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The 25th Volume: Role of the GATA Family of Transcription Factors in Andrology

Review

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Understanding how genes get turned on or off is central to the study of biological processes in both health and disease. Several regulatory mechanisms, which can be positive or negative, have been implicated in the control of tissue- and cell-specific gene expression. These include modulation of chromatin structure, DNA methylation, and the regulation of transcription and translation. Transcription factors are nuclear regulatory proteins that bind specific DNA sequences in the 5′ regulatory or promoter regions of target genes. They are involved in both basal and tissue-specific gene expression. There are several classes of transcription factors that have been defined based on similarities in the structure of the respective DNA-binding domains. They include zinc finger, helix-loop-helix, leucine zipper, and homeobox transcription factors. The GATA family of zinc finger transcription factors is named from the consensus nucleotide sequence (A/T*GATA*A/G) that these factors bind in the promoter regions of target genes. They were originally identified as crucial regulators of heart development and the differentiation of blood and immune cells. *GATA* expression, however, is not limited to these two systems. Indeed, reproductive tissues such as the testis and ovary are also prominent sites of *GATA* expression. As few as 5 years ago, the role of GATA factors in reproductive function was uncharted territory. With the recent contributions to the field, the scientific community has come a long way in filling this void. GATA factors have now been implicated in gonadal development, male sex determination and differentiation, and steroidogenesis. This review will provide a brief overview of the vertebrate family of GATA factors and how these factors have affected the field of andrology.

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The GATA Family of Transcription Factors

GATA regulatory elements and their prototypic binding protein were originally identified in studies of erythroid-specific gene expression more than a decade ago (Orkin, 1992; Weiss and Orkin, 1995a). A novel transcription factor that specifically bound to GATA *cis*-elements was cloned from erythroid cells and named GATA1 (Tsai et al, 1989). GATA1 was shown to contain a DNA-binding domain that consisted of two similar zinc fingers with the distinctive form C-X_2-C-(X_{17})-C-X_2-C (Tsai et al, 1989; Weiss and Orkin, 1995a). Since the cloning of the prototypic GATA1 factor, 5 additional vertebrate factors (named GATA2 to GATA6), having similar DNA-binding domains, have been identified (Weiss and Orkin, 1995a; Molkentin, 2000). The 6 vertebrate GATA factors can be separated into 2 subgroups based on sequence homology and tissue distribution: the hematopoietic (GATA1/2/3) and the cardiac (GATA4/5/6) GATA factors (Figure 1).

Expression and Role of GATA Factors Outside of the Reproductive System: GATA1/2/3—The *GATA1* gene is abundantly expressed in erythroid and megakaryotic cells (Orkin, 1992; Weiss and Orkin, 1995a). Consistent with this localization, consensus GATA-binding motifs are found in several genes specifically expressed in these cell lineages. Gene knockout experiments in mice have revealed that Gata1 is required for embryonic viability, since its expression is crucial for the terminal differentiation of erythroid precursors and the growth and maturation of megakaryocytes both in vitro and in vivo (Pevny et al, 1991; Fujiwara et al, 1996; Shivdasani et al, 1997). Erythroid precursor cells lacking Gata1 fail to mature and undergo either extensive apoptosis (Pevny et al, 1995; Weiss and Orkin, 1995b) or deregulated proliferation (Shivdasani et al, 1997). In addition to hematopoietic cells, the *GATA1* gene is also abundantly transcribed in the testis (described in detail later). Like GATA1, GATA2 is present in hematopoietic cells but is also found in several nonhematopoietic lineages such as endothelial cells,

