The role of TLF in mammalian spermatogenesis contrasts to its function in *Caenorhabditis elegans*, *Xenopus*, and zebrafish (*29*–*31*), where TLF appears to be critical in early embryogenesis—possibly acting as a surrogate of TBP at specific promoters whose expression is required at the onset of zygotic transcription in the developing embryo.

Specificity of Activation

The molecular and functional connections between the general transcription machinery and cell-specific activators have been extensively studied in somatic cells. Many genes activated postmeiotically contain CREs (cAMP-responsive elements), which recruit members of the CREB family of transcription factors (*32*).

In somatic cells, CREB binds to CREs and activates transcription when it becomes phosphorylated. The event of phosphorylation triggers the recruiting of a large coactivator, CBP (CREB-binding protein). CBP has a dual function. It contacts other elements of the transcription machinery and acetylates histones, and thus possibly contributes to chromatin decondensation events that precede transcription (*33*).

CREB is poorly expressed in testis. Instead, another member of the CREB family, CREM, is present at very high levels. In germ cells, CREM interacts with TFIIA and selected TAFs of the TFIID complex. CREM was thought to play a critical role during the postmeiotic transcriptional phase. Indeed, CREM-null mice display a complete block of the differentiation program at the first step of spermiogenesis (*32*). Thus, CREM appears to directly influence the fate of male germ cells. As TLF interacts with TFIIA, which interacts with CREM, a germ cell–specific complex appears to operate (Fig. 2). It is noteworthy that mice lacking either CREM or TLF display phenotypic similarities, including an important increase in germ cells apoptosis.

The complex including CREM has additional unique features. CREM does not seem to be phosphorylated in germ cells, and thus the classical signaling-dependent mechanism that uses CBP is not operating. Instead, CREM is activated by a tissue-specific coactivator, the LIM-only protein ACT, whose expression is exquisitely restricted to male germ cells (*34*). The study of the mechanism by which ACT operates is likely to reveal some important features of gene regulation in male germ cells.

Conclusion

The developmental process of spermatogenesis is governed by a unique genetic and molecular program. A fine-tuning of the regulatory mechanisms devoted to the differentiation of male germ cells is essential, because errors at any level could have dramatic consequences for the maintenance of the species. It is essential that we pursue a deeper understanding of the molecular processes regulating gene expression during spermatogenesis, as it will be highly valuable for biomedical and therapeutic work.

References and Notes

1. W. F. Crowley *et al.*, *Rec. Progr. Horm. Res.* **47**, 27 (1991).
2. K. C. Kleene, *Mech. Dev.* **106**, 3 (2001).
3. P. Sassone-Corsi, *Cell* **88**, 163 (1997).
4. E. M. Eddy *et al.*, *Curr. Top. Dev. Biol.* **37**, 140 (1998).
5. N. B. Hecht, *Bioessays* **20**, 555 (1998).
6. P. Cheung *et al.*, *Cell* **103**, 263 (2000).
7. B. M. Turner, *Bioessays* **22**, 836 (2000).
8. T. Jenuwein *et al.*, *Science* **293**, 1074 (2001).
9. M. L. Meistrich, in *Histones and Other Basic Nuclear Proteins*, L. Hnilica, G. S. Stein, J. L. Stein, Eds. (CRC Press, Boca Raton, FL), pp. 165–182.
10. N. C. Mills *et al.*, *Biol. Reprod.* **17**, 760 (1977).
11. Y. E. Yu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4683 (2000).
12. M. Zhao *et al.*, *Mol. Cell. Biol.* **21**, 7243 (2001).
13. K. Lee *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 12451 (1995).
14. A. R. Meetei *et al.*, *Biochemistry* **41**, 185 (2002).
15. H. P. Roest *et al.*, *Cell* **86**, 799 (1996).
16. J. Y. Wu *et al.*, *Nature Genet.* **25**, 448 (2000).
17. D. K. Palmer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 3734 (1991).
18. E. E. Schmidt *et al.*, *Development* **121**, 2373 (1995).
19. A. B. Upadhyaya *et al.*, *J. Biol. Chem.* **274**, 18040 (1999).
20. J. Ozer *et al.*, *J. Biol. Chem.* **275**, 122 (2000).
21. M. A. Hiller, T. Y. Lin, C. Wood, M. T. Fuller, *Genes Dev.* **15**, 1021 (2001).
22. P. J. Wang *et al.*, *Nature Genet.* **27**, 422 (2001).
23. M. A. Handel *et al.*, *Cytogenet. Cell Genet.* **66**, 83 (1994).
24. A. J. Solari, *Int. Rev. Cytol.* **38**, 273 (1974).
25. N. Ayoub *et al.*, *Chromosoma* **106**, 1 (1997).
26. D. Zhang *et al.*, *Science* **292**, 1153 (2001).
27. I. Martianov *et al.*, *Mol. Cell* **7**, 509 (2001).
28. I. Martianov *et al.*, *Development* **129**, 945 (2002).
29. C. Dantonel *et al.*, *Mol. Cell* **6**, 715 (2000).
30. F. Muller *et al.*, *Curr. Biol.* **11**, 282 (2001).
31. G. J. C. Veenstra *et al.*, *Science* **290**, 2312 (2000).
32. P. Sassone-Corsi, *Semin. Cell Dev. Biol.* **9**, 475 (1998).
33. B. Mayr *et al.*, *Nature Rev. Mol. Cell. Biol.* **2**, 599 (2001).
34. G. M. Fimia *et al.*, *Nature* **398**, 165 (1999).

