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Dynamic secretion during meiotic reentry integrates the function of the oocyte and cumulus cells

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The differentiation of the female gamete into a developmentally competent oocyte relies on the protected environment of the ovarian follicle. The oocyte plays a key role in establishing this microenvironment by releasing paracrine factors that control the functions of surrounding somatic cells. Growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) are secreted during follicle growth and play pivotal roles in this local regulation. The current view is that the function of these secreted factors declines in the periovulatory period when the oocyte reenters the meiotic cell cycle. Here, we provide evidence that oocyte reentry into meiosis is instead associated with a shift in the pattern of secretion with a new set of bioactive molecules synthesized before ovulation. Using interleukin 7 (IL7) as a prototypic secreted factor, we show that its secretion is dependent on activation of mRNA translation in synchrony with the cell cycle and that its translation is under the control of somatic cells. IL7 is part of a local feedback loop with the soma because it regulates cumulus cell replication. Similar conclusions are reached when IL7 secretion is measured in human follicular fluid during in vitro fertilization cycles. IL7 concentration in the follicular fluid correlates with the oocyte ability to reach the MII stage of maturation. These findings are consistent with the hypothesis that a new set of local factors is secreted by the oocyte during ovulation. These dynamic secretions are likely critical for promoting the final stages of maturation and oocyte developmental competence.

oocyte maturation | mRNA translation | oocyte secreted factors | interleukin-7

The differentiation of mammalian oocytes and the acquisition of developmental competence require a unique microenvironment generated by bidirectional regulation integrating the gamete function with that of surrounding somatic cells (1). Although the property of the oocyte to secrete factors that inhibit luteinization was suggested almost four decades ago (2), its critical role in directing somatic cell differentiation has been recognized more recently with the molecular identification of oocyte-secreted factors (OSFs), their receptors, and associated signals (3). In addition to the members of the TGFβ superfamily, which have been extensively studied, other factors secreted by the oocyte, such as FGF members, regulate cumulus cell function (4, 5). Specifically, these OSFs are essential for ovulation and fertility because they stimulate granulosa cell proliferation (6), function as antiapoptotic agents (7), and regulate cumulus expansion (8) and the metabolism of somatic cells surrounding the oocyte (9).

The oocyte/somatic cell dialogue is bidirectional because oocyte maturation and ovulation are regulated by a number of local factors secreted by somatic cells. A surge in luteinizing hormone (LH) induces reprogramming of the mural granulosa cells in the follicle and the expression of epidermal growth factor (EGF)-like peptides, including amphiregulin (AREG), epiregulin (EREG), and betacellulin (10). AREG and EREG are needed to propagate the LH stimulus from the mural granulosa cells to the cumulus cells, which are insensitive to direct LH stimulation (10).

Oocytes remain arrested at the prophase I of meiosis, the chromosomes are decondensed, and the chromatin is transcriptionally active throughout follicle growth. Toward the end of the folliculogenesis when the oocyte is fully grown, the transcriptional activity ceases and the chromatin in the germinal vesicle (GV) becomes condensed (11). After the ovulatory stimulus, meiosis resumes, and the oocyte nucleus undergoes the changes required for the correct ploidy of the gamete. These final stages of oocyte maturation, as well as fertilization and early embryo development, occur in the absence of transcription (12). Cell cycle progression and genome reprogramming rely on translation of stored maternal mRNAs until the embryo genome becomes activated at the early two-cell stage in mice or at four to eight cells in humans (12). The translation of stored mRNAs is, therefore, highly regulated, and the oocyte developmental competence depends almost exclusively on post-transcriptional events. Here, we have investigated how translational regulations contribute to oocyte secretion.

Functional and genetic studies have been used to characterize the secretory products of the oocyte (13, 14). Previous systematic approaches to identify these secreted factors have relied on mining of established databases (15). Proteomics profiling of the oocytes has also been used (16). In most cases, the sensitivity of the approach was insufficient to provide a genome-wide assessment of oocyte secretion. Oocyte transcriptomics had the potential to identify secretory products. However, given the fact that transcription is absent during the oocyte maturation, this approach cannot capture the dynamic nature of the synthesis/secretion by the oocyte.

It is generally accepted that the major secretory activity of the oocyte takes place during the growth phase of the follicle. Conversely, there is little information regarding the regulation of oocyte secretion during the final stages of oocyte maturation, and it is believed that oocyte secretion ceases as the oocyte reenters meiosis (3). Here, we have revisited this concept and hypothesized that translation and secretion of oocyte factors during oocyte maturation are actually highly dynamic and synchronized with the meiotic cell cycle. We have also explored whether somatic cells affect oocyte secretion via translational regulation in the oocyte, supporting the concept that these regulated translations/secretions are part of cross-talks or feedbacks between the gamete and surrounding cumulus

Significance

Oocyte fitness to support embryo development and pregnancy is dependent on an elaborate cross-talk with the surrounding environment of the ovarian follicle. Here, we show that this cross-talk continues during the periovulatory period when a new set of bioactive molecules is secreted by the oocyte in mice and humans. This shift in pattern of secretion is dependent on oocyte maturation and on paracrine factors secreted by somatic cells at the time of ovulation. These changes in pattern of secretion may be used to assess oocyte quality.

