}^-$ exchanger reversal upon external Cl^- removal.

Simultaneous Measurement of Histone H1 Kinase and MBP Kinase Activities

Histone H1 kinase (MPF) and MBP kinase (MAPK) activities were measured simultaneously in a single assay as previously described (Phillips *et al.*, 2002). For each measurement, seven eggs along with $\sim 1\text{--}1.5\ \mu\text{l}$ medium were added to $3.5\ \mu\text{l}$ lysis buffer and frozen at -80°C until the assay was performed. The lysis buffer used was identical to that described by Moos *et al.*, except that $10\ \mu\text{g/ml}$ leupeptin was added (Phillips *et al.*, 2002). The kinase assay reaction was initiated by thawing and addition of $5\ \mu\text{l}$ kinase buffer, which contained $500\ \mu\text{Ci/ml}$ $\gamma\text{-}^{32}\text{P}\text{-ATP}$ (Amersham). The kinase buffer used was identical to that described by Moos *et al.* for the Histone H1 kinase assay, except that it contained $0.5\ \text{mg/ml}$ MBP in addition to $2\ \text{mg/ml}$ histone type III-S (Phillips *et al.*, 2002). Phosphorylation of histone and MBP (transfer of $\gamma\text{-}^{32}\text{P}$) has been shown to increase linearly in this assay for at least 60 min (Phillips *et al.*, 2002), and the presence of phosphatase inhibitors prevented significant dephosphorylation during the assay period. After 30 min, the reaction was stopped by adding $10\ \mu\text{l}$ $2\times$ Laemmli sample buffer and boiling for 3 min. The extent of phosphorylation of MBP and histone was determined using SDS-PAGE (15% gels) quantified with a phosphorimager (Typhoon 8600 with ImageQuant software; Molecular Dynamics, Inc., Sunnyvale, CA). Background was determined from a reaction run without eggs. A sample of fresh unfertilized eggs was included in each gel, and H1 and MBP kinase activities were expressed relative to the values for eggs (set to 100%) within each gel. Histone H1 kinase and MBP phosphorylation were assumed to indicate MPF and MAPK activity, respectively, as previously demonstrated (Moos *et al.*, 1996a; Phillips *et al.*, 2002).

U0126, a specific inhibitor of the MAPK kinase, MEK, was used to decrease MAPK activity. We have extensively validated the use of

U0126 to inhibit MEK and hence MAPK in eggs, including demonstrating, by Western blot, a complete shift from the slower-migrating (active) forms of ERK1 and -2 to the faster-migrating (inactive) forms after U0126 treatment of eggs, and the complete loss of immunoreactivity with antiphospho-MAPK antibody after U0126 treatment (Phillips *et al.*, 2002). We previously found that $50\ \mu\text{M}$ U0126 reduced MAPK activity to background in unfertilized eggs (Phillips *et al.*, 2002). U0126 is somewhat more effective at preventing MEK activation than in inhibiting active MEK, and thus we found here that $20\ \mu\text{M}$ U0126 completely prevented MAPK activation during meiotic maturation (below). Therefore, these concentrations of U0126 were used here.

Okadaic acid was used in some experiments to maintain high MAPK activity. The induction of high MAPK activity in activated eggs and GV oocytes, where MAPK is normally inactive, by OA treatment was previously shown by both the kinase assay described here and by the expected mobility shifts of ERK by Western blot (Moos *et al.*, 1995; Lu *et al.*, 2002).

Statistics

Means of replicates were compared by *t* test (2 groups) or ANOVA (≥ 3 groups) using InStat (GraphPad, San Diego, CA). A parametric *t* test (Student's) or ANOVA (pANOVA) was used if variances were not significantly different by *F*-test or Bartlett's test, respectively, or else a nonparametric *t* test (Welch's) or ANOVA (nANOVA) was used. For ANOVAs, post hoc tests (Tukey-Kramer Multiple Comparisons test for pANOVA; Dunn's test for nANOVA) were performed to compare treatment groups. Plots and curve fits were done using SigmaPlot 5 (SPSS, Chicago, IL).

RESULTS

Development of $\text{HCO}_3^-/\text{Cl}^-$ Exchanger Activity after Sr^{2+} Activation of Eggs

To allow precise timing and to permit manipulation, we parthenogenetically activated eggs with Sr^{2+} . These activated eggs exhibited repetitive Ca^{2+}_i transients (Figure 1A), similar to those after IVF (Phillips and Baltz, 1999). The time courses of polar body emission and pronuclear development in Sr^{2+} activated eggs (Figure 1B) and in eggs after IVF (Phillips and Baltz, 1999) were similar. Furthermore, MPF (Histone H1 kinase) and MAPK activity (MBP kinase) decreased after Sr^{2+} activation (Figure 1C) with nearly identical time courses as after IVF (Phillips *et al.*, 2002). Sr^{2+} -activated eggs developed in culture past the two-cell stage (96%) to morulae (58%; $n = 60$, $N = 4$), but development to blastocysts was lower (15%), as is typical for parthenogenotes.

