Quantitative proteomics reveals the dynamics of protein changes during *Drosophila* oocyte maturation and the oocyte-to-embryo transition

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The onset of development is marked by two major, posttranscriptionally controlled, events: oocyte maturation (release of the prophase I primary arrest) and egg activation (release from the secondary meiotic arrest). Using quantitative mass spectrometry, we previously described proteome remodeling during *Drosophila* egg activation. Here, we describe our quantitative mass spectrometry-based analysis of the changes in protein levels during *Drosophila* oocyte maturation. This study presents the first quantitative survey, to our knowledge, of proteome changes accompanying oocyte maturation in any organism and provides a powerful resource for identifying both key regulators and biological processes driving this critical developmental window. We show that Muskelin, found to be up-regulated during oocyte maturation, is required for timely nurse cell nuclei clearing from mature egg chambers. Other proteins up-regulated at maturation are factors needed not only for late oogenesis but also completion of meiosis and early embryogenesis. Interestingly, the down-regulated proteins are predominantly involved in RNA processing, translation, and RNAi. Integrating datasets on the proteome changes at oocyte maturation and egg activation uncovers dynamics in proteome remodeling during the change from oocyte to embryo. Notably, 66 proteins likely act uniquely during late oogenesis, because they are up-regulated at maturation and down-regulated at activation. We find down-regulation of this class of proteins to be mediated partially by APC/C^{CORT}, a meiosis-specific form of the E3 ligase anaphase promoting complex/cyclosome (APC/C).

oocyte | embryo | meiosis | spindle | Muskelin

The change from oocyte to embryo marks the onset of development. Oocyte maturation is a prerequisite for the oocyte-to-embryo transition. In most animals, oocytes undergo a prolonged arrest in prophase I to permit oocyte growth, differentiation, and stockpiling of maternal components. This arrest is released at oocyte maturation, commonly in response to hormonal cues. The nuclear envelope then breaks down, followed by assembly of the meiotic spindle. A secondary meiotic arrest ensues, occurring at metaphase II in most vertebrates and metaphase I in insects (1). The oocyte-to-embryo transition initiates with egg activation, which causes the release of the secondary arrest and the completion of meiosis. In many organisms, egg activation requires fertilization, but egg activation is independent of fertilization in *Drosophila* (1). In all cases, the onset of embryogenesis requires sperm entry, fusion of the male and female pronuclei, and the start of the mitotic embryonic divisions.

How is this complex transition from differentiated oocyte into totipotent embryo regulated? Both oocyte maturation and egg activation occur in a transcriptionally silent context (2–4). These events are thus posttranscriptionally controlled, and translational regulation has been shown to play a crucial role. For example, resumption of meiosis in *Xenopus* depends on translational activation of *cyclin B* and *mos* mRNA by CPEB-mediated polyadenylation (4). Further work demonstrated that precisely timed translation of several other mRNAs is required for progression through the meiotic divisions (5, 6). Recent high-throughput studies highlight extensive translational changes accompanying oocyte maturation and egg activation in mice (7, 8). Moreover, genome-wide translational and quantitative proteomic analyses of *Drosophila* egg activation revealed that in addition to widespread translational regulation, posttranslational control, likely changes in protein stability, serve to remodel the proteome for the onset of embryonic development (9). At *Drosophila* egg activation, in addition to changes in protein levels, hundreds of proteins alter their phosphorylation status (10).

A comprehensive proteome analysis of oocyte maturation is lacking. Insights into protein changes accompanying *Xenopus*, murine, bovine, and porcine oocyte maturation have emerged from mass spectrometry analyses of 2D gels (11–16). In parallel, proteomic studies were conducted to semiquantitatively compare the protein composition of murine immature and mature oocytes (17). These approaches uncovered changes in factors as diverse as redox regulators, proteasome components, chaperones, and metabolic enzymes occurring during oocyte maturation (18). However, these studies lack the sensitivity, coverage, and quantitative information of currently available quantitative mass spectrometry

Significance

Oogenesis aberrations are an important cause of birth defects. During oocyte development, meiotic divisions are coordinated with intense transcription and translation, which produce maternally encoded RNAs and proteins that drive early embryonic development. Oocyte maturation and egg activation result in eggs competent for fertilization and embryogenesis, respectively, and these events occur in the absence of transcription or RNA degradation. Therefore, it is likely that changes in protein levels or activity govern these developmental transitions. By providing the first proteome-wide overview, to our knowledge, of the proteins whose levels change during oocyte maturation and combining these data with our previous description of proteome remodeling at egg activation, we describe critical events and factors driving the development of immature oocyte into an embryogenesis-competent egg.