Author contributions: H.C. and M.C. designed research; H.C., F.F., and A.M.Z. performed research; H.C., F.F., A.M.Z., M.I.C., and M.C. analyzed data; and H.C., F.F., and M.C. wrote the paper.

The authors declare no conflict of interest.

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cells. Such regulations may be crucial for oocyte final differentiation to sustain fertilization and early embryo development.

Results

Translational Regulation of mRNA Coding for Secreted Proteins During in Vivo Oocyte Maturation. Association of mRNA with the polysomes is an established approach to assess translation (17). This strategy was used to explore the pattern of translation of secreted proteins during oocyte maturation in a genome-wide fashion. We used two datasets generated by our laboratory, one assessing polysome association at GV, MI, and MII (18) and a second set comparing GV and MII (19). Of the ∼7,600 transcripts present in the oocyte, 170 were classified as transcripts coding for secreted proteins (see *Materials and Methods* for the criteria used). Inspection of the data indicated that the analysis indeed captures the pattern of secretion by the oocyte because growth differentiation factor 9 (GDF9), bone morphogenetic protein 5 (BMP5), plasminogen activator (PLAT), and zona pellucida glycoproteins (ZP1-3), all established oocyte secretory products, were among the transcripts identified. By comparing the polysome association during different stages of maturation, we identified three classes of transcripts of secreted proteins with distinct patterns of ribosome recruitment from GV to MII oocytes [false discovery rate (FDR) of <5%, q < 0.05] (Fig. 1*A*). Although no significant changes were observed for 45 transcripts, suggesting constitutive translation, one additional group of transcripts decreased (87 transcripts) and another increased (38 transcripts) in the polysome fraction during oocyte maturation (Fig. 1*A* and Dataset S1), suggesting that synthesis, and possibly secretion, of these proteins is dynamic. Consistent with previous reports (20), *Plat* translation increased approximately twofold (Fig. 1*B*). The translation of all three zona pellucida glycoproteins decreased dramatically during oocyte maturation, indicating that the turnover of the zona decreases as the oocyte matures (Fig. 1*C*). In addition to peptidases and extracellular matrix components, several additional classes of bioactive secretory products were identified. Consistent with the established production of members of the TGFβ superfamily by the murine oocyte, we identified *Gdf9*, *Bmp5*, *Bmp6*, *Bmp15*, *Tgfb2*, and *Tgfb3* as translated during oocyte maturation (Fig. 1*D*). Significant changes were observed in the pattern when comparing GV and MII: *Gdf9*, *Bmp15*, and *Tgfb3* translation decreased during oocyte maturation whereas translation of *Bmp5* increased, and *Bmp6* remained constant, suggesting a shift in the pattern of secretion of these factors around the time of ovulation (Fig. 1*D*). Among the FGF family, *Fgf1*, *Fgf3*, *Fgf7*, *Fgf8*, and *Fgf9* transcripts were present in the polysome fraction, with *Fgf8* being the most abundant (Fig. 1*E*). Although *Fgf7* translation increased and *Fgf8* translation showed a decrease of ∼50% (although statistically borderline) during oocyte maturation, the rest of the FGF family's association with polysomes did not change between GV and MII (Fig. 1*E*). In addition, the translation of multiple cytokines/chemokines was dynamic during oocyte maturation. Whereas the translation of *Csf1* increased, *Ik* and *Grem1* translation decreased during the transition from GV to MII (Fig. 1*F*).

This initial survey opens the possibility that the oocyte secretion pattern varies in phase with progression through the meiotic cell cycle and that different sets of signals are exchanged with the surrounding cellular environment while differentiating into a developmentally competent egg. To solidify this concept, we further analyzed the translation and secretion of some of these transcripts. Interleukin 7 (IL7), a cytokine that is expressed by the oocyte compared with other cells in the follicle (21), was selected as the prototype of transcripts that are increasingly translated during oocyte maturation (Fig. 2*A* and Dataset S1).