The $\text{HCO}_3^-/\text{Cl}^-$ exchanger became activated in eggs that were parthenogenetically activated with Sr^{2+} (Figure 1D). Half-maximal activation occurred at $\sim 5\ \text{h}$, and maximal activation at $7\ \text{h}$ post- Sr^{2+} activation. This time course is nearly identical to that obtained for $\text{HCO}_3^-/\text{Cl}^-$ exchanger activation after IVF, where $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity was half-maximal at $\sim 6\ \text{h}$ after sperm-egg incubation and maximal by $\sim 8\ \text{h}$ (Phillips and Baltz, 1999). Activation occurred just after pronuclear formation and the decrease in MAPK activity that follows egg activation.

Upregulation of $\text{HCO}_3^-/\text{Cl}^-$ Exchanger Does Not Depend on Ca^{2+}_i Transients

Exposing unfertilized eggs to a 5-min SrCl_2 (10 mM) pulse produced an immediate, single Ca^{2+}_i transient of $\sim 7\ \text{min}$

Figure 1. Sr²⁺ activation of mouse eggs and activation of HCO₃⁻/Cl⁻ exchanger. (A) Ca²⁺_i transients induced in individual eggs (n = 5) upon exposure to 10 mM Sr²⁺. (B) Timing of emission of second polar body (PB2; 65 eggs in 6 total replicates) and pronuclear development (PN; 155 eggs in 11 replicates) as a function of time after activation of eggs with Sr²⁺ (normalized to total number of eggs activated). (C) Mean (±SEM) MPF (H1 kinase) and MAPK (MBP kinase) activities as a function of time after activation of eggs with Sr²⁺. Each point represents 4–6 replicates. Values are normalized to freshly obtained eggs (100%). Inset: representative phosphorimager image of gel with positions of H1 and MBP as labeled, and time post-Sr²⁺ (numbers), pronuclear stage fertilized eggs (PN; obtained several hours after pronuclear formation) or unfertilized eggs (E) indicated below. (D) Mean (±SEM) HCO₃⁻/Cl⁻ exchanger activity as a function of time after activation of eggs with Sr²⁺ (●) or in unactivated eggs maintained in culture (○). Each point represents 4–6 replicates for activated eggs and 3–4 replicates for unactivated eggs. Eggs were activated with a 2-h exposure to Sr²⁺ (10 mM), starting at t = 0 (in B–D). Data in C and D were fit to sigmoidal curves by nonlinear least squares to facilitate visualization. (E) Examples of detection of HCO₃⁻/Cl⁻ exchanger activity by Cl⁻ removal. The presence of external Cl⁻-free solution is indicated by open boxes. The trace on the left is from unfertilized eggs, whereas that on the right is from activated eggs 7 h post-Sr²⁺. Traces show mean pH_i as a function of time in a group of eggs (10 unfertilized, 12 activated) measured simultaneously.

duration (unpublished data). This was sufficient to activate eggs (100%), which emitted a second polar body and developed pronuclei within 6 h (unpublished data). Activated eggs were found to develop HCO₃⁻/Cl⁻ exchanger activity after a single Ca²⁺ transient, with a mean activity of 0.050 ± 0.017 pH U/min (n = 69, N = 5) at 7–9 h after activation, as determined by the Cl⁻-removal assay.

To determine whether HCO₃⁻/Cl⁻ exchanger activity would develop in the absence of Ca²⁺_i transients, eggs were activated by exposure to cycloheximide, a protein synthesis inhibitor that activates eggs by disrupting the continuous cyclin synthesis required for MII arrest (Moos *et al.*, 1996a). Pronuclei developed after cycloheximide exposure with essentially the same time course as with Sr²⁺ activation (Figure 2A). Cycloheximide-induced egg activation occurs without Ca²⁺_i transients (Jones *et al.*, 1995; Moos *et al.*, 1996a), which we confirmed (Figure 2B). Cycloheximide-activated eggs developed HCO₃⁻/Cl⁻ exchanger activity to the same level (Figure 2C) as after Sr²⁺ activation (above) or IVF (Phillips and Baltz, 1999) at 7–9 h postactivation. This indicates that Ca²⁺_i transients are unnecessary for activation of HCO₃⁻/Cl⁻ exchange. Inhibition of HCO₃⁻/Cl⁻ exchanger activation after fertilization by chelation of Ca²⁺_i with BAPTA (Phillips and Baltz, 1999), must therefore reflect an