35. I apologize to all colleagues whose work could not be cited because of space limitation. I thank M. Parvinen, I. Davidson, D. Morse, and L. Monaco for valuable comments on the manuscript; S. Metz for help with the preparation of the figures; and all members of my laboratory for discussions.

REVIEW

Intercellular Communication in the Mammalian Ovary: Oocytes Carry the Conversation

Martin M. Matzuk,[1,2,3]* Kathleen H. Burns,[1,2] Maria M. Viveiros,[4] John J. Eppig[4]

The production of functional female gametes is essential for the propagation of all vertebrate species. The growth of oocytes within ovarian follicles and their development to mature eggs have fascinated biologists for centuries, and scientists have long realized the importance of the ovarian follicle's somatic cells in nurturing oogenesis and delivering the oocyte to the oviduct by ovulation. Recent studies have revealed key roles of the oocyte in folliculogenesis and established that bidirectional communication between the oocyte and companion somatic cells is essential for development of an egg competent to undergo fertilization and embryogenesis. The challenge for the future is to identify the factors that participate in this communication and their mechanisms of action.

Germ cells are uniquely specialized to transmit the genome to succeeding generations. In animal species, sexual reproduction requires meiotic division to produce haploid gametes (i.e., eggs and spermatozoa), which upon fertilization give rise to the totipotent embryo. In both sexes, interactions between the developing gametes and neighboring somatic cells are crucial for fertility (*1*). The importance of this communication in spermatogenesis is underscored by clinical cases of male infertility, transgenic mouse models, and xenogeneic germ cell transplantation experiments (*1, 2*). Similarly, in females, complex intercellular dialogs have evolved to regulate oogenesis in species as wide-ranging as fruit flies (*3*) and mice.

In the mammalian perinatal ovary, oocytes arrested in the diplotene stage of meiosis I become surrounded by a single, squamous layer of somatic cells to form a finite population of nongrowing primordial follicles (*4*) (Fig. 1). Primary follicles are recruited from the primordial pool as oocytes grow and the surrounding somatic cells (called granulosa cells) become cuboidal and proliferative. This transition is associated with a

commitment to subsequent stages of follicular development, and the measured recruitment of primordial follicles from the resting pool is critical for the continuity of folliculogenesis throughout the reproductive life-span of all mammals.