By using quantitative RT-PCR analysis of mRNAs recovered in the polysome fractions, we confirmed the maturation stage-dependent pattern of *Il7* mRNA recruitment to the translating pool of ribosomes, recapitulating the data obtained by microarray

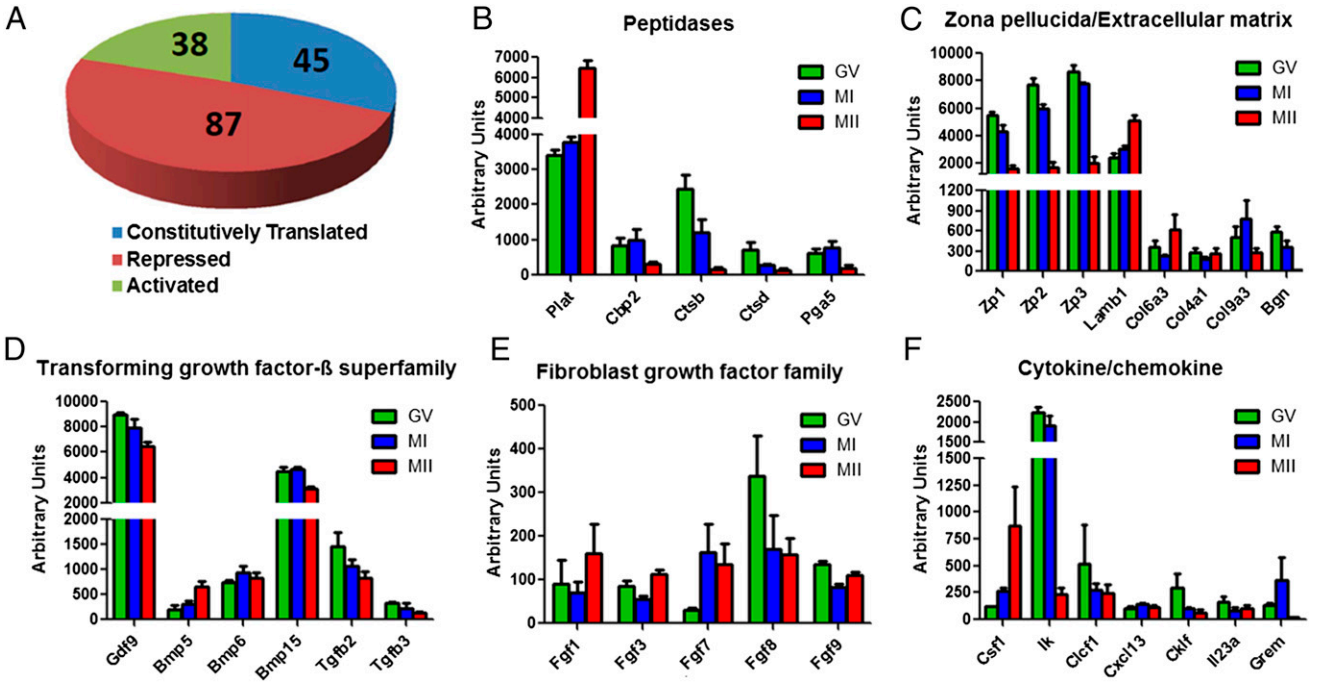

Fig. 1. Translational regulation of mRNA coding for secreted proteins during in vivo oocyte maturation. (*A*) Pie chart reporting different classes of transcripts for secreted proteins according to their polysome recruitment from GV to MII. The number of transcripts constitutively translated, those decreased (repressed) and increased (activated), is reported. See Dataset S1 for the full list. FDR < 5% and q < 0.05 were used in this analysis. Secreted proteins were grouped according to function: (*B*) peptidases, (*C*) zona pellucida and extracellular matrix proteins, (*D*) transforming growth factor β superfamily, (*E*) fibroblast growth factor family, and (*F*) cytokines/chemokines. Error bars correspond to the SEM, and data are from three biologically different samples. The dataset in ref. 18 was used.

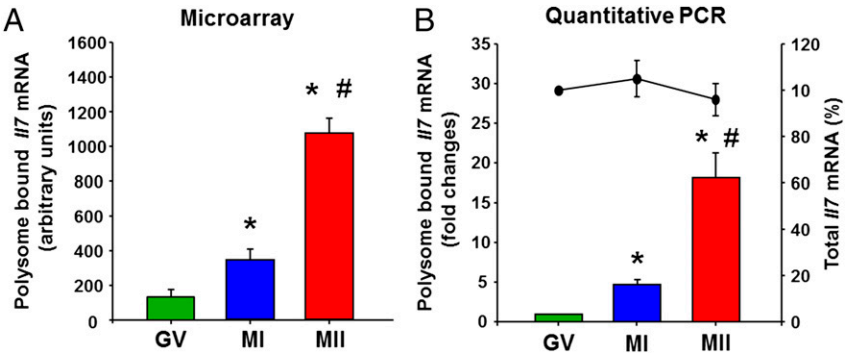

Fig. 2. *Il7* mRNA translation during in vivo oocyte maturation. (*A*) Microarray analysis of *Il7* mRNA in the polysome fraction during oocyte maturation. $^{*}P < 0.05$ vs. GV; $^{\#}P < 0.05$ vs. MI, ANOVA. (*B*) Quantitative PCR analysis of *Il7* mRNA in polysome fractions (bar graph) and whole-cell lysates (line plot) during oocyte maturation. $^{*}P < 0.05$ vs. GV; $^{\#}P < 0.05$ vs. MI, ANOVA. Error bars correspond to the SEM, and data are from three experiments from biologically distinct samples.

hybridization (Fig. 2 *A* and *B*). The level of polysome-bound *Il7* mRNA increased significantly in the MI oocyte compared with the GV oocyte and further increased with maturation to MII stage (Fig. 2*B*). Consistent with the absence of transcription in the fully grown oocyte, the total *Il7* mRNA level did not change during oocyte maturation (Fig. 2*B*). Thus, changes in transcript levels in the polysome fraction were due to transfer from or to the polysome fraction or destabilization, but not to de novo RNA synthesis.