effect of abnormally low Ca²⁺_i rather than any requirement for Ca²⁺_i transients. MPF inactivation after egg activation requires an intact metaphase spindle in mouse eggs (Kubiak *et al.*, 1993; Winston *et al.*, 1995). We arrested eggs in metaphase by disrupting the spindle (1 μg/ml demecolcine for 1 h) and then induced Ca²⁺_i transients with Sr²⁺ (2 h, as above). This prevented emission of the second polar body or pronuclear formation (Figure 3A) and maintained high MPF and MAPK activities (Figure 3B), whereas Ca²⁺_i transients occurred normally (unpublished data). HCO₃⁻/Cl⁻ exchanger activity did not develop despite the presence of Ca²⁺_i transients (Figure 3C). This indicates that Ca²⁺_i transients are not sufficient to induce activation of HCO₃⁻/Cl⁻ exchange.

HCO₃⁻/Cl⁻ Exchanger Is Inactivated during Meiotic Maturation

Under our conditions, release of GV oocytes from prophase I arrest (GVBD) was complete by ~3 h after removal from dbcAMP, and the transition from MI to MII, marked by emission of the first polar body, occurred at around 14 h (Figure 4A). Freshly obtained GV oocytes exhibited a high level of HCO₃⁻/Cl⁻ exchanger activity, which could be

Figure 2. Activation of $\text{HCO}_3^-/\text{Cl}^-$ exchange after egg activation by cycloheximide. (A) Timing of pronuclear development after cycloheximide exposure (109 eggs in 8 replicates, combined; normalized to total number of eggs activated). Eggs were activated with a 2-h exposure to cycloheximide (50 $\mu\text{g}/\text{ml}$), starting at $t = 0$. (B) Ca^{2+}_i in individual eggs ($n = 11$) during exposure to cycloheximide. (C) $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in eggs 7–9 h after activation by cycloheximide (3 replicates). For comparison, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in unfertilized eggs and Sr^{2+} -activated eggs is shown by lines as indicated.

maintained for more than 12 h in GV oocytes arrested with dbcAMP (Figure 4B). The alkalinization measured upon Cl^- removal in GV oocytes was confirmed to indicate $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity, because it was blocked by the anion transport inhibitor DIDS (100 μM ; unpublished data). dbcAMP itself has no effect on $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in eggs (Phillips and Baltz, 1999).

To determine whether the $\text{HCO}_3^-/\text{Cl}^-$ exchanger was deactivated during meiosis, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity was measured in oocytes as a function of time after they were released from prophase I (GV) arrest. After removal of dbcAMP, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity remained high in oocytes that still possessed a GV and in early MI eggs after GVBD (Figure 4B). Activity then decreased between 6 and 8 h after removal from dbcAMP, reached a minimum several hours before the transition to MII, and then remained low (Figure 4B). Thus, the $\text{HCO}_3^-/\text{Cl}^-$ exchanger is active in prophase I oocytes and is inactivated during first meiosis in the mouse oocyte.

Inactivation of the $\text{HCO}_3^-/\text{Cl}^-$ Exchanger Is Not a General Feature of Metaphase

Inactivation of $\text{HCO}_3^-/\text{Cl}^-$ exchange during meiotic metaphase might indicate that such inactivation is a feature of metaphase in general, at least during early development. Therefore, we examined $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity during the subsequent metaphase—the first mitotic metaphase at the 1- to 2-cell transition. $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity was measured in Sr^{2+} -activated eggs from just before pronuclear envelope breakdown (late G2 and prophase) through cytokinesis. Pronuclear envelope breakdown was complete in most parthenogenotes by ~ 18.5 h after egg activation, with cytokinesis ~ 3 h later (Figure 5A). We found no decrease in the very high $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity evident during this period (Figure 5B), indicating that activity does not decrease during first mitotic metaphase.

Metaphase-induced inactivation of the exchanger may require a longer period in metaphase than normally occurs during mitosis but that is typical of meiotic metaphase and MII arrest. We thus arrested parthenogenotes in first mitotic metaphase with demecolcine added at 12 h post- Sr^{2+} activation and then maintained them in demecolcine for 8 h. We found that $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity remained as high in activated eggs arrested in first mitotic metaphase as in activated eggs exposed to vehicle alone, which had progressed through metaphase and cleaved to the two-cell stage (Figure 5C). Thus, an inactive $\text{HCO}_3^-/\text{Cl}^-$ exchanger appears to be a specific feature of meiotic metaphase and is not a general consequence of metaphase.