In the mouse, an initial synchronous wave of follicular recruitment occurs within a few days of birth. By 10 to 12 days of postnatal life, a cohort of secondary-stage follicles develops, in which oocytes at midgrowth are surrounded by two or more layers of granulosa cells. Antral-stage follicles form between 14 and 24 days when fluid-filled cavities develop between the layers of somatic cells. Antrum formation subdivides the granulosa cells into two spatially and functionally distinct populations: Those with closest proximity to the oocyte are called the cumulus granulosa cells, and those lining the follicle wall are mural granulosa cells. During the preantral to antral follicle transition, the oocyte acquires the capacity to resume meiosis (*5*), and epigenetic modifications essential for fetal development are progressively established (*6*). Meiotic competence is associated with the accumulation of cell cycle regulatory factors (*7*), as well as reorganization of chromatin and microtubule configurations (*8*). The luteinizing hormone (LH) surge promotes substantial changes in gene expression in preovulatory granulosa cells (*9*) and indirectly stimulates oocyte meiotic maturation and ovulation of a metaphase II–stage egg that is competent to undergo fertilization.

Commencing with follicle formation and continuing throughout folliculogenesis, complex bidirectional communication between each oocyte and its surrounding somatic cells is essential for the coordinated development of both germ cell and somatic cell compartments (*10*). For example, defects in meiotic maturation are evident in mice lacking the granulosa cell oocyte junction protein connexin 37 (*11*). Although it has long been recognized that somatic cells support oocyte development, compelling evidence of the oocyte's active role in folliculogenesis as well as early embryogenesis has emerged primarily within the last decade. Oocytes promote granulosa cell proliferation and differentiation (*10*). In turn, the oocyte depends on somatic cells to support its growth and development (*12*), regulate meiosis (*13*), and modulate global transcriptional activity in the oocyte genome (*14*).

The crucial role of the oocyte in regulating the progression of follicular development was recently illustrated in experiments that exchange germ and somatic cell components of follicles at different stages of development (*15*). Oocytes at midgrowth were isolated from secondary-stage follicles of 12-day-old mice and combined with somatic cells from primordial follicles of newborn ovaries to produce reaggregated ovaries, which we refer to as (12/0) ovaries in Fig. 2. As compared to control reaggregated oocytes and somatic cells of newborn mice (0/0), 12-day oocytes accelerated follicle development, resulting in ovaries with large antral-stage follicles within 9 days (Fig. 2). These accelerated follicles contained granulosa cells that expressed functional characteristics of normal preovulatory follicles; furthermore, the resident oocytes were able to resume meiosis and undergo fertilization, producing embryos capable of development to the blastocyst stage. These experiments underscore the pivotal functions of the oocyte in mammalian folliculogenesis and suggest that a developmental program, intrinsic to the oocyte, controls the rate of follicle development in neonatal mice. If there is a "folliculogenesis clock," it appears to be set by the oocyte, at least in immature mice. Oocyte factors that participate in relaying communications to surrounding somatic cells are indicated in Fig. 1.

Oocyte-derived factors direct initial follicle formation in the newborn ovary, and knockout mice lacking factor in the germline α (FIGα), an oocyte-specific helix-loop-helix transcription factor, fail to develop primordial follicles (*16*). Factors that trigger the development of primary (one-layer) follicles are unknown, although somatic cell–derived anti-Müllerian hormone (AMH) (*17*) and activins (*18*) are implicated in the regulation of this process (Fig. 1).

