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At the Cutting Edge: The Structures that Underlie Normal Reproductive Function

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Abstract

The mechanisms and physiology of reproductive function have fascinated scientists throughout time. Recent cellular and molecular level structural studies have provided unprecedented insights into reproductive systems and signaling networks. This 'cutting edge' editorial provides a recent example in each of these areas, namely, the anatomical integrity of the follicle, the molecular structure of activin with its binding partners and the molecular regulation of inhibin. These three examples of structure informing function help explain reproductive health and may provide solutions to reproductive disease.

Keywords

activin; follicle; structure; ovary; inhibin; follistatin

Introduction

Since DaVinci and deGraf first struggled to understand then draw the female ovary, follicle unit and the egg, structure has played an essential role in understanding reproductive function. The past five years has been a particularly rich time in the discovery of the structural basis for normal follicle function and the hormones that control it. The use of flexible biomaterials that for the first time permit growth of fully functional follicles represents a significant breakthrough in ovarian biology (Kreeger et al., 2003,Kreeger et al., 2005,Kreeger et al., 2006,Pangas et al., 2003,Xu et al., 2006). Not only will the system permit a more detailed understanding of follicle development, it may also provide an approach necessary to preserving reproductive options for women and girls with cancer. Simultaneously, 3-D structures of key regulating hormones including FSH and its receptor (Fan and Hendrickson, 2005,Fox et al., 2001) and activin together with both its binding receptor (ActRIIA/B) (Thompson et al., 2003,Greenwald et al., 2004) and bioneutralizing regulating partner (follistatin) (Thompson et al., 2005,Harrington et al., 2006) have been solved giving new glimpses into the biology they

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control. Inside the granulosa cell, regulating enzymes such as the human C17 aromatase have been modeled and the co-regulators of aromatase and other genes, LRH-1 and SF-1 have been solved at the molecular level (Solomon et al., 2005,Little et al., 2005).

While structural biology has had a huge impact on establishing mechanisms of action of numerous factors relevant to the reproductive axis, we focus this editorial on a few relevant examples taken from our recent work, highlighting two critical areas of cutting edge research at the interface of structure-function research in reproductive science: the structure of the follicle and structures of key regulating and signaling hormones that control follicle function. These recent advances, which involve cell biology, endocrinology, biochemistry, biophysics and biomaterials, represent exciting interdisciplinary approaches that will not only provide answers to unsolved questions in reproductive science but also provide solutions to unmet needs in reproductive medicine.

I. The follicle as a 3-D structure

Proper follicle development involves maturation of the oocyte, which is surrounded by variable layers of granulosa cells, enveloped by theca cells (Drummond, 2005). Due to the requirement for spatially-determined interactions between these cell types, the spherical nature of the follicle is critical for proper development (Fig 1A). Granulosa cells provide physical support of the oocyte and mediate signals between the oocyte, outer theca cells and endocrine hormones. The traditional approach used to understand follicle function in vitro involved isolating ovarian cells and growing structures on two-dimensional plastic (Cortvrindt et al., 1996,Li et al., 1995,Smitz et al., 1996). While these cultures were ideal for studying the regulation of granulosa cell function, they did not support the development of good quality, highly fertilizable eggs from any species (Fig. 1B). Because the complete understanding of ovarian follicle function requires easily manipulable systems that support in vitro follicular development, a method was needed that could recapitulate all of the functions associated with normal follicular architecture. This included the expansion of the somatic cell layers, the secretion of appropriate hormones and the development of an egg that could be fertilized with high fidelity. To accomplish this goal, a 3-D system was developed that satisfied three key requirements: that the matrix permit free diffusion of hormones, growth factors, metabolic factors and oxygen into and out of the structure; that the material be easily manipulated to permit easy introduction and removal of the follicle and that it be sufficiently pliable to allow expansion of the immature, compact follicle into the large, antrum-containing Graffian follicle (Kreeger et al., 2006,Xu et al., 2006). Alginate, a product of algae, satisfied these criteria and has been successfully used to grow individual murine ovarian follicles *in vitro* (Fig. 1C and D). A gentle gelation process and low non-specific cell contacts provide a support for the follicle with a minimal level of physical stress. By monitoring the structural integrity, viability, growth and production of protein factors in developing cultured follicles, optimal matrix composition can be further developed for increased success in mature follicle formation and eventual fertilization.