Regulation of *Il7* mRNA Translation and Protein Synthesis/Secretion. To determine how the translation of *Il7* is activated during maturation, we constructed a reporter with the renilla luciferase ORF under the control of *Il7* 3′ UTR and polyadenylated firefly luciferase reporter as control. When injected into the oocyte, the accumulation of the *Il7* luciferase reporter paralleled the mRNA recruitment to the polysome pool during oocyte maturation (Fig. 3*A*). However, when oocyte maturation was prevented, the luciferase activity did not change (Fig. 3*A*). This experiment, as well as others reported below, shows that regulations impinging on the 3′ UTR of the mRNA are likely responsible for the regulation. The translation of the reporter indeed reflected the translation of the endogenous *Il7* mRNA and de novo IL7 synthesis, and its secretion was confirmed by measuring the levels of IL7 in the spent media of the oocyte culture (Fig. 3*B*). A significant increase in IL7 protein accumulation was observed in the spent media of either denuded or cumulus enclosed oocytes [cumulus oocyte complexes (COCs)] (Fig. 3*B*). Moreover, COCs secreted significantly higher amounts of IL7 during maturation compared with denuded oocytes (DOs), implying potential cumulus cell regulation of oocyte IL7 secretion (Fig. 3*B*). In agreement with translation data (Fig. 1*E* and Dataset S1), FGF8 secretion measured by ELISA decreased during oocyte maturation (Fig. 3*B*, *Inset*), confirming that polysome recruitment predicts pattern of translation.

We have shown that activation of translation is at least in part dependent on the progression through the cell cycle but that somatic cell inputs can also affect translation in the oocyte during maturation (19). To distinguish between these two mechanisms, COCs were incubated with or without the somatic cell-derived EGF-like growth factor AREG, which accumulates in response to LH surge and stimulates translation in the oocyte (19). Translation rates of the *Il7* reporter significantly increased as the oocytes progressed from GV to MII (Fig. 4*A*), consistent with recruitment of the endogenous *Il7* mRNA to the polysome fraction. However, *Il7* reporter translation in COC was further increased by supplementing the medium with AREG. When truncated *Il7* 3′ UTR reporter or a reporter lacking *Il7* 3′ UTR were injected to COCs, their translation did not change during oocyte maturation or after the addition of AREG (Fig. S1). This pattern suggests that full-length *Il7* 3′ UTR is required for the translation of *Il7* reporter. Although increase in the translation rate of *Il7* reporter was observed with meiotic maturation in DOs, AREG effects were not detected when oocytes were denuded before stimulation (Fig. 4*A*). This latter finding indicates that the stimulatory effect of AREG on *Il7* reporter translation requires somatic cells. As an additional control, we used 3′ UTR reporter of *Bmp6*, whose polysome recruitment is constant during oocyte maturation (Fig. 1*D* and Dataset S1). After injection in COCs and consistent with the polysome data, *Bmp6* reporter translation did not change with oocyte maturation or supplementing the medium with AREG (Fig. 4*A*, *Inset*).

To confirm the above data, GV oocytes obtained from pregnant mare's serum gonadotropin (PMSG)-primed mice were matured in vitro, and the IL7 levels in the culture supernatant were measured