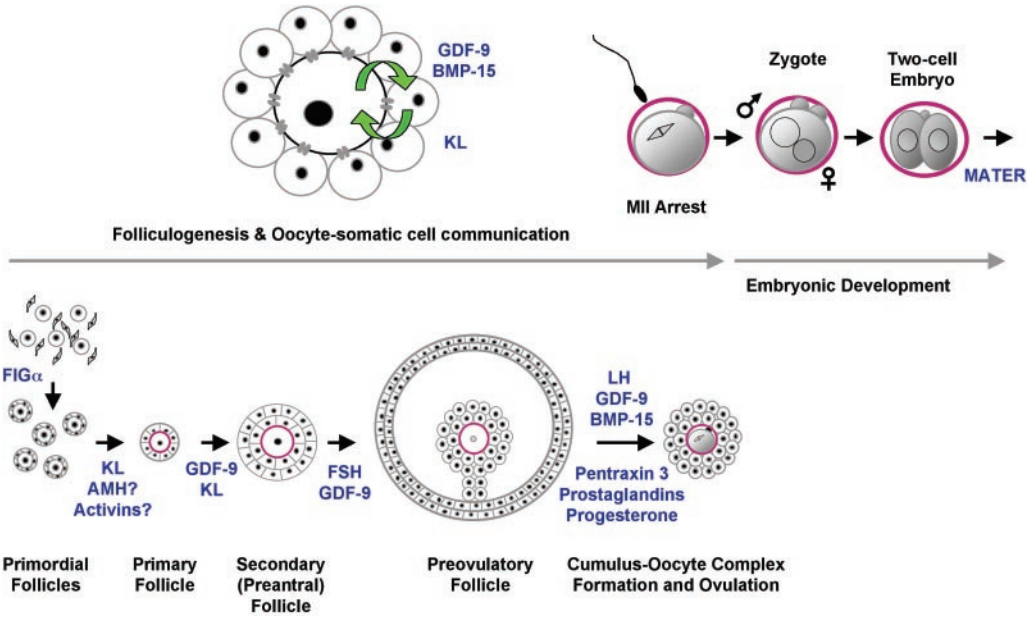

Fig. 1. The progression of folliculogenesis, oogenesis, and early embryonic development. Communication between oocytes and their associated somatic cells is established with primordial follicle formation. Granulosa cells proliferate during the ensuing stages of folliculogenesis, and oocyte–granulosa cell communication mediated by secreted paracrine factors and gap junctions is essential for the developmental progression of follicles. After the LH surge and immediately before COC release into the oviduct, meiosis I resumes, beginning with the dissolution of the nuclear membrane; this is termed "germinal vesicle breakdown." After fertilization, meiosis II is completed and male and female pronuclei undergo chromatin restructuring. Appropriate oocyte–somatic cell interactions during folliculogenesis are essential for female gametogenesis and likely have enduring effects on the potential for embryonic development after fertilization. Several factors (described in the text) function during specific stages in this progression.

Bidirectional signaling between oocytes and surrounding somatic cells is integral for the progression of preantral follicle development beyond the primary follicle stage. Studies in mouse models have identified specific protein participants in this oocyte–granulosa cell regulatory loop and revealed essential roles for these factors in folliculogenesis and oocyte development alike. Examples include (i) growth differentiation factor–9 (GDF-9), a transforming growth factor–β (TGF-β) superfamily member secreted by oocytes; (ii) KIT receptor, expressed on the oocyte surface; and (iii) KIT ligand (KITL), which is derived from granulosa cells. Mice homozy-

[1]Department of Pathology, [2]Department of Molecular and Human Genetics, [3]Department of Molecular and Cellular Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA. [4]The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA.

*To whom correspondence should be addressed. E-mail: mmatzuk@bcm.tmc.edu

gous for a null allele at the *Gdf9* locus (*19*) or for a hypomorphic *Kitl* allele (*20*) exhibit female infertility as a result of blocks in follicular development preceding formation of secondary follicles (Fig. 1). If defects in granulosa cell proliferation and differentiation in the *Gdf9* knockout ovary demonstrate the importance of the oocyte to follicular cell functions, oocyte abnormalities observed in this mouse model underscore the reciprocal—the necessary roles of somatic cells in oogenesis. In the absence of GDF-9, there is increased granulosa cell KITL expression and other granulosa cell anomalies that cause precocious oocyte growth and defects, eventually leading to oocyte demise (*21*).