Maintenance of MAPK Activity in Activated Eggs Prevents Activation of $\text{HCO}_3^-/\text{Cl}^-$ Exchanger

Both MPF and MAPK become activated during meiotic maturation, are active in MII eggs, and are inactivated after egg activation (Verlhac *et al.*, 1994, 1996; Moos *et al.*, 1995; Verlhac *et al.*, 1996), which is the converse of $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity. However, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity did not decrease again during first mitotic metaphase (Figure 5), where MPF is reactivated but MAPK activity is reported to remain low (Haraguchi *et al.*, 1998). $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity also appeared to more closely mirror MAPK activity. Therefore, we investigated whether MAPK

Figure 3. Effect of arrest with demecolcine on activation of $\text{HCO}_3^-/\text{Cl}^-$ exchanger. (A) Emission of second polar body (PB2, ■) and pronuclear development (PN, ●) assessed as a function of time after activation of eggs with Sr^{2+} , in the presence (deme) or absence (control) of demecolcine (1 $\mu\text{g}/\text{ml}$). For polar body determination, there were 43 eggs (3 replicates) in each group; for pronuclei, additional groups were assessed, with 213 and 216 eggs (8 replicates) in the absence and presence of demecolcine, respectively. (B) Mean ($\pm$ SEM) MPF (H1 kinase; at left) and MAPK (MBP kinase; at right) activities (4–6 replicates) in eggs 8 h after Sr^{2+} activation, in the presence of demecolcine (deme) or control exposed to vehicle alone (ctrl). $**p = 0.002$ (H1) and 0.004 (MBP) by Welch's t test. Inset: representative gel. (C) Mean ($\pm$ SEM) $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in eggs 7–9 h post- Sr^{2+} activation in the absence (control; vehicle alone) or presence (deme) of demecolcine. Demecolcine pretreatment significantly inhibited activity ($***p = 0.004$ by Student's t test; 5 replicates).

Figure 4. $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity during meiotic maturation. Oocytes were induced to undergo spontaneous GVBD and meiotic maturation by removal from dbcAMP ($t = 0$) or were maintained in GV arrest by the continuous presence of dbcAMP. (A) Proportion of oocytes that underwent GVBD (MI, ●) and emission of the first polar body (MII, ○) in oocytes as a function of time after removal from dbcAMP (351 eggs in 29 replicates). GV oocytes maintained in dbcAMP for the same period did not undergo GVBD (◇; 107 eggs in 11 replicates). (B) Mean ($\pm$ SEM) $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in eggs during GVBD and meiosis after removal from dbcAMP. $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity was measured in GV oocytes after removal from dbcAMP (◇), in MI oocytes after GVBD occurred (●), and in MII oocytes after emission of first polar body (○). Activity was compared with that in eggs maintained in GV arrest with dbcAMP (◇). Each point represents 3–8 replicates. Data were fit to sigmoidal curves by nonlinear least squares to facilitate visualization. The slight activation of $\text{HCO}_3^-/\text{Cl}^-$ exchange in MII eggs after extended in vitro culture (at 16.5 h) was also seen in ovulated MII eggs maintained after extended in vitro culture (our unpublished data).

activity affects $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in mouse oocytes.

OA, an inhibitor of protein phosphatases PP1 and PP2A (Cohen *et al.*, 1990), has been used as a tool to maintain high MAPK activity in fertilized eggs (Schwartz and Schultz, 1991; Moos *et al.*, 1995). When OA (2.5 μM) was added 15 min before (Figure 6A) or coincident with (unpublished data) Sr^{2+} , it prevented the development of pronuclei. This is consistent with previous reports that the decrease in MAPK activity after fertilization regulates the formation of pronuclei (Moos *et al.*, 1995, 1996b), and OA thus prevents formation of pronuclei (Schwartz and Schultz, 1991; Moos *et al.*, 1995). Introduction of OA immediately after the 2-h period of Sr^{2+} exposure permitted only a transient (~ 2 h) appearance of pronuclei (unpublished data), whereas OA introduced to the pronucleate stage or two-cell stage parthenogenotes (Sr^{2+} -activated eggs after cleavage to the 2-cell stage) caused the pronuclei to disappear after ~ 4 h (Figure 6A). We confirmed that OA, added 15 min before Sr^{2+} , maintained high MAPK activity in activated eggs (Figure 6B). In contrast, OA had no effect on MPF activity, which was similarly low in OA-treated and control eggs 8 h after Sr^{2+} activation (Figure 6B). Therefore, OA can be used to produce activated eggs with low MPF activity and inappropriately high MAPK activity.

An additional strategy for maintaining high MAPK activity by expressing exogenous constitutively activated MEK in eggs has been reported previously (Moos *et al.*, 1995). How-

ever, we have been unable to obtain sufficient expression to prevent pronuclear formation after activation by Sr^{2+} (unpublished data; constitutively-activated MEK was a gift of S. Gutkind, NIH). Thus, we have relied on OA here to maintain high MAPK activity after egg activation.