Three key observations have been made from the 3-D culture system. First, the matrix can provide structural support to maintain critical connections between somatic cells and the egg, which are likely enriching the competency for fertilization. Second, the follicle can survive and support oocyte development in a completely autonomous manner with minimal hormonal supplementation. Finally, the significance of maintaining follicle structure was firmly established in this system by the *in vitro* fertilization of 3D cultured follicles resulting in live birth of fertile offspring (Xu et al., 2006). This technology, which recapitulates *in vivo* follicle development, now permits questions to be asked about follicle dynamics, oocyte cyotplasmic maturation and signaling pathways necessary for follicle development. Moreover, the technology can now be moved toward human applications including the preservation of fertility

for women and girls with cancer, for the elimination of current IVF strategies as well as for oocytes used to derive stem cells. These developments will require additional creative solutions but will continue, no doubt to rely on the structural context of the cells in an in vitro organ-like environment.

II. Structures and signals that regulate local and endocrine control of follicle development

Maturation of ovarian follicles is a dynamic process that involves tight regulation of numerous signaling factors, many of which belong to the TGF-β superfamily. Recent advances in understanding such regulation in the maturation process focuses on a key member of the TGF-β superfamily, activin. Activin is necessary to the regulation of pituitary follicle stimulating hormone biosynthesis as well as the formation of early follicles. In the pituitary gland, activin acts by binding a receptor (RIIA/B) with constitutive serine-threonine kinase activity. Receptor occupancy results in the recruitment of a second membrane-spanning receptor (ALK4) and the proximity of the second receptor activates its endogenous serine-threonine kinase function. The downstream signaling co-activator, Smad3, is phosphorylated and binds a co-mediator, Smad4. This complex translocates to the nucleus where it binds a pituitary specific co-activator, Pitx2 and then binds to a consensus binding element on the FSH-β gene to activate transcription. FSH is then secreted from the pituitary cell and stimulates the recruitment of immature ovary follicles.

The structure of activin together with its receptor has been solved (Thompson et al., 2003,Greenwald et al., 2004). These structures show that activin binds to its type II receptors through the ‘knuckle’ region, which is different from TGF-β, yet similar to that of BMPs (Lin et al., 2006). Surprisingly, activin was found to have flexibility about the dimer interface (Fig. 2–1), which has not been seen in any of the BMP proteins, and may reflect a difference in relative signaling ability. Subsequent studies have verified the specific amino acids required for activin:ActRIIB interactions (Cook et al., 2005), and suggest a binding site for ALK4, activin’s type I receptor (Harrison et al., 2004), allowing for a model of a hexameric activin signaling complex (Fig. 2–2). SMAD proteins, consisting of an MH1 and an MH2 domain, have also been characterized structurally, demonstrating that promoter regions on target genes associate with the MH1 domain (Fig. 2–3), while the MH2 domain maintains the oligomeric state of SMAD complexes (Chai et al., 2003,Wu et al., 2002,Wu et al., 2001,Shi et al., 1998,Chacko et al., 2004,Qin et al., 1999). The most recent of these structures shows that two SMAD3 molecules and one SMAD4 form a trimeric complex in the activated state. The solution structure of the Pitx2 homeodomain is the first to characterize a native K50 class homeodomain and elaborates on the specificity of this protein with its consensus DNA binding sequence (Chaney et al., 2005). Smad3/4, along with Pitx2, activate transcription of FSHβ (Suszko et al., 2005), which acts in an endocrine fashion to stimulate follicle development (Fig 2–4). While the full cooperative effects of the SMAD3/4 complex and Pitx2 are being unraveled, these advances demonstrate the structural intricacies that are maintained in the activin signaling pathway.