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techniques, warranting a comprehensive study, which we decided to conduct in *Drosophila*.

Although much remains to be discovered about oocyte maturation in *Drosophila*, several regulators have already been recovered through genetic screens and biochemical approaches. The timing of maturation likely is controlled by keeping Cyclin B/CDK1 activity at bay through inhibition of Polo kinase by Matrimony (Mtrm) and downstream activation of the PP2A phosphatase inhibitor Endos (19–21). After a still elusive signal initiates maturation, an oocyte-specific cytoplasmic poly(A) polymerase, the product of the *wispy* gene, leads to extension of poly(A) tails (22, 23). This cytoplasmic polyadenylation results in increased protein levels of Mos and Cyclin B, two maturation regulators conserved in vertebrates, and the *Drosophila*-specific protein Cortex, an activator of the APC/C (22–25). It has been shown that thousands of mRNAs are polyadenylated in a *wispy*-dependent manner during *Drosophila* oogenesis and egg activation (22, 23, 26). However, the scale of consequent induction of translation and the number of up-regulated proteins during oocyte maturation remain unknown.

Here, we define the proteome changes accompanying maturation of the *Drosophila* oocyte by quantitative mass spectrometry. By combining these data with our previous study of egg activation, we define the patterns of proteome remodeling through the developmental window that starts with immature oocytes and ends with embryogenesis-competent activated eggs. This approach uncovered an interesting subset of proteins enriched from oocyte maturation until egg activation and likely composed of important regulators of the completion of oogenesis.

Results

Proteome Remodeling During *Drosophila* Oocyte Maturation. Oocyte maturation is a late event of oogenesis. *Drosophila* oogenesis unfolds through 14 morphologically distinct stages that can be recovered by dissection (Fig. 1*A*) (27). Recovered egg chambers are composed of three cell types: Germ-line–derived oocyte and nurse cells and somatic follicle cells. Nurse cells are connected to their sister oocyte via cytoplasmic bridges, and they support oocyte development by transcribing maternal mRNAs and synthesizing maternal proteins until stage 11 of oogenesis. At stage 11, the nurse cells transfer their cytoplasm to the oocyte in a process called dumping, and the nuclei die (28). Follicle cells encapsulate developing egg chambers and secrete the protective coverings of the oocyte (Fig. 1*A*).

To define the proteome changes accompanying *Drosophila* oocyte maturation, we compared protein levels in prematuration and postmaturation egg chambers by quantitative mass spectrometry. Because of the lack of consensus on whether oocyte maturation occurs during stage 12 or 13 (20, 29), we isolated as immature egg chambers, stage 11, which are unequivocally arrested in prophase I. As mature oocytes, we isolated stage 14, which have attained the secondary arrest point at metaphase I.

Peptides obtained after trypsin digestion of three independent preparations of stage 11 and stage 14 egg chambers were differentially labeled with stable isotopes via reductive methylation and further processed for mass spectrometry analysis. The peptide ratios were calculated from MS signal intensities of the differentially labeled peptide pairs by using MaxQuant software (30). The total number of proteins quantified in the three replicates ranged from 3,181 to 3,803. Based on our previous transcriptome analysis of this developmental window, which identified ~6,135 mRNAs as expressed, we estimate that our mass spectrometry measurements quantified ~50% of potentially encoded proteins (9). The ratios of protein levels at oocyte maturation between the three replicates were well correlated (Pearson $R = 0.55$–0.77) (Fig. 1*B*).

The proteome undergoes substantial remodeling at oocyte maturation. Approximately 30% of detectable proteins significantly change in levels: 536 proteins increase and 512 decrease (Dataset S1). Statistical analysis with the Limma package, and not protein ratios alone, was used to define these significantly changed proteins ($P < 0.05$; Fig. 1*C*). The even distribution of summed MS signal intensities for proteins significantly up-regulated (shown in magenta) and down-regulated (shown in blue) indicates that proteins across all detected abundances are affected (Fig. 1*D*). These protein changes were validated for selected candidates by immunoblots (*SI Results* and Fig. S1 *A* and *B*).