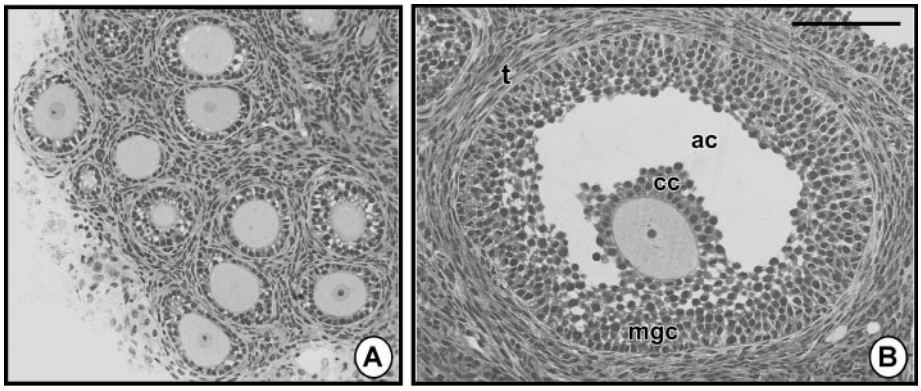

Fig. 2. The rate of follicle development is regulated by the oocyte. Follicle development is shown in 0/0 (control) and 12/0 reaggregated ovaries, 9 days after grafting to the renal bursa. Whereas 0/0 ovaries (**A**) contain no follicles beyond the secondary stage, large antral follicles are clearly evident in 12/0 ovaries (**B**), indicating accelerated follicular development. The precocious antral follicles in the 12/0 ovaries exhibit a normal morphology, with an oocyte surrounded by cumulus cells (cc), an antral cavity (ac), and mural granulosa cells (mgc). A distinct layer of theca cells (t) also surrounds the follicle. Scale bars, 100 μm.

Multilayered follicles become subject to regulation by extraovarian factors, namely the gonadotropin hormones, and require follicle-stimulating hormone (FSH) ligand and receptor to become large antral preovulatory follicles (Fig. 1) (*22, 23*). Knockout mice lacking oocyte-derived zona pellucida protein 3 (ZP-3) or ZP-2 display defects in early antral and preovulatory follicle development, cumulus-oocyte complex (COC) formation, and ovulation (*24*)—evidence that oocytes control somatic cell functions at these stages. Blastocysts derived from in vitro maturation and fertilization of eggs from *Zp2* or *Zp3* knockout females are not capable of completing development after transfer to wild-type pseudopregnant recipients; this finding suggests that zona matrix proteins are important in mediating granulosa cell signals and connections to oocytes that optimize their later developmental potential (*24*).

Oocyte-derived paracrine factors are essential for efficient ovulation. For example, GDF-9 (i) promotes the formation and integrity of the COC by inducing hyaluronan synthase 2, pentraxin 3, and tumor necrosis factor–induced factor 6, and suppressing urokinase plasminogen activator (*21, 25*); (ii) stimulates prostaglandin and progesterone synthesis and/or signaling pathways in preovulatory cumulus granulosa cells (*21*); and (iii) suppresses the luteinization of cumulus granulosa cells by inhibiting LH receptor expression (*21*). A related oocyte-secreted TGF-β superfamily protein, bone morphogenetic protein 15 (BMP-15), functions in a cooperative manner with GDF-9 to maintain the integrity of the COC and maximize female fertility in mice (*26*). Point mutations in the *BMP15* coding sequence in *Fecundity X Inverdale* and *Hanna* sheep cause defects beyond the primary follicle stage and infertility in homozygote ewes, but enhanced ovulation in heterozygotes (*27*). A dominant activating mutation in the TGF-β superfamily receptor ALK6 is responsible for increased ovulation rates and litter sizes the in *Fecundity B Booroola* sheep (*28*), although the importance of this receptor in GDF-9 and BMP-15 signaling pathways is yet to be determined. Many other factors participate in the response to LH in the periovulatory period and have been characterized in knockout mouse models. These include mediators of prostaglandin and progesterone pathways (e.g., CCAAT/enhancer binding protein β, cyclooxygenase 2, and progesterone receptor) (*9*).

During follicle development, oocytes accumulate maternal effect factors necessary to support early embryogenesis, which occurs in the absence of de novo transcription of either parental genome (*29*). Two such factors have been characterized in knockout mouse models. MATER ("maternal antigen that embryos require"), which is necessary for development beyond the two-cell stage, has been implicated in establishing embryonic genome transcription patterns (*30*), and DNMT1o, an oocyte-specific DNA methyltransferase, maintains genomic imprinting crucial for viability of the developing fetus (*31*). In both cases, the oocyte-expressed gene product is stored during oocyte growth and follicular development and is dispensable until after ovulation. As we continue to discover factors that coordinate the oocyte–granulosa cell regulatory loop and follicular development, it will be important to define their roles as determinants not only of ovarian function, but also of embryonic development.