Activin is made in a constitutive fashion throughout the reproductive cycle. Therefore, negative regulation of this FSH agonist is critical. Activin is controlled by the endocrine hormone inhibin and by a locally produced bioneutralizing binding protein called follistatin (Fig 2–5). The structure of inhibin has not yet been solved, however, activin in complex with follistatin has been solved and reveals important interactions between the proteins that explains the remarkable ability of follistatin to quickly and efficiently block activin action (Thompson et al., 2005,Harrington et al., 2006). First, follistatin was shown to interact with several residues that compose activin’s type II receptor interface. By occupying this interface, the ligand is sequestered from association with its high affinity receptor. In addition to this interface, the N-terminal domain of follistatin occupies the putative type I receptor binding site on activin

in a striking display of molecular mimicry. Using a modified TB domain, F47 of follistatin is buried into a hydrophobic pocket on activin, a strategy that is spatially conserved in the type I receptor (BRIA) interaction with BMP2 (Kirsch et al., 2000). By blocking the type I receptor binding site, we can see how follistatin could also act to inhibit BMPs, which tend to bind type I receptors with higher affinity (Iemura et al., 1998). Finally, as two follistatin proteins encircle an activin dimer, specific interactions with both type II and type I receptor binding sites on activin, as well as potential cooperativity between follistatin chains provide a molecular understanding of this inhibitor's robust bioneutralizing ability.

In the follicle, activin stimulates follicle formation (Bristol-Gould and Woodruff, 2006) and FSH sensitivity by enhancing FSH receptor expression. Activin, (dimers of two β-subunits) also induces its antagonist inhibin (αβ heterodimers). While activin is locally restricted by follistatin, inhibin acts in an endocrine fashion adverse to that of FSH. By traveling back to the pituitary, inhibin restricts activin signaling (Fig 2–6), completing this extraordinary cycle of follicular regulation.

The regulation of inhibin in the follicle is absolutely necessary to normal ovarian function and the control of this hormone is equally dynamic (Weck and Mayo, 2006). Two structurally related NR5A family nuclear receptors, steroidogenic factor-1 (SF-1) and liver receptor homologue-1 (LRH-1) play distinct roles in regulating inhibin-α expression, yet bind to identical promoter regions. Both of these proteins can act synergistically with CREB to enhance expression levels, yet the functional determinant of each factor lies in temporal association with the promoter (Ito et al., 2000). In the basal state, SF-1 is the primary NR5A protein associated with the DNA, whereas activation of the cAMP pathway, by mimicry of the FSH signal with forskolin, results in rapid dissociation of this factor from the DNA. A short time later, the amount of LRH-1 bound to the promoter increases significantly, followed by a sharp increase in inhibin levels (Fig. 2–7). Dynamic switching of these nuclear receptors as a result of FSH signaling can be attributed to an increase in LRH-1 level, while SF-1 levels remain consistent. Additionally, switching may be driven by association with other factors that may modify nuclear receptor function or phosphorylation, which might drive dissociation of the SF-1:DNA complex.

Structural characterization of the SF-1 (Little et al., 2005) and LRH-1 (Solomon et al., 2005) DBDs has shown that these nuclear receptors contain a unique Ftz-F1 helix carboxy-terminal to the core DBD and C-terminal extensions observed in most nuclear receptors. This helix acts as a secondary determinant for DNA affinity and is critical for proper function. This helical region also enables these nuclear receptors to function as monomers, potentially giving these proteins a lower affinity for DNA than traditional dimeric nuclear receptors. The SF-1 binding site (SBS) that is occupied by either SF-1 or LRH-1 is an atypical binding site, in that it deviates from the consensus SBS. The recent solution structure of the DNA-binding domain (DBD) of SF-1 in complex with the inhibin-α SBS demonstrates that a G:C to A:T change in the SBS would likely provide a higher affinity interaction between SF-1 and this stretch of DNA via an arginine side chain (Little et al., 2005). It is plausible that the dissociation of SF-1 would occur more slowly if this were the case. Clearly, studies geared towards understanding the molecular basis for this dynamic switching event will clarify this vital process in follicular development.

Final Thoughts

The quest to understand reproductive function has advanced since the times of DaVinci and deGraf, and the importance of structure has been central in this progression. The control of female reproduction requires the integration of endocrine and paracrine acting hormones, explosive growth of the somatic layers of selected ovarian follicles and the coordinated development of a meiotically dormant oocytes into fully developed eggs that can be fertilized

and developed into a new generation of progeny. The relationships between structure and function in reproductive science are crucial to our understanding of follicle biology and may have important implications to clinical preservation of fertility as well as the development of new drugs for fertility control.