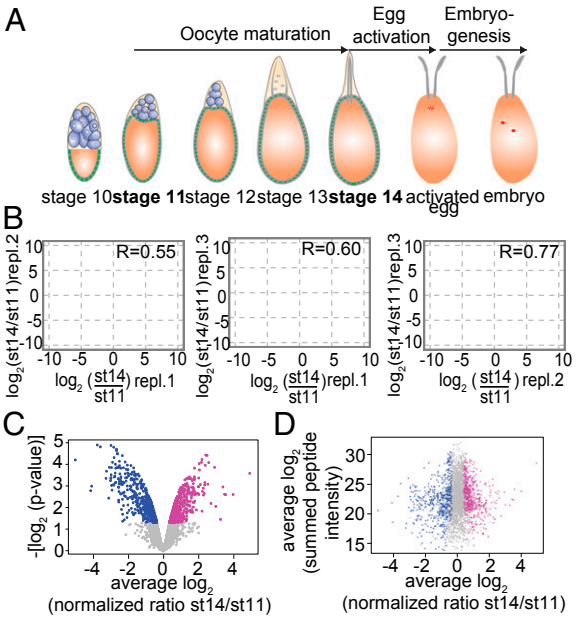

Fig. 1. Protein changes during oocyte maturation. (*A*) Schematic representation of *Drosophila* oocyte maturation, egg activation, and early embryonic development. The oocyte is shown in orange, nurse cells in blue, and follicle cells in green. Gray protrusions in stage 13 and 14 oocytes and embryos represent dorsal appendages in the egg shell. DNA (within polar body in the activated egg or nuclei in the embryo) is shown in red. The stages used in the quantitative mass spectrometry experiment are shown in bold. (*B*) Scatterplots showing the correlation between the $\log_2$ of normalized protein ratios for three biological replicates of experiments comparing protein levels in stage 11 (st11) versus stage 14 (st14) egg chambers. In *B*–*D*, Limma analysis (53) was used to define proteins significantly up-regulated (magenta), unchanged (gray), and down-regulated (blue) during oocyte maturation ($P < 0.05$). Pearson correlation is displayed on each graph. A total of 3,147, 2,879, and 2,759 data points are shown in total for *Left*, *Center*, and *Right*, respectively. (*C*) Volcano plot showing, for 3,477 proteins, *P* values ($-\log_2$) versus average of normalized protein ratios in stage 14 compared with stage 11 egg chambers in three replicate experiments. The color scheme is the same as for *B* with 536 proteins shown in magenta and 512 shown in blue. (*D*) Scatterplot showing a $\log_2$ of normalized ratios for 3,477 proteins (*C*) in stage 14 compared with stage 11 egg chambers (*x* axis) versus $\log_2$ of a summed peptide Intensity (*y* axis). The average of three biological replicates is represented.

We note that some of the protein changes during stages 11–14 of *Drosophila* oogenesis could occur in the nurse or follicle cells. Because nurse cell contents are dumped into the oocyte, the nurse cell and oocyte proteome can be viewed as shared. Examination of the transcript levels in follicle cells suggests that the majority of proteins identified as significantly up-regulated during oocyte maturation in our proteome measurements stem from the germ line and not follicle cells (*SI Results* and Fig. S2 *A* and *B*).

Functional Classes of Proteins Changed During Oocyte Maturation. We expected that proteins changing in levels during oocyte maturation would play important roles in oogenesis and, thus, are likely to be encoded by genes with a developmental pattern of maternal expression. Indeed, we observed that the mRNAs encoding the vast majority of proteins up-regulated in levels

during oocyte maturation are present predominantly in adult females and 0–2 h embryos (Fig. S3*A*). Although mRNAs encoding proteins down-regulated during maturation are abundant in adult females, their relative levels compared with other developmental stages are not as prominent in early (0–2 h) embryos as those for mRNAs encoding up-regulated proteins (Fig. S3*B*). The maternal expression pattern of both of these sets of mRNAs is a specific subset of the developmental expression pattern of all 15,577 *Drosophila* mRNAs (Fig. S3*C*).

We next identified biological processes and molecular functions characteristic of proteins down-regulated or up-regulated during oocyte maturation. Gene ontology (GO) analysis of the 512 proteins whose levels decrease during oocyte maturation revealed a significant enrichment of factors involved in ribosome biogenesis and RNA processing [false discovery rate (FDR) < 0.001, Fig. 2*A* and Dataset S1]. RNA helicases, known to support various aspects of the complex life of RNAs, also were enriched among proteins down-regulated during oocyte maturation (Fig. 2*A*). We hypothesize that the majority of these proteins are derived from nurse cells, which are engaged in RNA transcription and translation before oocyte maturation to provide the maternal mRNAs and protein for the oocyte. The levels of these proteins may decline after dumping as the nurse cell nuclei are degraded. Strikingly, the levels of six factors involved in the RNAi pathway (AGO1, ARMI, SpnE, Sqd, DCR-2, RM62, and MAEL) decrease during oocyte maturation, possibly linked to the absence of RNAi competence in mature *Drosophila* oocytes (31).