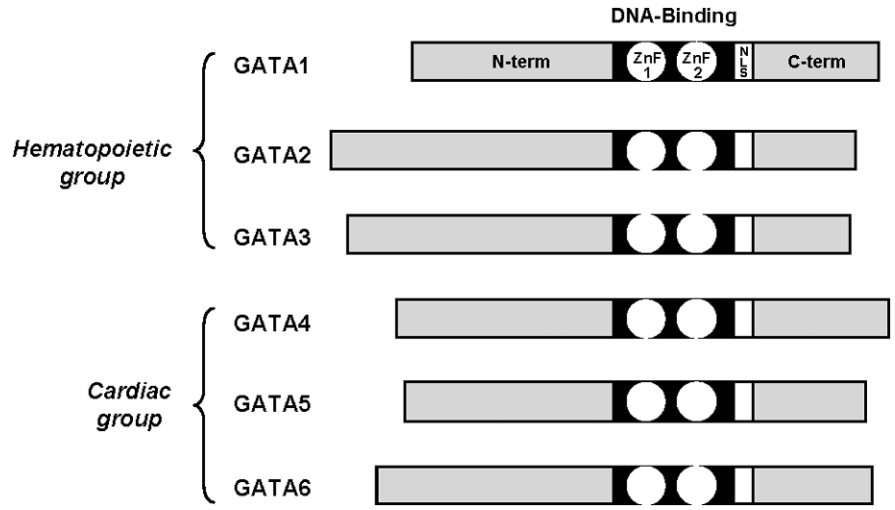

Figure 1. Structure of the vertebrate family of GATA proteins. All 6 vertebrate GATA factors share a conserved DNA-binding domain consisting of 2 zinc fingers (ZnF), a feature that defines this family of transcription factors. The different GATA factors can be divided into 2 subgroups based on amino acid sequence homology and tissue distribution: the hematopoietic subgroup (GATA 1/2/3) and the cardiac subgroup (GATA 4/5/6). Transactivation domains are found in either the N-terminal (N-term) and/or C-terminal (C-term) portions of the different GATA proteins. NLS, nuclear localization signal.

fibroblasts, Wolffian ducts, pituitary, and embryonic brain and liver cells (Yamamoto et al, 1990; Lee et al, 1991; Gordon et al, 1997; Zhou et al, 1998; Dasen et al, 1999). Similarly, *GATA3* expression is not limited to hematopoietic cells (T-lymphocytes and definitive erythroid cells) but is also found in several other embryonic tissues, including the placenta, brain, kidney, and thymus (Leonard et al, 1993; Kornhauser et al 1994; George et al, 1997). Although GATA1/2/3 have overlapping expression patterns in hematopoietic cell lineages, the knockout of their corresponding genes produce distinct phenotypes in mice (Tsai et al, 1994; Pandolfi et al, 1995; Fujiwara et al, 1996; Ting et al, 1996). The absence of the Gata2 factor produces a defect in which early hematopoietic cells fail to proliferate (Tsai et al, 1994). Interestingly, a rescue of the embryonic-lethal hematopoietic defect in the *Gata2* knockout mouse using yeast artificial chromosomes (YAC) has revealed a critical role for this factor in the development of tissues that derive from the Wolffian duct, such as the seminal vesicles and vasa deferentia (described further below)(Zhou et al, 1998). Finally, mice embryos containing homozygous mutations in the *Gata3* gene die between 11 and 12 days postcoitum as a result of massive internal hemorrhaging and severe brain and spinal cord deformities (Pandolfi et al, 1995). Mice lacking the *Gata3* gene also exhibit a hematopoietic defect in which the development of mature T-lymphocytes is arrested (Ting et al, 1996).

GATA4/5/6—Unlike their counterparts in hematopoietic cells, members of this GATA factor subfamily exhibit strong expression in the stomach, gut epithelium, heart, and gonads (Arceci et al, 1993; Kelley et al, 1993; Tamura et al, 1993; Grépin et al, 1994; Heikinheimo et al, 1994, 1997; Laverriere et al, 1994; Morrisey et al, 1996, 1997; Bossard and Zaret, 1998; Viger et al, 1998; Ketola et al, 1999; Robert et al, 2002; Nemer and Nemer, 2003). The *GATA4* gene is abundantly expressed in the developing heart (Kelley et al, 1993; Heikinheimo et al, 1994). Consequently, GATA4 has been indicated as a key regulator of cardiac-specific gene expression during development. Indeed, functional GATA-binding elements have been identified in the promoters of several cardiac-specific genes that are activated by GATA4 in noncardiac cells (Grépin et al, 1994; Ip et al, 1994; Molkentin et al, 1994; Parmacek et al, 1994). The characterization of *Gata4* knockout mice, which die between embryonic days 6.5 to 8.0 because of defects in heart tube formation, has confirmed the importance of this factor in heart development (Kuo et al, 1997; Molkentin et al, 1997). Moreover, GATA4 is associated with human heart disease, in which case it acts as a novel transcriptional regulator of calcineurin-dependent cardiac hypertrophy (Molkentin et al, 1998), and mutations in the *GATA4* gene have been recently linked to congenital heart defects (Garg et al, 2003). In addition to the heart, GATA4 is also prominently expressed in the developing gonads, including Sertoli and Leydig cells of the testis (described below). During embryogenesis, GATA5 is first expressed in the developing heart and subsequently in the lung, vasculature, and genitourinary system (Morrisey et al, 1997; Molkentin et al, 2000; Nemer and Nemer, 2003). GATA6 is expressed in multiple cell lineages derived from lateral mesoderm, including the heart, gut, and gonads (Morrisey et al, 1996; Ketola et al, 1999; Robert et al, 2002; Nemer