We thus determined whether $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity developed in Sr^{2+} -activated parthenogenotes where elevated MAPK activity was maintained by OA. OA, added 15 min before Sr^{2+} , completely prevented the appearance of $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity at 7–9 h after egg activation, by which time maximal $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity had developed in control groups of Sr^{2+} -activated eggs (Figure 6C). Similarly, no $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity developed in Sr^{2+} -activated eggs at 7–9 h after egg activation when OA was added immediately after the 2-h period of Sr^{2+} exposure, 1.5 h later or 3 h later (unpublished data).

To determine if OA was capable of inactivating the fully active $\text{HCO}_3^-/\text{Cl}^-$ exchanger in pronucleate stage eggs, we added OA to eggs 10 h after Sr^{2+} activation, several hours after full $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity had developed. There was no significant decrease in $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity 4 h after OA addition (unpublished data). However, 8 h after OA addition, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity had been reduced to background levels (Figure 6C). Similarly, 8 h of OA exposure was able to eliminate the robust $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in two-cell parthenogenotes (Figure 6C).

Figure 5. $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity during first mitotic metaphase. (A) Timing of pronuclear envelope breakdown (NEBD) and cytokinesis (to 2-cell stage) in Sr^{2+} -activated eggs as a function of time after activation (60 eggs in 3 replicates). (B) Mean ($\pm$ SEM) $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity during mitotic metaphase of Sr^{2+} -activated eggs (each point represents 4–6 replicates, with 13–18 eggs in each, except 2 replicates at 26 h). (C) Mean ($\pm$ SEM) $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in Sr^{2+} -activated eggs arrested in first mitotic metaphase with demecolcine (deme; 1 $\mu\text{g}/\text{ml}$) compared with control (vehicle only). See text for details. Mean $\text{HCO}_3^-/\text{Cl}^-$ exchanger was not significantly different in eggs arrested in first mitotic metaphase with demecolcine compared with vehicle controls, which had cleaved to the two-cell stage (NS; $p > 0.05$ by Student's t test; 3 replicates).

Figure 6. HCO₃⁻/Cl⁻ exchanger activity after okadaic acid (OA) treatment of activated eggs. (A) Proportion with pronuclei or nuclei assessed as a function of time after OA addition (OA addition at $t = 0$ in each case), with OA added either just before Sr²⁺ activation (–15 min; 127 eggs in 5 replicates), to activated eggs with pronuclei (10 h; 107 eggs in 7 replicates), or to activated eggs which had cleaved to the two-cell stage (24 h; 67 eggs in 6 replicates). Vehicle (DMSO) alone did not affect pronuclear formation, and pronuclei or nuclei persisted throughout the experimental period in the absence of OA

(unpublished data). (B) Mean ($\pm$ SEM) MPF (H1) and MAPK (MBP) activities measured 8 h after egg activation with Sr²⁺, in control eggs (ctrl; vehicle only) or eggs where OA was added 15 min before Sr²⁺ (OA). Differences were not significant between control and OA for H1 (NS, $p = 0.6$) and significant (** $p = 0.005$) for MBP by t tests. Each bar represents 4–6 replicates. Inset: lanes from same representative gel. (C) Mean ($\pm$ SEM) HCO₃⁻/Cl⁻ exchanger activity measured 8 h after egg activation with Sr²⁺. OA ($\square$) or control (vehicle only, $\blacksquare$) were added as described in A. HCO₃⁻/Cl⁻ exchanger activity was significantly lower in OA-treated eggs vs. control eggs exposed to vehicle only in each case (** $p < 0.001$, **** $p < 0.0001$ by Student's t test; each bar represents 4–6 replicates).

Inactivation of MAPK in Unfertilized Eggs Activates HCO₃⁻/Cl⁻ Exchange

We used U0126, a specific inhibitor of the MAPK kinase MEK, to decrease MAPK activity in unfertilized eggs. We previously showed (Phillips *et al.*, 2002) that U0126 (50 μ M) rapidly and completely inactivates MAPK in unfertilized eggs (<1 h versus 5-h post-IVF or -Sr²⁺ activation). The loss of CSF activity in the presence of U0126 in turn caused MPF activity to decrease, with a time course similar to that after IVF or Sr²⁺ activation (1–2 h; Phillips *et al.*, 2002). We found here that exposure of eggs to U0126 (50 μ M) caused them to develop HCO₃⁻/Cl⁻ exchanger activity (Figure 7). Activity appeared to begin to develop immediately, without the 3–4-h lag time as seen with Sr²⁺ (Figure 1D, 7) or after IVF (Phillips and Baltz, 1999), and was maximal by 5 h after U0126 was added.