GO analysis of the 536 proteins whose abundance increases at oocyte maturation showed significant enrichment of categories consistent with the key biological events of resumption of meiosis (Fig. 2*B* and Dataset S1). Observed categories such as cell cycle, M phase, meiosis, nuclear division, and spindle and microtubule organization are noteworthy, because during maturation, the meiotic spindle assembles as the oocyte exits the primary arrest. Twenty-seven established microtubule-associated proteins including nine molecular motors increase in abundance, consistent with roles in microtubule reorganization into the meiotic spindle. Contained in this group are also CNN and at least four additional centrosomal proteins (Table S1 and Dataset S1). The second category of interest comprises proteins involved in chromosome architecture such as IPL (Aurora B, part of the Chromosome Passenger Complex), the Cohesin subunits SMC1 and SA, regulators of Cohesin loading (PDS5 and WAPL), and the Condensin subunits SMC2, CAP-D2, and CAP-H (Barren) (Table S1 and Dataset S1). These proteins are likely to contribute to proper meiotic chromosome segregation and could potentially also support chromosome segregation during subsequent mitotic embryonic divisions.

Factors required at egg activation also are up-regulated during maturation. This group of proteins includes components and regulators of the APC/C, such as CDC27, CDC16, and FZY, which promotes the metaphase-to-anaphase transition and, therefore, exit out of meiosis (Table S1 and Dataset S1). The subunits of the PNG kinase complex, which is a major regulator of translational changes occurring at egg activation (9), and Fs(1) Ya, a protein required for the first mitotic division (32), become elevated during oocyte maturation (Table S1 and Dataset S1).

Several proteins required for early embryogenesis, after egg activation, already become up-regulated during maturation. This observation was surprising because egg activation is in itself a transition characterized by prominent translational changes and proteome remodeling (9). Proteins involved in DNA replication, e.g., ORC1, DUP, Geminin, MCM2, MCM3, and MCM5-7, are up-regulated during maturation, most likely in preparation for the fast and frequent S phases during early embryonic cycles (Table S1 and Dataset S1). Furthermore, proteins involved in embryonic patterning, such as CACT, PAR-6, Dl, PUM, CRK, and CSW, increase in levels during oocyte maturation (Table S1 and Dataset S1).

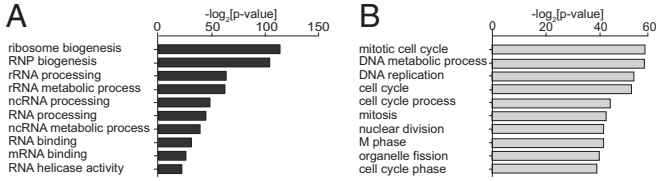

Fig. 2. GO analysis of proteins identified as changed in levels during oocyte maturation. (*A*) GO term categories for 512 proteins identified as significantly down-regulated during oocyte maturation. Top 10 GO categories with false discovery rate (FDR) $P < 0.001$ are shown. (*B*) Same as *A*, only GO term categories for 536 proteins identified as significantly up-regulated during oocyte maturation are shown.

Identification of New Regulators. Our proteomic analysis provides a valuable tool to identify regulators of late oogenesis or the oocyte-to-embryo transition. As a proof of principle, we investigated the role of an evolutionarily conserved, cytoskeletal C-terminal LisH and Kelch-beta-propeller domain-containing protein named Muskelin that is up-regulated during maturation. Muskelin was implicated in regulating adherens junctions in the *Drosophila* embryo epithelium (33). Interestingly, the founding Kelch domain protein is required for dumping because it is a pivotal constituent of ring canals between the oocyte and its sister nurse cells (34). To test whether Muskelin is required during late oogenesis, we used UAS-GAL4 to express RNAi against *muskelin* in the female germ line and successfully reduced its transcript levels (Fig. 3*A*).

The absence of *muskelin* did not visibly affect earlier stages of oogenesis, but resulted in persistence of nurse cell nuclei in stage 14 egg chambers. Approximately 65% of stage 14 egg chambers in the control lack nurse cell nuclei altogether or contain only one small, condensed nurse cell nucleus (Fig. 3 *B* and *C*). However, ∼79% of stage 14 egg chambers with *muskelin* RNAi contained at least one large nurse cell nucleus compared with ∼35% in the control (Fisher test $P < 0.0001$; Fig. 3 *B* and *C*). Despite the presence of nurse cell nuclei in stage 14 egg chambers depleted of *muskelin*, the oocyte nevertheless assumed the secondary metaphase I arrest. The metaphase I plate had a normal configuration in 98.6% of control stage 14 oocytes compared with 96.8% in *muskelin* RNAi ($n = 70$ and $n = 95$ stage 14 oocytes for control and *muskelin* RNAi, respectively, measured in the total of three replicates; paired t test $P = 0.41$).