and Nemer, 2003). Targeted inactivation of the *Gata5* and *Gata6* genes has revealed that these factors serve distinct physiological roles in vivo (Morrisey et al, 1998; Koutsourakis et al, 1999; Molkentin et al, 2000). Inactivation of the *Gata6* gene causes early embryonic lethality shortly after implantation as a result of a lack of endoderm differentiation and/or extraembryonic tissue (Morrisey et al, 1998; Koutsourakis et al, 1999). Although loss of Gata5 function does not lead to embryo death, female *Gata5*$^{-/-}$ mice exhibit pronounced genitourinary abnormalities that include vaginal and uterine defects and hypospadias (Molkentin et al, 2000). Taken together, the mouse knockout data have revealed that GATA factors play critical developmental roles. Indeed, aberrations in GATA function have now been recently linked with human disease, where a mutation of the *GATA1* gene has been associated with dyserythropoietic anemia and thrombocytopenia (Nichols et al, 2000), *GATA3* haplo-insufficiency with human hypoparathyroidism, sensorineural deafness, renal anomaly (HDR) syndrome (Van Esch et al, 2000), and *GATA4* mutations with congenital heart defects (Garg et al, 2003).

Functional Specificity of GATA Factors—All vertebrate GATA proteins contain a conserved DNA-binding domain composed of two multifunctional zinc fingers. The C-terminal zinc finger is required for site-specific recognition and DNA-binding to the core GATA motif, whereas the N-terminal zinc finger contributes to the specificity and stability of the DNA-binding (Martin and Orkin, 1990; Yang and Evans, 1992; Omichinski et al, 1993). Since members of the GATA family share a highly conserved DNA-binding domain, they all exhibit similar DNA-binding properties (Ko and Engel, 1993; Merika and Orkin, 1993). This contrasts, however, with their rather specific roles in vivo (Pevny et al, 1991; Tsai et al, 1994; Blobel et al, 1995; Pandolfi et al, 1995; Kuo et al, 1997; Molkentin et al, 1997, 2000; Morrisey et al, 1998; Zhou et al, 1998; Koutsourakis et al, 1999; Takahashi et al, 2000). The specificity of GATA action is controlled, at least in part, via protein-protein interactions with other transcriptional partners (Charron and Nemer, 1999; Molkentin, 2000). Indeed, there is now an extensive list of ubiquitously expressed or cell-restricted factors that are known to cooperate with GATA factors to control tissue-specific transcription in the hematopoietic system, the heart, the pituitary, the adrenal, and the gonads (Kawana et al, 1995; Merika and Orkin, 1995; Osada et al, 1995; Gregory et al, 1996; Durocher et al, 1997; Gordon et al, 1997; Lee et al, 1998; Ono et al, 1998; Rekhtman et al, 1999; Tremblay and Viger, 1999; Morin et al, 2000; Nerlov et al, 2000; Tremblay et al, 2002; Jimenez et al, 2003). Of the different GATA-interacting factors, the most notable are the multitype zinc finger proteins termed Friend of GATA1 (FOG1) and Friend of GATA2 (FOG2), because the FOG proteins were originally identified as GATA-specific cofactors through their ability to interact with the N-terminal zinc fingers of the different GATA factors (Tsang et al, 1997; Holmes et al, 1999; Lu et al, 1999; Svensson et al, 1999; Tevosian et al, 1999). Like *GATA1, FOG1* is highly expressed in hematopoietic cell lineages (Tsang et al, 1997). Similarly, *FOG2* is coexpressed with *GATA4* in the heart, brain, and gonads (Lu et al, 1999; Tevosian et al, 1999; Laitinen et al, 2000; Ketola et al, 2002; Robert et al, 2002; Anttonen et al, 2003). Mouse knockout studies have revealed that FOG proteins, like their GATA counterparts, have crucial developmental functions in vivo. Thus, the lack of Fog1 leads to a block in erythroid and megakaryocytic differentiation (Tsang et al, 1998), while genetic ablation of Fog2 leads to defects in heart morphogenesis and coronary vascular development (Svensson et al, 2000b; Tevosian et al, 2000; Crispino et al, 2001), as well as impaired gonad development (Tevosian et al, 2002). Although the FOG proteins do not appear to directly bind to DNA, they act as either enhancers or repressors of GATA transcriptional activity depending on the cell context and promoter being studied (Tsang et al, 1997; Fox et al, 1999; Holmes et al, 1999; Lu et al, 1999; Svensson et al, 1999, 2000a; Tevosian et al, 1999; Robert et al, 2002). The role of FOG proteins in modulating GATA-dependent transcription in the testis will be discussed later.