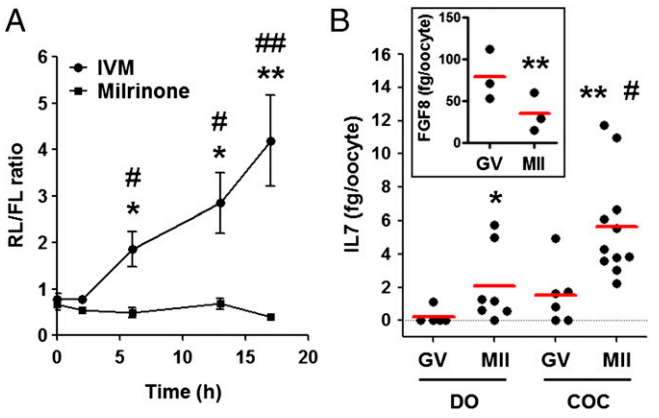

Fig. 3. *Il7* 3′ UTR reporter translation and IL7 protein secretion during oocyte maturation. (*A*) Ratio of renilla luciferase (RL) to firefly luciferase (FL) activity in oocytes cultured as COCs. Oocytes still in complex with cumulus cells were injected with two reporters, one coding for RL under the control of *IL7* 3′ UTR and one coding for FL with a polyadenylated 3′ UTR as the control. After 3 h of preincubation in the presence of milrinone (2 μM) to maintain the meiotic arrest, a group of injected COCs were washed free of inhibitor and in vitro matured (IVM) or kept in milrinone to block maturation. Both groups were incubated for 2, 6, 13, or 17 h. At the end of the incubation, oocytes were dissected free of cumulus cells, and luciferase activity was measured in oocyte extracts. Error bars correspond to the SEM, and data are from at least three experiments from biologically different samples. $^{*}P < 0.05$ vs. 0 h; $^{**}P < 0.01$ vs. 0 h; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. milrinone counterparts, *t* test and ANOVA. (*B*) IL7 protein levels in spent media during oocyte maturation. Denuded oocytes (DOs) and COCs were incubated with milrinone (2 μM) or in maturing media for 20 h; culture supernatant was collected, and IL7 levels were measured by ELISA. Error bars correspond to the SEM, and data are from at least three experiments from biologically different samples. $^{*}P < 0.05$ vs. DO GV; $^{**}P < 0.05$ vs. COC GV; $^{\#}P < 0.05$ vs. DO MII, ANOVA. (*Inset*) FGF8 secretion during oocyte maturation. COCs were incubated with milrinone (2 μM) or in maturing media for 20 h; culture supernatant was collected, and FGF8 levels were measured by ELISA. $^{**}P < 0.01$ vs. GV, paired *t* test.

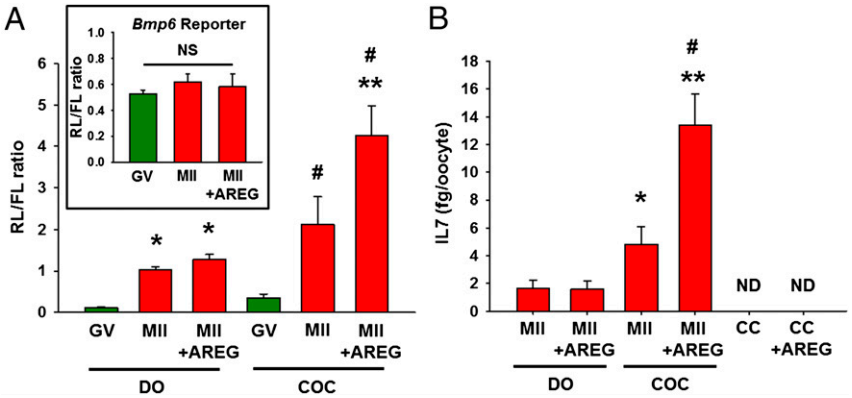

Fig. 4. Somatic cells regulate *Il7* mRNA translation and IL7 protein secretion. (*A*) Ratio of renilla luciferase (RL) to firefly luciferase (FL) activity in oocytes cultured as denuded oocytes (DOs) or cumulus oocyte complexes (COCs). Oocytes were injected with two reporters and cultured as detailed in Fig. 3 with or without amphiregulin (AREG, 100 nM) for 17 h. At the end of the incubation, luciferase activity was measured in oocyte extracts. Error bars correspond to the SEM, and data are from at least three experiments from biologically different samples. $^{*}P < 0.05$ vs. DO GV; $^{\#}P < 0.05$ vs. COC GV; $^{**}P < 0.05$ vs. COC MII, two way ANOVA. (*B*) IL7 protein levels in spent media during oocyte maturation. Denuded oocytes (DOs), COCs, and cumulus cells (CCs) were incubated in maturing media with or without amphiregulin (AREG, 100 nM) for 20 h; culture supernatant was collected, and IL7 levels were measured by ELISA. Error bars correspond to the SEM, and data are from at least three experiments from biologically different samples. ND, not detectable. $^{*}P < 0.05$ vs. DO MII; $^{**}P < 0.05$ vs. DO MII+AREG; $^{\#}P < 0.05$ vs. COC MII, ANOVA. (*A*, *Inset*) *Bmp6* 3′ UTR reporter translation during oocyte maturation. Ratio of RL to FL activity in oocytes cultured as COCs. Oocytes still in complex with cumulus cells were injected with two reporters, one coding for RL under the control of *Bmp6* 3′ UTR and one coding for FL with a polyadenylated 3′ UTR as the control. COCs were cultured as detailed in Fig. 3 with or without amphiregulin (AREG, 100 nM) for 17 h. At the end of the incubation, luciferase activity was measured in oocyte extracts. Error bars correspond to the SEM, and data are from at least three experiments from biologically different samples. NS, Not significant, two way ANOVA.

by ELISA. AREG enhanced IL7 secretion only in COCs, but not in DOs (Fig. 4*B*). This result confirmed that the stimulatory effect of AREG on IL7 protein secretion requires somatic cells. Non-detectable levels of secreted IL7 in cumulus cell-only cultures (Fig. 4*B*) with or without AREG treatment and at least 30-fold higher total *Il7* mRNA in oocytes compared with cumulus cells in the same treatment group (Fig. S2) are consistent with the view that IL7 measured in the culture supernatant is secreted only by the oocytes. After fertilization, IL7 secretion by the embryo ceases (Fig. S3*A*).