The *muskelin* RNAi phenotype cannot be categorized as "dumpless," because these stage 14 oocytes reach the appropriate size, indicating the nurse cells released their contents into the oocyte. The average length of control stage 14 oocytes is 523.3 ± 31.8 μm, and *muskelin* RNAi stage 14 oocytes are on average 520.7 ± 28.7 μm ($n = 80$ and $n = 127$ stage 14 oocytes for control and *muskelin* RNAi, respectively, measured in the total of three replicates; Welch two sample t test $P = 0.06$). Although distinct from "dumpless" mutants, the effect of *muskelin* RNAi on the mature egg chambers is comparable to the loss of apoptotic caspases or autophagy players (35). Loss of Muskelin may cause a delay rather than a complete block of nurse cell nuclear degradation, as the number of persisting nurse cell nuclei is reduced in *muskelin* RNAi-treated females with aged mature egg chambers. Eggs resulting from *muskelin* RNAi treatment are fertile, producing viable embryos.

Dynamics of Protein Levels During the Oocyte-to-Embryo Transition. We previously delineated the protein changes during *Drosophila* egg activation, a developmental stage following oocyte maturation. This analysis revealed 365 and 291 proteins as significantly down-regulated and up-regulated, respectively (9). Here, we merge our data on protein changes during consecutive developmental transitions of oocyte maturation and egg activation to gain insight into

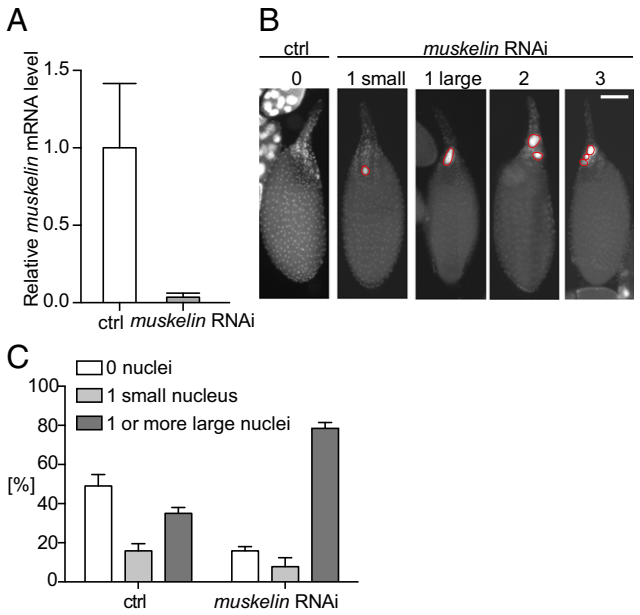

Fig. 3. *muskelin* RNAi leads to the persistence of nurse cell nuclei in stage 14 egg chambers. (*A*) Real time quantitative reverse transcription-PCR (qRT-PCR) analysis of *muskelin* mRNA levels in stage 14 egg chambers dissected from control females (progeny of cross between *maternal-αtubulin-Gal4* driver and wild-type) or *muskelin* germ-line RNAi females (progeny of cross between *maternal-αtubulin-Gal4* driver and *BL51405 muskelin* RNAi line). *muskelin* mRNA levels in the control are set to 1. *Actin5c* was used for normalization. Error bars indicate the range of expression levels measured (minimum and maximum) for triplicate reactions performed within one representative qRT-PCR experiment. (*B*) Representative images of DAPI-stained stage 14 egg chambers. The first image (control) shows stage 14 egg chambers with no nurse cell nuclei, and the remaining images show stage 14 egg chambers where *muskelin* is knocked down by germ-line–specific RNAi. Nurse cell nuclei are encircled with red lines. Nurse cell nuclei designated as small appear as small round fragments and often do not stain brightly with DAPI. (Scale bar: 100 μm.) (*C*) Quantification of categories shown in *B* in stage 14 egg chambers dissected from control females or females with germ-line knockdown of *muskelin* (n = 186 in control and 193 stage 14 egg chambers in *muskelin* RNAi). Shown is mean ± SD from three independent experiments. Comparison of the number of stage 14 egg chambers that have zero nurse cell nuclei to those that have one or more large cell nuclei shows a highly statistically significant difference (Fisher test $P < 0.0001$).

the dynamics of protein expression during this crucial window of development.