HCO₃⁻/Cl⁻ Exchanger Inactivation during Meiotic Maturation Requires MAPK Activity

MI oocytes cultured with U0126 (20 μ M) after GVBD developed normally high MPF (H1 kinase) activity. However, unlike normal MI oocytes, MAPK activity remained mini-

mal and was still not detectable at 8 h after release from prophase arrest (Figure 8A). MI oocytes treated with U0126 exhibited high HCO₃⁻/Cl⁻ exchanger activity at 8 h, which was not significantly different from that of GV oocytes maintained in prophase arrest (Figure 8B). In contrast, control

Figure 7. Effect of U0126 on HCO₃⁻/Cl⁻ exchanger activity in eggs. Mean ($\pm$ SEM) HCO₃⁻/Cl⁻ exchanger activity in unfertilized eggs treated with the MEK inhibitor U0126 (50 μ M; $\bullet$) or in control eggs (vehicle only; $\circ$). HCO₃⁻/Cl⁻ exchanger activity after Sr²⁺ is shown for comparison (gray curve, taken from curve fit in Figure 1D). Each point represents 3–6 replicates.

Figure 8. Effect of U0126 on HCO₃⁻/Cl⁻ exchanger activity in first meiotic metaphase oocytes. (A) Mean ($\pm$ SEM) MPF (H1 kinase) and MAPK (MBP kinase) in GV oocytes maintained in GV arrest with dbcAMP, or in MI oocytes that had been allowed to undergo GVBD (3-h incubation in the absence of dbcAMP) and then cultured in U0126 (20 μ M) or with vehicle alone (ctrl = control). In each case, measurements were made after 8 h in culture (4 replicates each). Different letters (a vs. b) above bars indicate significant difference between the three treatments for MPF (H1 kinase; $p < 0.05$), or MAPK (MBP kinase; $P < 0.01$), by ANOVA and Tukey-Kramer Multiple Comparisons Test. Inset: representative gel (GV, U = U0126, and ctrl = control). (B) Mean ($\pm$ SEM) HCO₃⁻/Cl⁻ exchanger activity in oocytes treated as described in A. Different letters (a vs. b) above bars indicate significant difference ($P < 0.001$ by ANOVA and Tukey-Kramer Multiple Comparisons Test; 6 replicates).

Figure 9. Effect of activation of MAPK by OA pulse on HCO₃⁻/Cl⁻ exchanger activity in GV oocytes. (A) Mean (±SEM) MPF (H1 kinase) and MAPK (MBP kinase) activities in oocytes. Oocytes were maintained in GV arrest with dbcAMP (GV), maintained in dbcAMP after a 1-h pulse of OA (OA-p), or released from arrest after 1 h treatment with vehicle for OA alone (MI). Kinase activity was then measured after 9 h in culture. Each bar represents four replicates. Different letters (a vs. b) above bars indicate significant difference between treatments for MPF (H1; $p < 0.001$) or MAPK (MBP; $p < 0.01$) by ANOVA and Tukey-Kramer Multiple Comparisons Test. Inset: a representative gel. (B) Mean (±SEM) HCO₃⁻/Cl⁻ exchanger activity in oocytes treated as in A. Activity was measured after 9 h in culture. Different letters (a vs. b) indicate significant difference ($p < 0.0001$; ANOVA, Tukey-Kramer's Multiple Comparisons Test; 6 replicates each). (C) Mean (±SEM) MPF (H1 kinase; filled bars) and MAPK (MBP kinase) activities in oocytes 9 h after a 1-h OA pulse, in the continuous presence (OA-p + U0126) or absence (OA-p) of U0126 (50 μ M); only GV-intact oocytes were included in the OA-p + U0126, as described in the text. dbcAMP was present throughout. Each bar represents four replicates. ** $p = 0.001$ for H1, 0.004 for MBP by t tests. Inset shows a representative gel. D. Mean (±SEM) HCO₃⁻/Cl⁻ exchanger activity in oocytes treated as in C. *** $p = 0.008$ by Student's t test; 4 replicates each).

oocytes treated in parallel had the low HCO₃⁻/Cl⁻ exchanger activity expected for late MI oocytes (Figure 8B).

HCO₃⁻/Cl⁻ Exchanger in GV Oocytes Can Be Inactivated by MAPK Activation in the Absence of MPF Activation

It has recently been shown that the treatment of dbcAMP-arrested GV oocytes with a brief pulse of OA induces the irreversible activation of MAPK and breakdown of the GV, but MPF does not become activated (de Vantery Arrighi *et al.*, 2000; Lu *et al.*, 2002). When we treated GV oocytes with an OA pulse (2.5 μ M, 1 h), they exhibited the characteristic "ruffled" appearance (Schwartz and Schultz, 1991; Moos *et al.*, 1995; Zernicka-Goetz *et al.*, 1997) and underwent breakdown of the GV despite the presence of dbcAMP. We confirmed that this treatment produced oocytes with low MPF activity and high MAPK activity at 10 h post-GVBD (Figure 9A). As expected, at the same time post-GVBD, MPF, and MAPK were still inactive in GV oocytes maintained in arrest with dbcAMP and were both maximally active in MI oocytes exposed to a pulse of vehicle (DMSO) alone (Figure 9A). After an OA pulse, oocytes maintained in dbcAMP exhibited very low HCO₃⁻/Cl⁻ exchanger activity at 10 h, which was not significantly different from that in MI oocytes and was significantly lower than that in oocytes maintained in GV arrest for the same period (Figure 9B).