Expression of GATA Factors in the Male Reproductive System

As previously mentioned, GATA factors are not unique to the hematopoietic and cardiac systems but rather are expressed in a wide variety of tissues. This includes tissues of both the male and female reproductive tracts, with the predominant sites of expression being the testis and ovary. GATA-like DNA-binding proteins are found in the gonads of species ranging from worms to humans (Spieth et al, 1991; Tamura et al, 1993; Drevet et al, 1994; Laverriere et al, 1994; Singh et al, 1994; Yomogida et al, 1994; Lossky and Wesink, 1995; Heikinheimo et al, 1997; Viger et al, 1998; Ketola et al, 1999; De Santa Barbara et al, 2000), suggesting that a functional role for GATA factors in the gonads has been conserved during evolution. Of the 6 vertebrate GATA factors, 4 are expressed in the mammalian gonads: GATA1 (Ito et al, 1993; Yomogida et al, 1994; Viger et al, 1998), GATA2 (Siggers et al, 2002), GATA4 (Heikinheimo et al, 1997; Viger et al, 1998; Ketola et al, 1999; McCoard et al, 2001), and GATA6 (Heikinheimo et al, 1997; Ketola et al, 1999; Robert et al, 2002). As a general rule, GATA factors label the major somatic cell types of the gonads. The exceptions are GATA2, which is expressed specifically in germ cells of the mouse ovary during a very discrete period of

Expression of GATA factors in the male reproductive system

Tissue	Cell Type	GATA Factor	Developmental Expression	Putative GATA Target Genes*
Testis	Sertoli	GATA1	Prepubertal, adult	*Mis/Amb, Sf1, Inha, Inhbb, Fshr, Dmrt1, Sry†*
		GATA4	Fetal, neonatal, adult	
		GATA6	Fetal, neonatal, adult	
	Leydig	GATA1	Adult (purified cells)	*Star, Sf1, Cyp17, Cyp19, HSD17B1, HSD3B2†*
		GATA4	Fetal, adult	
		GATA6	Adult (human)	
	Germ	GATA4	Fetal, prepubertal (human)	Unknown
Prostate		GATA2	Fetal, neonatal, adult, LNCaP cells	*KLK3* enhancer
		GATA3	Fetal, neonatal, adult, LNCaP cells	
		GATA6	PC-3, ALVA-31 cells	
Seminal vesicle		GATA2	Fetal, neonatal, adult	Unknown
		GATA3		
Coagulating gland		GATA2	Fetal, neonatal, adult	Unknown
		GATA3		
Wolffian duct		GATA2	Fetal	Unknown
		GATA3		
Genital tubercle		GATA3	Fetal	Unknown

* References provided in text.
† Our unpublished data.

early fetal development (Siggers et al, 2002), and GATA4, which, in addition to labeling somatic cells, has also been reported to be strongly expressed in fetal and prepubertal germ cells of the human testis (Ketola et al, 2000). The significance of GATA expression in germ cells, however, has yet to be demonstrated.

GATA Factors in the Testis: GATA1—The mammalian testis expresses 3 GATA factors: GATA1, GATA4, and GATA6 (Table). Because of the functional importance of GATA proteins in other systems, these factors have emerged as a group of potentially important regulators of testicular gene expression and function. GATA1 was the first GATA factor shown to be expressed in the testis (Ito et al, 1993; Yomogida et al, 1994). To date, it is also the only GATA factor whose expression in the testis is known to be driven by a testis-specific promoter (Onodera et al, 1997). In the mouse, testicular *Gata1* expression is restricted to Sertoli cells of postnatal animals (Yomogida et al, 1994; Viger et al, 1998; Ketola et al, 2002). Expression begins in the prepubertal testis, where Sertoli cells uniformly express the protein (Yomogida et al, 1994). After puberty, however, the number of *Gata1*-expressing seminiferous tubules show a stage-specific decline, so that by adulthood, only Sertoli cells in stage VII, VIII, and IX tubules exhibit Gata1 immunoreactivity (Yomogida et al, 1994). In addition to postnatal Sertoli cells, recent data by Chen et al have suggested that Leydig cells might also express GATA1 (Zhang et al, 2002). This was based on Northern blot data of RNA obtained from MA-10 Leydig tumor cells and an enriched population of 21-day-old rat progenitor Leydig cells (Zhang et al, 2002). Further in situ hybridization or immunohistochemistry data is needed, however, to confirm that GATA1 is indeed expressed in Leydig cells in vivo.