We found that subsets of proteins show opposite changes in abundance at maturation versus activation, whereas others exhibit synergistic changes. Twenty-three proteins are down-regulated during oocyte maturation but increase at egg activation (Fig. 4*A* and Dataset S2). Their functions may interfere with meiotic progression, but the proteins may be needed for early embryogenesis. For example, the RNAi components Spn-E and RM62 fall into this class, coincident with the reappearance of RNAi competence (31).

There are 43 proteins up-regulated both during oocyte maturation and egg activation (Fig. 4*A* and Dataset S2). Among them are proteins involved in DNA replication, such as several ORC and MCM subunits, DNApol-δ, DNApol-ε, RnrL, and RnrS. After a prolonged period without DNA replication during meiosis, a supply of these proteins may be required in preparation for the rapid S phases that occur in 4 to 5 min during the first 13 early embryonic cycles. In contrast, the levels of 78 proteins decreased during oocyte maturation are further decreased at egg activation (Fig. 4*A* and Dataset S2). Low levels of these proteins may be needed for meiosis and early embryogenesis.

A notable pattern is that the level of 66 proteins increases significantly during oocyte maturation but decreases at egg activation (Fig. 4*A* and Dataset S2). The restricted expression of these proteins is consistent with key roles in late oogenesis. Therefore, we investigated mechanisms responsible for the decrease in levels for this class of proteins at egg activation. The degradation of proteins at egg activation is, at least partially, mediated by the APC/C and its activator CORT (24, 36). Interestingly, as previously shown, CORT protein itself increases in levels at maturation and decreases at egg activation (24). Moreover, at egg activation, CORT was shown to be required for proper down-regulation of one of the proteins in our subset of 66, CycB3 (24, 36).

To determine whether CORT targets include proteins in the subset of 66, we compared protein levels in wild-type versus *cort* mutant-activated eggs by quantitative mass spectrometry in two independent replicates (Dataset S3). Our analysis showed that among the proteins that are normally down-regulated at egg activation, the levels of only about 22% (42 proteins) remain higher in *cort* than in wild-type–activated eggs (Fig. 4*B*). Of the 66 proteins of interest, 44 also were detected in the *cort* dataset (Fig. 4*C*). Of these 44 proteins, ~40% have higher levels in *cort* versus wild-type eggs, suggesting that they may be APC/C^{CORT} substrates (Fig. 4*C*). This analysis indicates that CORT has a limited and selected set of substrates during egg activation, with a slight preference for substrates (Fisher test P = 0.0008) that are present only during the window between oocyte maturation and egg activation.

Discussion

Defining the proteome changes during oocyte maturation is a means to identify candidate regulators required for late

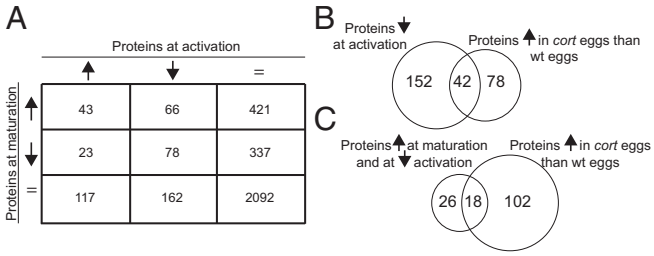

Fig. 4. Protein levels dynamically change during the oocyte-to-embryo transition, partly dependent on the female meiosis-specific APC/C activator, CORTEX. (*A*) Table showing the number of proteins that belong to any of the possible nine categories: increase, decrease, or remain unchanged in levels during maturation and subsequently increase, decrease, or remain constant at egg activation. Only the proteins whose levels are quantified both during maturation and activation are presented. Consequently, although 536 proteins are up-regulated at maturation, 530 of these were quantified in the sample comparing protein levels during egg activation (shown in the three cells at *Top*). Similarly, of 512 proteins down-regulated at maturation, 448 were detected at activation and presented in *Middle*. (*B*) Venn diagram showing the overlap between the proteins statistically down-regulated in levels at egg activation and statistically higher in *cort* eggs versus wild-type eggs. Presented are only the proteins whose levels are quantified in all three samples: during maturation, activation, and in comparison of protein levels in *cort* mutant versus wild-type activated eggs. For example, of 365 proteins quantified as down-regulated at egg activation (9), 306 were also detected at maturation (middle column of *A*), and 194 of these 306 proteins were also quantified in the sample comparing protein levels in *cort* versus wild-type activated eggs (left portion of the Venn diagram). (*C*) Same as *B* with the exception that shown Venn diagram presents the overlap between the proteins up-regulated during maturation and subsequently down-regulated at activation with proteins whose levels are higher in *cort* eggs than wild-type eggs. From 66 proteins identified as up-regulated at maturation and down-regulated at activation (*A, Top Middle*), 44 were quantified in the sample comparing protein levels in *cort* versus wild-type activated eggs and presented in the left portion of the Venn diagram.