To show that the decrease in HCO₃⁻/Cl⁻ exchanger activity was due specifically to MAPK activity induced by the OA pulse, we blocked activity of the MAPK kinase, MEK, in GV oocytes before the OA pulse by pretreating them with 50 μ M U0126 for 30 min in the continuous presence of dbcAMP. Consistent with previous reports (de Vantery Arrighi *et al.*, 2000), U0126 inhibited GVBD in OA-treated oocytes by

~50% (unpublished data), and we assumed here that the continued presence of a GV indicated successful reversal of the effects of the OA pulse by U0126. As before (Figure 9A), MAPK activity was high in OA pulse-treated oocytes, whereas MPF activity was low (Figure 9C), although a minor but statistically significant activation of MPF was evident. Treatment with U0126 eliminated MAPK activity, thereby producing an OA-treated oocyte with low MPF and MAPK activities. In these oocytes, HCO₃⁻/Cl⁻ exchanger activity was significantly higher compared with oocytes treated with an OA pulse alone (Figure 9D).

DISCUSSION

We report here that the HCO₃⁻/Cl⁻ exchanger in mouse oocytes is inactivated during meiotic metaphase. Activity decreases from maximal in the prophase I-arrested GV oocyte as the oocyte proceeds through first meiotic metaphase and reaches a minimum approximately 2 h before the emission of the first polar body and entry into second meiotic metaphase. Reactivation does not occur until the end of meiosis, after the second polar body is emitted and pronuclei have developed, as the embryo enters interphase.

In the mouse, MAPK activation and the development of CSF activity occurs about 2 h after MPF activation and GVBD (Verlhac *et al.*, 1994). High MAPK activity persists through meiosis, remaining high in the MII-arrested egg. Inactivation of MAPK does not occur until several hours after egg activation, at the time of pronuclear formation (Moos *et al.*, 1995; Zernicka-Goetz *et al.*, 1995). In contrast, MPF deactivation occurs quickly after egg activation, preceding second polar body formation. Thus, MAPK activity appeared to be inversely correlated with HCO₃⁻/Cl⁻ ex-

Figure 10. Schematic diagram of $\text{HCO}_3^-/\text{Cl}^-$ exchanger, MAPK, and MPF activities in oocytes from GV to pronuclear stage. MPF and MAPK activities are taken from data of Verlhac *et al.* (1994) for meiotic maturation (0–10 h), Kubiak *et al.* (1992) for the MI to MII transition, and from Figure 1C for activated eggs (from 16 h). MPF activity is reported to recover only to 85% after the MI to MII transition (Kubiak *et al.*, 1992), as shown here. $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity is taken from Figures 1 and 4. Timing of major events is shown at top. Activation of unfertilized eggs was assumed to occur at 18 h, but the interval between 10 and 18 h may be of varying length, depending on the actual timing of egg activation or fertilization. Because the various time courses arise from separate sets of experiments and from previous reports, the relative timing will not correspond exactly. Thus, this figure indicates general correlations rather than the exact timing and sequence of events.

changer activity in the mouse oocyte, whereas changes in MPF activity preceded changes in $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity (Figure 10). We therefore hypothesized that MAPK negatively regulates $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity in mouse oocytes.

Several pieces of evidence support this hypothesis (summarized in Table 1). First, $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity did not develop in eggs when high MAPK activity was maintained with OA for an extended period after egg activation, even although the eggs were activated and MPF decreased. In addition, $\text{HCO}_3^-/\text{Cl}^-$ exchange could be inactivated by OA in pronuclear stage or two-cell stage parthenogenotes. Second, the rapid inactivation of MAPK using the MEK inhibitor U0126 in unfertilized eggs (Phillips *et al.*, 2002) resulted in accelerated $\text{HCO}_3^-/\text{Cl}^-$ exchanger activa-

tion. Third, oocytes in which the normal activation of MAPK after GVBD was prevented with U0126 did not exhibit decreased $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity, even although GVBD occurred and MPF was activated. Fourth, activation of MAPK in GV oocytes with an OA pulse induced GVBD and suppressed $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity without activating MPF, and $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity could be at least partially restored when MAPK activity was inhibited by U0126 in oocytes treated with an OA pulse. Thus, in each case where MAPK activity was low, $\text{HCO}_3^-/\text{Cl}^-$ exchange was activated, and vice versa (Table 1), consistent with negative regulation of $\text{HCO}_3^-/\text{Cl}^-$ exchanger by MAPK in the mouse oocyte.