References and Notes

1. K. H. Burns, F. J. DeMayo, M. M. Matzuk, in *Molecular Biology in Reproductive Medicine,* B. C. J. M. Fauser, Ed. (Parthenon, Lancashire, UK, ed. 2, in press).
2. R. L. Brinster, *Science* **296**, 2174 (2002).
3. W. Deng, H. Lin, *Int. Rev. Cytol.* **203**, 93 (2001).
4. H. Peters, *Acta Endocrinol.* **62**, 98 (1969).
5. R. A. Sorensen, P. M. Wassarman, *Dev. Biol.* **50**, 531 (1976).
6. T. Kono, Y. Obata, T. Yoshimzu, T. Nakahara, J. Carroll, *Nature Genet.* **13**, 91 (1996).
7. F. Chesnel, J. J. Eppig, *Mol. Reprod. Dev.* **40**, 503 (1995).
8. D. Wickramasinghe, K. M. Ebert, D. F. Albertini, *Dev. Biol.* **143**, 162 (1991).
9. J. S. Richards, D. L. Russell, S. Ochsner, L. L. Espey, *Annu. Rev. Physiol.* **64**, 69 (2002).
10. J. J. Eppig, *Reproduction* **122**, 829 (2001).
11. M. J. Carabatsos, C. Sellitto, D. A. Goodenough, D. F. Albertini, *Dev. Biol.* **226**, 167 (2000).
12. P. T. Brower, R. M. Schultz, *Dev. Biol.* **90**, 144 (1982).
13. F. Chesnel, K. Wigglesworth, J. J. Eppig, *Dev. Biol.* **161**, 285 (1994).
14. R. De la Fuente, J. J. Eppig, *Dev. Biol.* **229**, 224 (2001).
15. J. J. Eppig, K. Wigglesworth, F. L. Pendola, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2890 (2002).
16. S. M. Soyal, A. Amleh, J. Dean, *Development* **127**, 4645 (2000).
17. A. L. Durlinger *et al.*, *Endocrinology* **140**, 5789 (1999).
18. H. Mizunuma *et al.*, *Endocrinology* **140**, 37 (1999).
19. J. Dong *et al.*, *Nature* **383**, 531 (1996).
20. P. J. Donovan, M. P. de Miguel, in *Transgenics in Endocrinology,* M. M. Matzuk, C. W. Brown, T. R. Kumar, Eds. (Humana, Totowa, NJ, 2001), pp. 147–163.
21. J. A. Elvin, C. Yan, M. M. Matzuk, *Mol. Cell. Endocrinol.* **159**, 1 (2000).
22. T. R. Kumar, Y. Wang, N. Lu, M. M. Matzuk, *Nature Genet.* **15**, 201 (1997).
23. A. Dierich *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13612 (1998).
24. M. Zhao, J. Dean, *Rev. Endocr. Metab. Disord.* **3**, 19 (2002).
25. S. Varani *et al.*, *Mol. Endocrinol.* **16**, 1154 (2002).
26. C. Yan *et al.*, *Mol. Endocrinol.* **15**, 854 (2001).
27. S. M. Galloway *et al.*, *Nature Genet.* **25**, 279 (2000).
28. T. Wilson *et al.*, *Biol. Reprod.* **64**, 1225 (2001).
29. J. Y. Nothias, S. Majumder, K. J. Kaneko, M. L. DePamphilis, *J. Biol. Chem.* **270**, 22077 (1995).
30. Z. B. Tong *et al.*, *Nature Genet.* **26**, 267 (2000).
31. C. Y. Howell *et al.*, *Cell* **104**, 829 (2001).
32. We apologize to colleagues whose work is not described or referenced here because of space limitations. Studies in the Matzuk and Eppig laboratories on oogenesis and folliculogenesis have been supported by NIH grants CA60651, HD32067, HD33438, HD07495, CA62392, and HD23839. K.H.B. is a Medical Scientist Training Program student supported by NIH grants GM07330 and EY07102.