IL7 Enhances Cumulus Cell Proliferation During Oocyte Maturation. IL7 receptor is a heterodimer membrane receptor and has a specific α-chain (*Il7r*) and γ-chain (*Il2rc*) that is shared by the receptors for IL2 and IL4 (21). To determine whether IL7 secreted by the oocyte functions as part of a feedback, we investigated the expression of *Il7r* in cumulus cells during oocyte maturation. COCs obtained from PMSG-primed mice were matured in vitro, cumulus cells were stripped, and *Il7r* mRNA levels in cumulus cells were assessed by quantitative RT-PCR. The level of *Il7r* mRNA in cumulus cells increased with oocyte maturation and was further augmented with addition of AREG (Fig. 5*A*). Thus, during oocyte maturation, cumulus cells become more sensitive to the IL7 ligand.

Lastly, we determined whether ligation of the IL7 receptor on cumulus cells has a biological effect on these cells. IL7 is a growth factor that stimulates the proliferation of immune cells (21). During in vitro maturation of COCs obtained from PMSG-primed mice, cell replication measured as EdU incorporation was enhanced by recombinant IL7 and was attenuated by adding IL7 blocking antibody to the culture media (Fig. 5 *B* and *C*). Because *Il7r* mRNA levels were enhanced with AREG, all of the experiments were performed in the presence of this ligand. In contrast, the modulation of IL7 activity did not impact the cumulus cell expansion (Fig. S4 *A* and *B*). Moreover, blocking IL7 activity did not change the expression of *Has2* and *Ptgs2* mRNA in the cumulus cells, both transcripts necessary for cumulus expansion (Fig. S4*C*).

IL7 Levels in the Follicular Fluid Increase with Oocyte Maturation and Correlate with Follicular AREG Levels in Human. To investigate whether IL7 secretion increases with oocyte maturation in vivo in human as we have shown in the mouse in vitro, follicular fluid was obtained from a single dominant follicle before or after human chorionic gonadotropin (hCG) administration during controlled ovarian stimulation cycles. Eight samples taken before hCG administration were from follicles yielding GV oocytes and 42 samples obtained 36 h after hCG contained MII oocytes. IL7 levels were significantly higher in follicles containing MII oocyte (with normal fertilization) compared with those containing GV oocyte (Fig. 6*A*). Moreover, in egg donors, oocytes that were normally fertilized (two-pronuclei formation) after intracytoplasmic sperm injection had significantly higher follicular levels of IL7 compared with those with abnormal fertilization (Fig. 6*B*). Additionally, IL7 levels positively correlated with AREG levels in the follicular fluid ($P < 0.001$, $\sigma = 0.488$), suggesting that AREG may be the factor inducing IL7 secretion from COCs also in human (Fig. 6*C*). In the culture system, IL7 was detectable in human COC spent media (Fig. S3*B*). After fertilization, similar to mouse, IL7 secretion diminished in the human embryo (Fig. S3*B*).

Discussion

Communication between the oocyte and the surrounding somatic cells is critical for follicle growth, oocyte maturation, and ovulation of a developmentally competent egg. It is generally accepted that oocyte-secreted factors modulate the intrafollicular environment until the formation of a graafian follicle and cease during the periovulatory period. Here, we propose the novel concept that the pattern of oocyte protein secretion during the transition from transcriptionally quiescent oocyte in GV to an MII oocyte ready to be ovulated is instead highly dynamic. Our genome-wide analysis predicts changes in secretion of 125 proteins, including synthesis/secretion of extracellular matrix proteins, cytokines, chemokines, and growth factors. Regulated translation of the maternal mRNAs coding for these putative oocyte-secreted proteins is a molecular mechanism underlying this timed secretion. More importantly, we show that secretion is also sensitive to somatic cues, supporting the concept that these interactions are part of a feedback between the gamete and surrounding cumulus cells, crucial for oocyte development and survival. We propose that cumulus cells respond to ovulatory stimuli by sending signals that promote translation and