oogenesis, egg activation, and early embryogenesis. Moreover, it serves to highlight different requirements for important molecular functions and biological processes, such as RNAi, spindle assembly, cell cycle regulation, chromatin organization, transport of molecules, proteolysis, and changes in redox state during this remarkable developmental transition. It is striking that among ~500 proteins we found as significantly down-regulated between stages 11 and 14, there is a significant enrichment of factors involved in RNA processing, translation, or RNAi. This down-regulation may reflect the cessation of the factory function of nurse cells in stockpiling the oocyte with nutrients, proteins, and organelles. Proteins that play an integral role in meiotic divisions are among ~530 factors whose abundance significantly increases during oocyte maturation. This group includes microtubule motors and other components of the spindle, cell cycle proteins, and proteins such as Cohesins and Condensins that regulate chromosome architecture. Studies performed in mouse similarly identified proteins involved in spindle assembly as increased in levels during oocyte maturation (13). The inhibitor of the APC, Rca1, is up-regulated at maturation, possibly facilitating the secondary arrest at metaphase I.

In addition to proteins involved in the resumption of meiosis, proteins necessary for the completion of meiosis also were up-regulated. Interestingly, meiotic exit occurs only during the subsequent developmental window, egg activation. In contrast to egg activation, which occurs during a brief 20-min window (37), oocyte maturation lasts for about 2 h (27), and the secondary arrest in metaphase I can be extended to several days. We speculate that this elongated time period permits proteins required for the meiotic divisions to be newly translated, whereas the proteins needed for egg activation need to be present already at its onset. Surprisingly, during maturation, the oocyte already gears up for the rapid rounds of embryonic DNA replication and mitosis that only occur after egg activation. Proteomic analysis of oocyte maturation in the mouse also indicated that this developmental window serves as preparation for embryogenesis (13, 17, 18).

Unexpectedly, we found that about 18 transmembrane transporters, including a xenobiotic and several oligopeptide transporters, are up-regulated during *Drosophila* oocyte maturation. Their possible function in oogenesis is intriguing. They may establish communication with follicle cells, given the importance of follicle cells in relaying information to the oocyte during maturation recently shown in the mouse (38). In the case of *Drosophila*, however, a vitelline membrane separates the oocyte and the follicle cells (39). Given that the vitelline membrane does not become cross-linked until egg activation (1), the transporters could take up amino acids and other cargo provided by the follicle cells.

A striking similarity between oocyte maturation in *Drosophila* and several vertebrate species is the increase in levels of several proteins that control redox state, such as thioredoxin (12, 15, 16). The thioredoxin DHD previously was shown to be required for exit from meiosis and proper early embryonic development in *Drosophila* (40). In mouse, egg activation, i.e., fertilization, marks the onset of changes to the energy source used by the egg, likely resulting in a need for the observed change in levels of redox homeostasis regulators (41). These proteins additionally could maintain *Drosophila* oocyte health by controlling the amount of oxidative damage present in the oocyte, as demonstrated in mammals (42). These conserved pathways may reveal essential processes that must occur during oocyte maturation in an array of organisms.

The power of our approach in uncovering previously unidentified regulators of the oocyte-to-embryo transition has been confirmed by the functional analysis of a Kelch-beta propeller and C-terminal LisH domain-containing protein Muskelin. We found Muskelin to be up-regulated in levels during oocyte maturation and required for timely clearing of nurse cell nuclei from mature egg chambers, most likely independently of nurse cell dumping. It still remains to be discovered how Muskelin contributes to this aspect of egg chamber remodeling during late oogenesis. Phagocytosis by follicle cells is thought to be responsible for the removal of apoptotic fragments of nurse cell nuclei (43), and phagocytosis is a process that often depends on actin fibers (44). Interestingly, human Muskelin was described as a mediator of actin cytoskeleton reorganization in response to the cell-cell or cell-matrix interaction factor, thrombospondin I (45). Therefore, the delay in nurse cell nuclei loss upon germ-line knockdown of *muskelin* could be attributed to perturbation of the interactions between germ-line and follicle cells or disruption of the actin cytoskeleton, resulting in diminished or delayed phagocytosis of the nurse cell nuclei remnants.