GATA4—Testicular expression of the GATA4 transcription factor has been well-documented in 3 different species: mouse, pig, and human (Viger et al, 1998; Ketola et al, 1999, 2000; McCoard et al, 2001). In the mouse and pig, GATA4 is abundantly expressed from the onset of gonadal development, where it labels virtually all cell types present with the notable exception of germ cells (Viger et al, 1998; McCoard et al, 2001). After gonadal differentiation, *GATA4* expression is maintained at high levels in the fetal testis in both Sertoli cells and interstitial (Leydig) cells (Viger et al, 1998; McCoard et al, 2001). In the mouse, Sertoli cells continue to express *Gata4* after birth, but levels decline in adulthood, when Gata1 becomes the predominant GATA factor (Viger et al, 1998). GATA4 is also abundantly expressed by the MSC-1 Sertoli cell line (Heikinheimo et al, 1997; Robert et al, 2002). Much like Sertoli cells, GATA4 expression in Leydig cells persists in the developing mouse and porcine testis through to adulthood (Ketola et al, 1999; McCoard et al, 2001), and GATA4 is a marker of several transformed and/or immortalized Leydig cell lines (Ketola et al, 1999; Robert et al, 2002; Zhang et al, 2002). In the human testis, *GATA4* expression parallels what has been observed in the mouse and pig, with one exception: germ cells (Ketola et al, 2000). Unlike the mouse and pig, GATA4 appears to be a major GATA factor of both fetal and prepubertal human male germ cells (Ketola et al, 2000).

GATA6—Along with GATA1 and GATA4, GATA6 is the third GATA factor shown to have predominant expression in Sertoli cells. In the mouse, *Gata6* expression

overlaps with *Gata4* in Sertoli cells during fetal and early postnatal development (Viger et al, 1998; Ketola et al, 1999; Robert et al, 2002). All 3 GATA factors (Gata1/4/6), however, are coexpressed in Sertoli cells of the adult testis (Yomogida et al, 1994; Viger et al, 1998; Ketola et al, 1999; Robert et al, 2002;). In rodents, *Gata6* expression appears to be specific to Sertoli cells, whereas in humans, GATA6 is detected in both Sertoli and Leydig cells at least in the fetal testis (Ketola et al, 2003).

Other Androgen-Dependent Tissues—Aside from the testis, little attention has been given to the expression of GATA factors in the other tissues that compose the male reproductive tract. At present, it is unclear whether the absence of such reports can be construed as a lack of expression or merely a lack of investigation. Nonetheless, there is at least one report of *GATA* expression in the prostate, coagulating gland, and seminal vesicle (Perez-Stable et al, 2000). Normal mouse and human prostate were shown to express both GATA2 and GATA3 (Perez-Stable et al, 2000). Although no other GATA factor was detected in normal prostate, weak *GATA6* expression was found in the human PC-3 and ALVA-31 prostate cancer cell lines (Perez-Stable et al, 2000). The *GATA2* gene was also shown to be abundantly transcribed in the androgen-dependent LNCaP prostate cancer cell line (Perez-Stable et al, 2000). The same group also identified several GATA elements in the human *KLK3* (prostate-specific antigen) gene enhancer, suggesting that GATA factors might be involved in the androgen-dependent regulation of the *KLK3* gene in the prostate.

The two other sites of significant GATA expression in the male reproductive system are the Wolffian ducts (precursors of the seminal vesicles, epididymides, and vasa deferentia) and the genital tubercle. *Gata3* expression in the developing genital tubercle (primordium of the penis in males) was found serendipitously while studying the temporal and spatial control of the murine *Gata3* promoter in transgenic mice (Lieuw et al, 1997). Although targeted inactivation of the *Gata2* in mice leads to mid-gestational death (Tsai et al, 1994), rescue of the lethal hematopoietic defect revealed an expected role for Gata2 in urogenital development (Zhou et al, 1998). Despite normal appearing testes and epididymides, the Gata2 mutant males frequently had hypoplastic seminal vesicles that were aberrantly fused to the vasa deferentia (Zhou et al, 1998). This interesting finding prompted the investigators to look more closely at Gata2 expression in the developing urogenital system. Consistent with their observed phenotype, Gata2 expression in males was found to be strongly expressed in the Wolffian ducts. However, unlike the prostate and especially the testis, which is described next, target genes for GATA2 in the Wolffian duct derivatives have yet be identified.