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References

Bristol-Gould S, Woodruff TK. Theriogenology 2006;66:5–13. [PubMed: 16620931]

Chacko BM, Qin BY, Tiwari A, Shi G, Lam S, Hayward LJ, De Caestecker M, Lin K. Mol Cell 2004;15:813–23. [PubMed: 15350224]

Chai J, Wu JW, Yan N, Massague J, Pavletich NP, Shi Y. J Biol Chem 2003;278:20327–31. [PubMed: 12686552]

Chaney BA, Clark-Baldwin K, Dave V, Ma J, Rance M. Biochemistry 2005;44:7497–511. [PubMed: 15895993]

Cook RW, Thompson TB, Kurup SP, Jardetzky TS, Wookdruff TK. J Biol Chem 2005;280:40177–86. [PubMed: 16186117]

Cortvrindt R, Smitz J, Van Steirteghem AC. Hum Reprod 1996;11:2656–66. [PubMed: 9021369]

Drummond AE. Cell Tissue Res 2005;322:107–15. [PubMed: 15983782]

Fan QR, Hendrickson WA. Nature 2005;433:269–77. [PubMed: 15662415]

Fox KM, Dias JA, Van Roey P. Mol Endocrinol 2001;15:378–89. [PubMed: 11222739]

Greenwald J, Vega ME, Allendorph GP, Fischer WH, Vale W, Choe S. Mol Cell 2004;15:485–9. [PubMed: 15304227]

Harrington AE, Morris-Triggs SA, Ruotolo BT, Robinson CV, Ohnuma S, Hyvonen M. Embo J 2006;25:1035–45. [PubMed: 16482217]

Harrison CA, Gray PC, Fischer WH, Donaldson C, Choe S, Vale W. J Biol Chem 2004;279:28036–44. [PubMed: 15123686]

Iemura S, Yamamoto TS, Takagi C, Uchiyama H, Natsume T, Shimasaki S, Sugino H, Ueno N. Proc Natl Acad Sci U S A 1998;95:9337–42. [PubMed: 9689081]

Ito M, Park Y, Weck J, Mayo KE, Jameson JL. Mol Endocrinol 2000;14:66–81. [PubMed: 10628748]

Kirsch T, Sebald W, Dreyer MK. Nat Struct Biol 2000;7:492–6. [PubMed: 10881198]

Kreeger PK, Deck JW, Woodruff TK, Shea LD. Biomaterials 2006;27:714–23. [PubMed: 16076485]

Kreeger PK, Fernandes NN, Woodruff TK, Shea LD. Biol Reprod 2005;73:942–50. [PubMed: 15987824]

Kreeger PK, Woodruff TK, Shea LD. Mol Cell Endocrinol 2003;205:1–10. [PubMed: 12890562]

Li R, Phillips DM, Mather JP. Endocrinology 1995;136:849–56. [PubMed: 7867593]

Lin SJ, Lerch TF, Cook RW, Jardetzky TS, Woodruff TK. Reproduction 2006;132:179–90. [PubMed: 16885528]

Little TH, Zhang Y, Matulis CK, Weck J, Zhang Z, Ramachandran A, Mayo KE, Radhakrishnan I. Mol Endocrinol. 2005

Pangas SA, Saudye H, Shea LD, Woodruff TK. Tissue Eng 2003;9:1013–21. [PubMed: 14633385]

Qin B, Lam SS, Lin K. Structure 1999;7:1493–503. [PubMed: 10647180]

Shi Y, Wang YF, Jayaraman L, Yang H, Massague J, Pavletich NP. Cell 1998;94:585–94. [PubMed: 9741623]

Smitz J, Cortvrindt R, Van Steirteghem AC. Mol Reprod Dev 1996;45:466–75. [PubMed: 8956285]

Solomon IH, Hager JM, Safi R, McDonnell DP, Redinbo MR, Ortlund EA. J Mol Biol 2005;354:1091–102. [PubMed: 16289203]