Our integration of quantitative analyses of proteome changes during oocyte maturation and egg activation in *Drosophila* is, to our knowledge, the first description of the dynamics of proteome remodeling during the oocyte-to-embryo transition. We observed that a substantial fraction of proteins experience a change in protein levels first during oocyte maturation and again during egg activation. A notable class of about 60 proteins increases at oocyte maturation but declines markedly at egg activation, a profile consistent with these proteins being important for the completion of oogenesis and meiosis but needing to be removed before the onset of embryogenesis and mitosis. The presence of factors known to be required for a successful oocyte-to-embryo transition, such as DHD (thioredoxin) and the PNG kinase-activating subunit GNU, shows that this list highlights important regulators of this crucial developmental transition.

We previously demonstrated that an oocyte-specific form of the APC/C, APC/C^{CORT}, arises after oocyte maturation (24). Furthermore, we previously showed that at least one of its substrates, the Polo kinase inhibitor Matrimony, must be removed for proper embryogenesis (46). By defining the proteome of *cortex* mutant eggs, we identified proteins dependent on the CORT form of the APC for removal at egg activation. Some, but not all, of the proteins present solely during the maturation window require CORT for their decline. Thus, it is likely that multiple proteolysis mechanisms contribute to removal of proteins at egg activation. Indeed, it is striking that ~15 peptidases as well as several members of ubiquitination pathways and several proteasome components increase in abundance at oocyte maturation. This change in levels of components of the proteolytic machinery may be a conserved feature of oocyte maturation, because a smaller-scale study in mouse also reported an increase in levels of a proteasome component during oocyte maturation (14). The presence of peptidases in our dataset also is intriguing, because in *Caenorhabditis elegans*, peptidases such as the aminopeptidase PAM-1 were shown to mediate completion of meiosis (47).

Our unbiased, quantitative definition of the proteome changes accompanying oocyte maturation has provided key insights into the critical developmental transition from oocyte to embryo. The protein changes highlight the induction of spindle, cell cycle, chromosome architecture, DNA replication, and proteolytic components that play integral roles not only in the completion of meiosis but also in the early embryonic divisions. Thus, oocyte maturation both puts the proteins in place to complete oogenesis while setting the stage for embryogenesis. In addition, this study presents a set of candidate regulators of the oocyte-to-embryo transition. Our model system, *Drosophila*, will provide a powerful toolkit to delineate the function of these proteins.

Materials and Methods

***Drosophila* Stocks.** *Oregon R* (OrR) was used as a wild-type control. The $cort^{RH65}$ allele has been described (48). Flies were kept at 22 °C or 25 °C according to standard procedures (49). The *muskelin* RNAi line (BL51405) from the Transgenic RNAi project (TRiP) and the *maternal-tubulin-Gal4 P{matα4-GAL-VP16}V37* driver were obtained from the Bloomington Drosophila Stock Center.

Quantitative Mass Spectrometry. Egg chambers were hand-dissected in Grace's Unsupplemented Insect Media (Gibco) from 3-d-old flies fattened for 2 d with wet yeast at 22 °C. Comparison of the *cort* mutant versus wild-type proteome

was done by collecting activated eggs from *cn cort*^{RH65} *bw* females (mated to *cn cort*^{RH65} *bw* males and/or *OrR* males) and from *OrR* females (mated to sterile *twine*^{HB5} males) as in ref. 9. Samples were lysed and extracts prepared as described (9). Digestion of the proteins and stable isotope labeling of the peptides (peptide dimethylation) were performed as described (50, 51). Mass spectrometry and statistical analyses were as detailed in Kronja et al. (9). Several follicle cell proteins were removed from the list of identified proteins before proceeding with the statistical analysis and are listed in a separate sheet of Dataset S1.

Quantitative PCR. To measure the efficacy of *muskelin* RNAi, total RNA was isolated from mature oocytes by homogenizing them in TRIzol (Invitrogen) according to manufacturer's instructions. These mature oocytes were dissected from females that were the progeny of the following crosses: *maternal-tubulin-Gal4* driver virgin females mated either to *OrR* males (control) or *muskelin* RNAi (BL51405) males. Synthesis of cDNA and quantitative PCR (qPCR) were performed as described in ref. 9. The sequences of *muskelin* and *actin5c* primers that were used for qPCR normalization are available upon request.

Immunofluorescence of Egg Chambers. Ovaries were hand-dissected in Grace's Unsupplemented Insect Media (Gibco) from 3-d-old flies fattened for 2 d with wet yeast at 25 °C. Then they were fixed and stained with DAPI as described, with the exception that the fixation was done in 4% (vol/vol) formaldehyde in Grace's unsupplemented insect medium (52).

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