GATA Factors in the Testis: Functional Roles and Target Genes

Although gene inactivation experiments have identified crucial roles for GATA factors in early vertebrate development, they have been less useful for the study of their later recruitment as regulators of tissue-specific gene expression in vivo, since 5 out of the 6 *Gata*$^{-/-}$ mice are embryonic lethal. This includes the *Gata1/4/6* gene knockouts, in which embryo death occurs prior to testis development (Pevny et al, 1991; Kuo et al, 1997; Molkentin et al, 1997; Morrisey et al, 1998). To assess the role of these factors in the testis, a selective inactivation or knockdown of GATA function is therefore essential. These types of experiments have begun to be used to gain insights into the in vivo testicular roles played by GATA1 and GATA4 (Tevosian et al, 2002; Lindeboom et al, 2003). As previously mentioned, the *Gata4* gene is abundantly expressed in the somatic cell population of the developing mouse genital ridge around the time of sex determination. Thus, based on its expression pattern, GATA4 was proposed to play a central role in sex determination and, hence, testis differentiation. This hypothesis has been recently confirmed in the mouse, in which in vivo disruption of one of Gata4's functions (interaction with its co-factor Fog2) via an elegant knock-in mutation of the *Gata4* gene leads to a block in testis development and a marked down-regulation of expression of the testis-determining gene, *Sry* (Tevosian et al, 2002). Thus, GATA4 appears to function as an upstream regulator of *SRY* expression in the developing testis. Although the latter has yet to be conclusively demonstrated, the presence of multiple GATA regulatory motifs in the mouse, human, and pig *SRY* promoters strongly supports this notion.

By creating a conditional knockout allele of the *Gata1* gene and using the *Desert Hedgehog* promoter driving Cre recombinase to excise the floxed *Gata1* gene in Sertoli cells, Lindeboom et al (2003) recently reported the first testis-specific knockout of a GATA gene. Surprisingly, *Gata1* null testes were morphologically and functionally normal (Lindeboom et al, 2003). The absence of an overt testicular phenotype was likely due to compensation by Gata4 and Gata6 in Sertoli cells, which masked the loss of Gata1. A global knockout or knockdown of GATA activity would therefore be needed to assess the functional role of GATA factors in postnatal Sertoli cells in which overlapping expression of multiple GATA factors poses a problem.

The lack of conditional knockout models has not stopped investigators from gaining insights into the potential role played by GATA factors in the testis. The fact that GATA factors recognize specific regulatory motifs (WGATAR) in the promoter regions of genes has been exploited to identify novel targets for these factors in

many tissues, including the testis. The Müllerian inhibiting substance (MIS or AMH) promoter was identified as the first known target for GATA4 in Sertoli cells (Viger et al, 1998). The MIS hormone is the earliest marker of testis formation. It regulates male sex differentiation by triggering regression of the Müllerian ducts (the precursors of the female reproductive tract) in males (Teixeira et al, 2001). *MIS* gene expression is tightly regulated during gonadal development; lack of expression in humans causes persistent Müllerian duct syndrome, a condition in which affected males exhibit both male and female internal reproductive organs. Through an analysis of the conserved 5′ regulatory elements of the *MIS* gene, several transcription factors have been proposed to participate in *MIS* transcription, such as the nuclear receptor steroidogenic factor 1 (SF1) and the high mobility group box-containing protein SOX9 (Shen et al, 1994; Giuili et al, 1997; Arango et al, 1999). However, since SOX9 and SF1 are colocalized in several tissues that do not express *MIS*, other factors must act to restrict *MIS* expression to the gonads. Indeed, cooperation between transcription factors has been shown to contribute to tissue-specific *MIS* expression (Teixeira et al, 2001); this includes GATA4 (Tremblay and Viger, 1999; Watanabe et al, 2000; Tremblay et al, 2001). GATA4 has been shown to regulate both the mouse and human *MIS* promoters through a transcriptional cooperation with SF1 (Tremblay and Viger, 1999; Watanabe et al, 2000). Although no human *GATA4* gene mutations have yet been linked to gonadal defects, recent evidence suggests that disruption of GATA4/SF1 synergism may account for some cases of abnormal human male sex differentiation involving insufficient *MIS* expression (Tremblay and Viger, 2003a).

The overlapping expression of multiple GATA factors in Sertoli cells is a strong indication that these GATA factors are key regulators of Sertoli cell–specific gene expression and function during ontogeny. Indeed, in addition to *MIS*, GATA factors have been shown to regulate several Sertoli-cell promoters (Table). These include the inhibin α (*Inha*; Feng et al, 1998; Ketola et al, 1999; Tremblay and Viger, 2001a), inhibin/activin βB (*Inhbb*; Feng et al, 2000), *Sf1* (Tremblay and Viger, 2001a), follicle-stimulating hormone (FSH) receptor (*Fshr*; Kim and Griswold, 2001), Doublesex- and Mab-3–related transcription factor (*Dmrt1*; Lei and Heckert, 2004), and *Sry* (Viger et al, unpublished data) promoters. In vitro studies by Feng et al have shown that the inhibin α promoter is preferentially activated by GATA1 despite the colocalization of GATA1 and GATA4 in Sertoli cells (Feng et al, 1998, 2000). However, since all GATA factors are known to have similar DNA-binding properties (Merika and Orkin, 1993), selectivity based on transactivation properties alone would seem unlikely. Rather, whether a Sertoli cell gene responds or not to regulation by a particular GATA factor would likely depend on cooperative interactions with specific transcriptional partners acting as either coactivators or corepressors.