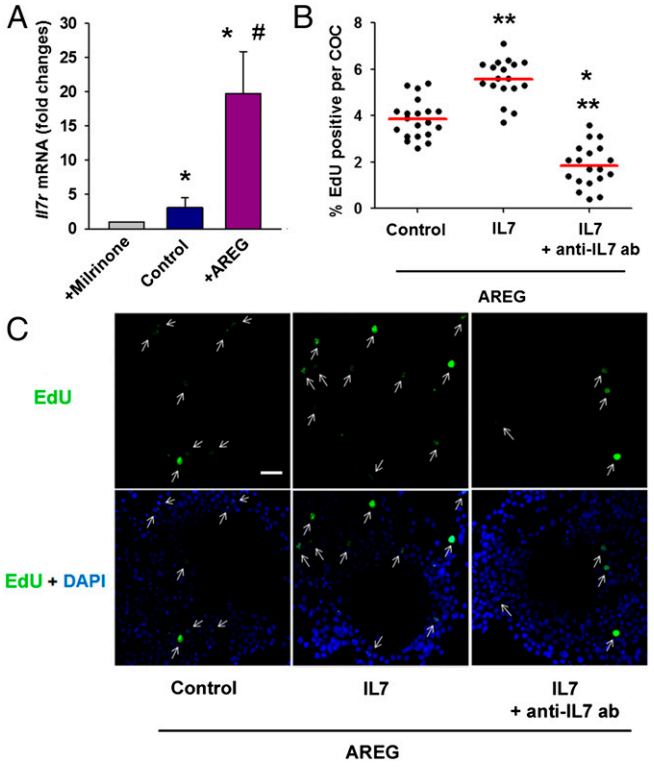

Fig. 5. IL7 enhances cumulus cell proliferation during oocyte maturation. (*A*) COCs obtained from PMSG-primed mice were matured in vitro, cumulus cells were stripped, and *Il7r* mRNA levels in cumulus cells were assessed by quantitative RT-PCR. $^{*}P < 0.05$ vs. maturing media + milrinone; $^{\#}P < 0.05$ vs. maturing media, ANOVA. Error bars correspond to the SEM, and data are from at least three experiments. Cumulus cell proliferation was measured as EdU incorporation during in vitro maturation of COCs with or without recombinant IL7 ± anti-IL7 blocking antibody. (*B*) Percentage of EdU-positive cells in individual COCs. $^{*}P < 0.05$ vs. IL7, $^{**}P < 0.05$ vs. control, ANOVA. The red line represents the average. (*C*) Representative COCs labeled with EdU (green) and nuclear stained with DAPI (blue). Arrows show EdU-positive cumulus cells. (Scale bar: 20 μm.)

secretion of bioactive molecules by the oocytes (Fig. S5). The secreted factors, in turn, act on cumulus cells, regulating their function, thus completing a feedback regulatory loop (Fig. S5). This timed oocyte secretion before ovulation likely modulates crucial molecular events in cumulus cells that are necessary for the development of a competent oocyte and are possibly necessary for communication with the other cellular environments the oocyte becomes in contact with after ovulation.

Our conclusions on dynamic translation are based on genome-wide data documenting polysome recruitment of mRNA coding for secreted protein increases or decreases during the transition from GV to MII. Using IL7 as a prototype of oocyte-secreted factors, we confirm that *Il7* mRNA is recruited to the polysomes during maturation and that the translation of reporter constructs containing the 3′ UTR of *Il7* mRNA increases three- to fivefold as the oocyte matures. The activation of translation of the endogenous *Il7* mRNA is confirmed by the finding that IL7 secretion is marginally detectable in GV oocytes and increases significantly when oocytes reach the MII stage. A similar pattern can be detected in the follicular fluid of human follicles before and after hCG stimulation in vivo. Of note, IL7 secretion becomes undetectable after fertilization, further supporting major fluctuations in the concentration of this interleukin in the environment surrounding the cumulus oocyte complex.

We established significant changes in mRNA association with the polysomes for 125 secreted proteins. These transcripts include proteins involved in the formation/maintenance of the extracellular matrix and the zona pellucida. The translation of all three major components of the zona (ZP1, -2, and -3) are greatly decreased during oocyte maturation, which is consistent with the view that the zona proteins accumulate during oocyte growth but that their synthesis ceases as the oocyte reenters the cell cycle. A classical prototype of translationally induced oocyte secretion is the tissue plasminogen activator PLAT, an endopeptidase that promotes fibrinolysis. In our survey, we found that other transcripts that regulate blood coagulation, such as *Tfpi* and *Serpine2*, show an increased polysomal association whereas several activators of blood coagulation and platelet degranulation decreased (*Cpb2*, *Calu*, *Scg3*). This observation, together with the finding that many of the changed transcripts are involved in the extracellular matrix organization, suggests that the oocyte actively participates in the remodeling of its surroundings to facilitate the ovulatory process. We also observed major changes in translation of growth factors that belong to the TGFβ superfamily whereas synthesis of FGF family members seems to be constitutive, except for *Fgf7 and Fgf8*. Specifically, an increase in *Bmp5* and decrease in *Tgfb3* mRNA translation, as well as a decrease in *Gdf9* and *Bmp15*, were detected. The decrease in *Gdf9* mRNA translation is consistent with a report of GDF9 protein concentration in porcine oocytes during maturation (22) and on the commonly held idea that production of these factors is decreased in MII oocytes (3). In addition to *Il7*, other transcripts encoding for molecules that have cytokine/chemokine activity, including *Csf1* and *Tnfsf11*, showed an increased polysome association. In contrast, other cytokine mRNAs, such as *Il34*, *Il23a*, and *Ik*,