The data do not support regulation of the $\text{HCO}_3^-/\text{Cl}^-$ exchanger by MPF. OA treatment of activated eggs or OA pulse treatment of GV oocytes did not activate MPF but did suppress $\text{HCO}_3^-/\text{Cl}^-$ exchanger activity (Table 1). Conversely, oocytes undergoing meiotic maturation in the presence of U0126 and parthenogenotes in mitotic metaphase possess high MPF activity, whereas the $\text{HCO}_3^-/\text{Cl}^-$ exchanger was not inactivated (Table 1).

There is some precedent for a role for MAPK in the control of pH_i -regulatory transporters. MAPK activity mediates growth factor and arginine vasopressin activation of Na^+/H^+ antiporter activity in mammalian cells (Sardet *et al.*, 1991; Aharonovitz and Granot, 1996; Bianchini *et al.*, 1997), whereas in *Xenopus* oocytes upregulation of Na^+/H^+ antiporter activity during meiotic maturation is dependent on RAF (Kang *et al.*, 1998) and can be induced by MOS (Rezai *et al.*, 1994). These examples, however, involve positive regulation of pH_i -regulatory mechanisms by the MAPK pathway. In contrast, the $\text{HCO}_3^-/\text{Cl}^-$ exchanger appears to be negatively regulated by MAPK or downstream effectors in mouse oocytes during meiosis.

Similar to the $\text{HCO}_3^-/\text{Cl}^-$ exchanger, the Na^+/H^+ antiporter is quiescent in MII eggs and only becomes activated a number of hours after fertilization (Lane *et al.*, 1999b). Thus, pH_i -regulatory mechanisms in general may prove to be inactive during meiotic metaphase. The physiological reason for specific inactivation of pH_i -regulatory mechanisms during meiosis and reactivation after fertilization remains unclear, since pH_i in mouse oocytes does not change upon fertilization (Kline and Zagray, 1995) in contrast to, e.g., sea urchin. Reactivation could be explained by a requirement to robustly regulate pH_i as the egg becomes more metabolically active after fertilization, as has been found for activation of somatic cells (Ganz *et al.*, 1989). However, this would not explain the initial inactivation of pH_i regulation during meiosis. At present, we can only speculate that it may reflect a need to restrict transmembrane ion transport during meiosis or during fertilization, but further work is clearly needed.

More generally, a picture is emerging of regulation of transport and homeostatic mechanisms in oocytes, eggs, and early embryos by the cell cycle interacting with developmental clocks, to which pH_i -regulatory mechanisms conform. The cell swelling-activated anion channel, which functions in cell volume regulation, was shown to become inactivated during prophase and inactive in metaphase after the two-cell stage in mouse embryos but is not affected by previous mitotic or meiotic metaphases (Kolajova *et al.*, 2001). In addition, a large conductance K^+ channel in mouse

Table 1. Regulation of $\text{HCO}_3^-/\text{Cl}^-$ exchanger

Treatment	MAPK	MPF	$\text{HCO}_3^-/\text{Cl}^-$ exchanger
Sr^{2+} -activated eggs	–	–	+
OA + Sr^{2+} -activated eggs	+	–	–
Unfertilized egg	+	+	–
U0126 + unfertilized eggs	–	–	+
	(quick)		(accelerated)
GV oocyte	–	–	+
U0126 + GVBD oocytes	–	+	+
OA pulse + GV oocytes	+	–	–
OA pulse and U0126 + GV oocytes	–	–	+
Mitotic metaphase	–	+	+

oocytes of unknown function is regulated by a cytoplasmic cell cycle, becoming activated during meiotic metaphase and each mitotic metaphase during early embryo development (Day *et al.*, 1998a). The T-type Ca²⁺ channel in mouse embryos is activated upon exit from metaphase at the end of the one-cell stage and then deactivated before entry into the next metaphase, maintaining high activity during interphase of the two-cell stage (Day *et al.*, 1998b).

Like these ion channels, the HCO₃⁻/Cl⁻ exchanger is under cell cycle control in the mouse oocyte. Inactivation during metaphase is restricted to meiosis, demonstrating an interaction with a developmental clock, similar to such control of the K⁺ and T-type Ca²⁺ channels in oocytes and embryos. Unlike these channels, however, a possible regulatory pathway for the HCO₃⁻/Cl⁻ exchanger has been identified, with MAPK or its downstream effectors implicated. Given the similar behavior of the Na⁺/H⁺ antiporter in hamster eggs, suppression of pH-regulatory mechanisms during meiosis may prove to be a novel function of MAPK, and hence CSF activity, in the mammalian oocyte.

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