Besides Sertoli cells, GATA are also prominently expressed in the interstitial cell (steroidogenic) compartment of the testis (Table). Although both GATA4 and GATA6 are expressed in ovarian steroidogenic cells throughout development (Heikinheimo et al, 1997; Laitinen et al, 2000), in the testis, GATA4 is the predominant GATA factor of both fetal and postnatal Leydig cells (Viger et al, 1998; Ketola et al, 1999, 2000). Thus, GATA factors, and in particular GATA4, have been proposed to be key regulators of steroidogenesis in the testis (Tremblay and Viger, 2003b). In support of this hypothesis, the promoters of several steroidogenic enzyme-encoding genes have been reported to contain one or more consensus GATA regulatory motifs (Tremblay and Viger, 2003b). Using in vitro transactivation experiments, several laboratories have confirmed that some of these steroidogenic promoters are indeed targets for GATA factors. Those studied to date include the promoters for *Sf1* (Tremblay and Viger, 2001a), 17β-hydroxysteroid dehydrogenase type 1 (*HSD17B1*; Piao et al, 1997), P450 aromatase (*Cyp19*; Tremblay and Viger, 2001a), steroidogenic acute regulatory protein (*Star*; Silverman et al, 1999; Wooton-Kee and Clark, 2000; Tremblay and Viger, 2001a; Tremblay et al, 2002), and 3β-hydroxysteroid dehydrogenase type 2 (*HSD3B2*; Viger et al, unpublished data). In addition to their ability to directly stimulate transcription of target steroidogenic promoters, GATA factors also contribute to the tissue-specific activity and hormone dependence of some of these promoters via synergistic interactions with the orphan nuclear receptor SF1 and the CCAAT enhancer binding protein C/EBPβ (Tremblay and Viger, 2003b).

Regulation of GATA Expression and Transcriptional Activity in the Testis

Although the list of target genes for GATA factors in testicular cells continues to grow, much less is known about how GATA expression itself is regulated in the testis. Yomigida et al (1994) observed that Sertoli cell expression of *Gata1* is increased in germ cell–deficient mouse models, suggesting that GATA1 expression in the testis is negatively regulated by one or more paracrine factors produced by germ cells. The concept of a ''negative regulation'' of GATA1 in the testis has resurfaced recently with the demonstration that *Gata1* expression in both MA-10 Leydig cells and primary Sertoli cell cultures is markedly down-regulated by FSH/cAMP treatment (Zhang et al, 2002). Interestingly, the inhibitory effect was specific for testicular cells, since Gata1 expression in erythroid cells was unaffected under similar conditions. At present, however, the physiological significance of this gonadotropin/cAMP–mediated regulation remains un-

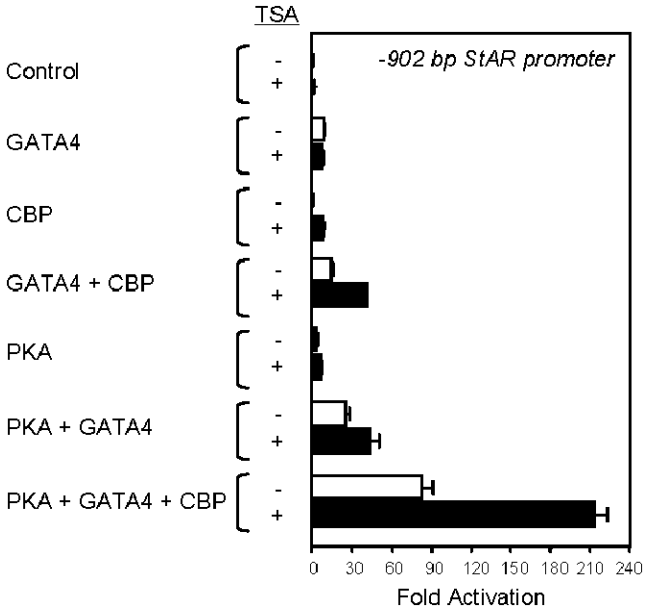

Figure 2. Inhibition of histone de-acetylase activity potentiates the PKA-dependent transcriptional cooperation between GATA4 and CBP. CV-1 cells were cotransfected with the -902-bp murine *StAR* promoter along with an empty expression vector (control) or expression vectors for GATA4, CBP, PKA, or different combinations of the 3 factors in the absence (−) or presence (+) of 80 ng/mL of the histone de-acetylase inhibitor trichostatin A (TSA). Promoter activities are shown as fold activation over control (±SEM). The marked enhancement of the GATA4/CBP or GATA4/PKA/CBP transcriptional cooperation elicited by TSA treatment indicates that GATA4 may be a target for CBP-mediated acetylation.