Suszko MI, Balkin DM, Chen Y, Woodruff TK. Mol Endocrinol 2005;19:1849–58. [PubMed: 15761025]

Thompson TB, Lerch TF, Cook RW, Woodruff TK, Jardetzky TS. Dev Cell 2005;9:535–43. [PubMed: 16198295]

Thompson TB, Woodruff TK, Jardetzky TS. Embo J 2003;22:1555–66. [PubMed: 12660162]

Weck J, Mayo KE. Mol Endocrinol. 2006

Wu JW, Hu M, Chai J, Seoane J, Huse M, Li C, Rigotti DJ, Kyin S, Muir TW, Fairman R, Massague J, Shi Y. Mol Cell 2001;8:1277–89. [PubMed: 11779503]

Wu JW, Krawitz AR, Chai J, Li W, Zhang F, Luo K, Shi Y. Cell 2002;111:357–67. [PubMed: 12419246]

Xu M, Kreeger PK, Shea LD, Woodruff TK. Tissue Eng. 2006

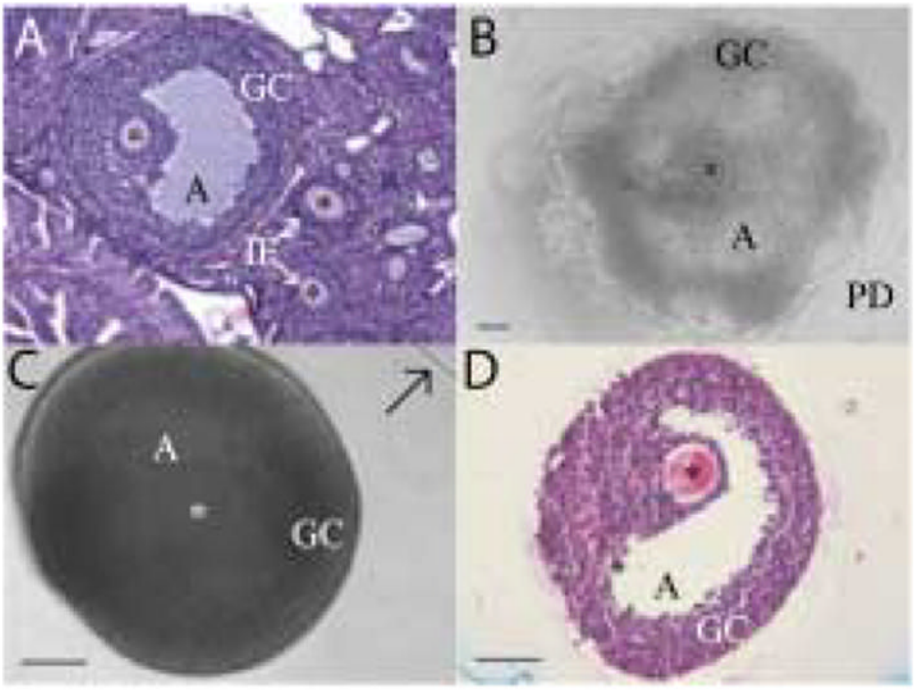

Figure 1. Preservation of follicular architecture

(A) Normal, healthy follicles progress from immature follicles to mature, antrum-containing Graffian follicles. Follicular architecture is crucial to this maturation, as follicles cultured on two- dimensional petri dishes lose their structure (B). Granulosa cells adhere to the plastic surface and no longer support a healthy oocyte (*). The low quality oocyte unsuccessfully attempts to reconstruct the native follicular design and is therefore rarely competent for fertilization. (C) Follicles cultured in three-dimensional alginate beads maintain support of the oocyte by the granulosa cells and grow from pre-antral to mature, antrum-containing follicles. The edge of the alginate bead (arrow) demonstrates the spherical nature of this culture system. (D) A cross section of a follicle cultured in alginate further displays the overwhelming preservation of structure when comparing this system to *in vivo* matured follicles. Scale bar = 100μm. Abbreviations: IF (immature follicle), A (antrum), PD (petri dish), GC (granulosa cells).