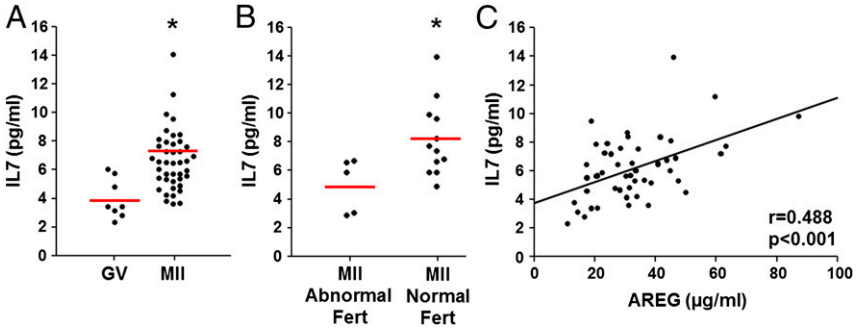

Fig. 6. IL7 in follicular fluid increases with oocyte maturation and correlates with follicular AREG levels in human. (*A*) IL7 levels in follicles yielding GV ($n = 8$) or MII ($n = 42$) oocyte. $^{*}P < 0.01$, Student *t* test. The red line represents the average. (*B*) Comparison of follicular levels of IL7 in follicles yielding oocytes with normal ($n = 12$) or abnormal ($n = 5$) fertilization after intracytoplasmic sperm injection $^{*}P < 0.05$, Student *t* test. (*C*) IL7 levels positively correlated with AREG levels in follicular fluid ($n = 50$). $P < 0.001$, $\sigma = 0.488$, Spearman's correlation test.

decreased their association with the polysomal fraction. Although the biological significance of these changes needs to be determined, it is likely that the surrounding somatic cells sense and adjust their function to support the maturing oocyte.

In humans, IL7 protein was detectable in follicles where oocytes are still at the GV stage. It is likely that IL7 from the circulation accounts at least in part for the detection in the follicular fluid. It is established that the follicular fluid is transudate from the plasma and to some extent in equilibrium with the circulation system (23). One could envisage that increased secretion by the oocyte before ovulation may lead to increased IL7 in the circulation. However, in our pilot experiments this increase was not detected.

We propose two mechanisms of regulation of translation/synthesis of secreted proteins. One mechanism is dependent on progression through the cell cycle and may involve regulation of RNA binding proteins such as cytoplasmic polyadenylation element binding protein 1 (CPEB1) or deleted in azoospermia-like (DAZL) (24). An additional mechanism involves somatic cell activation that indirectly leads to increased translation in the oocyte. Paracrine factors released at the time of ovulation, such as AREG, likely mediate this regulation. AREG induced activation of EGF receptor signaling in the cumulus cells, which indirectly activates phosphatidylinositol-3-phosphate-kinase (PI3K) in the oocyte and plays a role in the regulation of translation (19).

The importance of bidirectional communications between oocytes and follicular somatic cells for the acquisition of oocyte developmental competence is well-established (3, 25). Decreased proliferation and increased apoptosis in cumulus cells have been associated with immaturity of human oocytes, impaired fertilization (26), suboptimal blastocyst development, and poorer in vitro fertilization (IVF) outcomes (27). IL7 was previously shown to suppress apoptosis of cultured granulosa cells by activating the PI3K/AKT pathway (28). Here, we demonstrated that IL7 stimulates the proliferation of cumulus cells, providing additional evidence that timely oocyte secretion regulates cumulus cell function, and in turn, potentially improves its own developmental competence. It has been reported that a lower IL7 level in pooled follicular fluid samples is associated with lower egg/embryo quality and lower in vitro fertilization success rates (29). In the same vein, we have demonstrated that normally fertilized oocytes had significantly higher follicular levels of IL7 compared with those with no or abnormal fertilization in egg donors. These findings further support the concept of the oocyte determining its own fate by regulating its own microenvironment with secreted factors.

As an extension of the above concepts, noninvasive biomarkers monitoring the oocyte secretion pattern during oocyte maturation may be prognostic of the "fitness" of an oocyte to develop into an embryo that successfully implants and sustains pregnancy. Further studies are required to assess whether these OSFs can be used as a tool to select the most competent oocytes in assisted reproductive technologies.

Materials and Methods

Human follicular fluid samples and COC/embryo culture media were obtained from patients undergoing controlled ovarian stimulation/in vitro fertilization treatments at the University of California, San Francisco (UCSF) Center for Reproductive Health. The study was approved by the UCSF Committee on Human Research, and informed consent was obtained from all patients involved in this study. C57BL/6 female mice (22–24 d old) were used in animal experiments. All animal experiments were approved by the UCSF Animal Care and Use Committee.

Refer to *SI Materials and Methods* for the rest of the materials and methods.

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