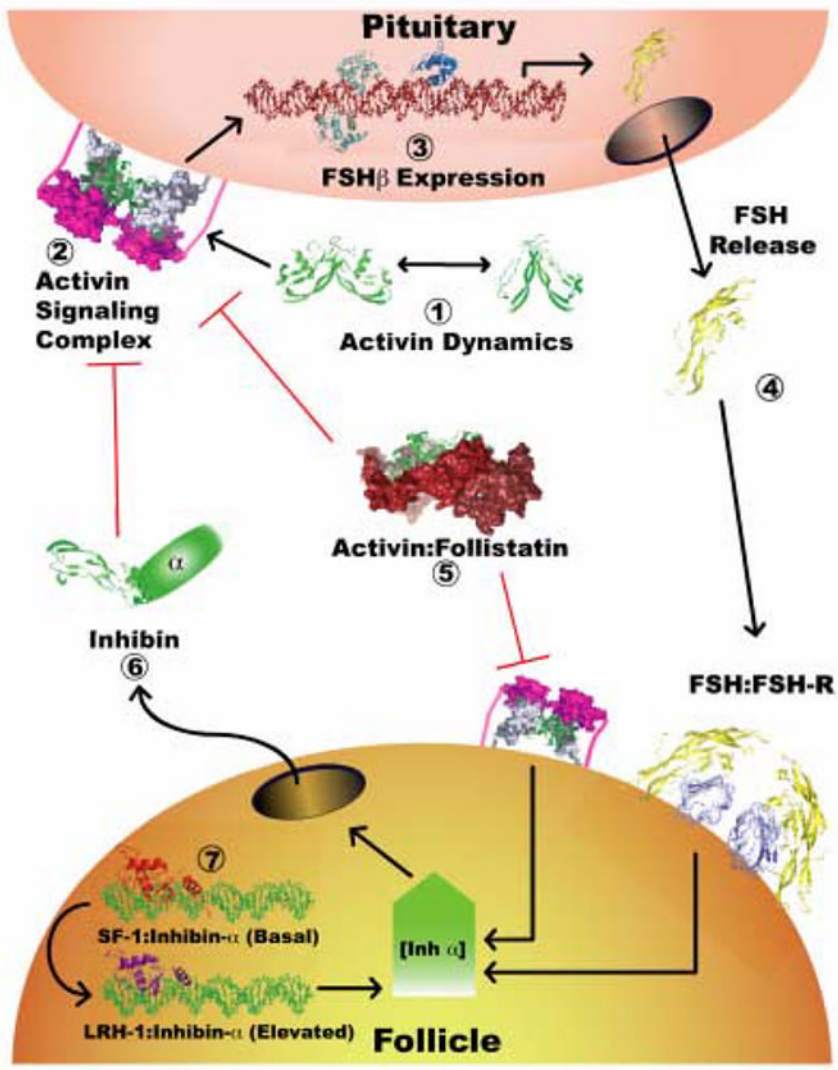

Figure 2. Regulation of activin signaling is vital for proper follicle development

Activin is a flexible dimer (1) which signals in an autocrine fashion via type II and type I receptors (2). In pituitary cells, the signal is propagated intracellularly via SMADs 3 and 4, which, together with the transcription factor Pitx2, promote FSHβ synthesis (3). FSH is released from the pituitary, and interacts with FSH receptors expressed on ovarian granulosa cells (4). FSH stimulates inhibin α-subunit and follistatin expression. Activin is locally inhibited by follistatin (5), which embraces the ligand, sequestering it from receptor binding. Thus, local activin activity decreases, as ovarian inhibin becomes the dominant non-steroidal hormone produced by the follicle. Follicular activin promotes the production of inhibin, which is released into circulation, completing the endocrine loop to inhibit pituitary activin signaling (6). Inhibin levels are also controlled by the dynamic exchange of SF-1 and LRH-1 on the promoter of the inhibin α gene (7). SF-1 is primarily associated in the basal state, while LRH-1 levels vary dramatically, increasing for enhanced expression of inhibin α. The ability to correlate structure with function in the control of pituitary FSH and ovarian follicle development is an exciting advance in reproductive biology.