clear. In contrast to GATA1, gonadotropins and/or cAMP agonists have been shown to induce *Gata4* and/or *Gata6* expression in several gonadal cell lines, including MSC-1 Sertoli cells and mLTC-1 Leydig cells (Heikinheimo et al, 1997; Ketola et al, 1999). In humans, the stimulatory role of FSH in the regulation of GATA4 is supported, at least in the ovary, by the association of an inactivating mutation of the FSH receptor with greatly diminished *GATA4* expression (Vaskivuo et al, 2002).

The FOG Proteins in the Testis: Friend or Foe?—In the mouse testis, Fog1 and Fog2 are coexpressed with Gata1/4/6 in Sertoli and Leydig cells during development (Ketola et al, 2002; Robert et al, 2002). Fog1 is first detected in Sertoli cells on embryonic day 15.5 (E15.5) (Ketola et al, 2002). Testicular *Fog1* expression is maintained throughout development but eventually becomes restricted to stage VII–XII tubules (Ketola et al, 2002). In contrast to Fog1, Fog2 can be detected as early as the undifferentiated (bipotential) gonad stage in both sexes (Anttonen et al, 2003). After sex determination, Fog2 expression is maintained in the ovary but is rapidly down-regulated in the testis, so that by E17.5 only the testis capsule and Leydig cells show any significant Fog2 expression (Ketola et al, 2002). Abundant Fog2 expression then reinitiates after birth, and like Fog1, eventually becomes restricted to stage VII–XI tubules in the adult testis (Ketola et al, 2002).

Although the FOG proteins do not bind directly to DNA, in vitro studies indicate that they function as either enhancers or repressors of GATA transcriptional activity depending on the cell and promoter context being studied (Tsang et al, 1997; Fox et al, 1999; Holmes et al, 1999; Lu et al, 1999; Svensson et al, 1999, 2000a; Tevosian et al, 1999). Therefore, it has been suggested that the FOG proteins act as bridging molecules that link GATA proteins with other transcriptional regulators involved in either activation or repression. On GATA-dependent gonadal promoters, however, recent in vitro data have shown the FOG proteins to play a purely repressive role (Robert et al, 2002). The repressive nature of FOG proteins is due in part to their association with the potent transcriptional corepressor C-terminal binding protein-2 (CtBP-2) (Turner and Crossley, 1998; Holmes et al, 1999; Svensson et al, 2000a). Recently, a non–CtBP-2-dependent repression domain has also been identified in the N-terminal regions of the FOG proteins (Svensson et al, 2000a). Thus, FOG repression is likely an important mechanism for modulating the transcription of GATA-dependent genes in the testis in vivo. Indeed, targeted inactivation of the *Fog2* gene in the mouse leads to a block in testis development and the absence of several testicular markers, including Mis, P450scc, and 3β-HSD (Tevosian et al, 2002). At present, however, it remains unclear as to whether the lack of testis-specific markers in these animals is the result of a primary defect in Sertoli and/or Leydig cell gene expression or a secondary defect due to a block in Sertoli cell differentiation and/or fetal Leydig cell development.

The abundance of FOG1 and FOG2 in Sertoli cells indicates that these two proteins play a key role in fine-tuning GATA-dependent gene expression in Sertoli cells. To date, the best-studied GATA target gene in Sertoli cells is *MIS* (Viger et al, 1998; Tremblay and Viger, 1999, 2001b; Watanabe et al, 2000; Tremblay et al, 2001). Although the sexually dimorphic expression of *MIS* is largely controlled by Sox9 (Arango et al, 1999), how MIS levels are specifically down-regulated in postnatal Sertoli cells remains unclear. It has been suggested that the FOG proteins may play such a role (Robert et al, 2002). The fact that overexpression of Fog2 in primary Sertoli cell cultures has been shown to markedly decrease *Mis* promoter activity is consistent with this hypothesis (Tremblay et al, 2001). The sexually dimorphic expression of *Fog2* in the fetal mouse gonad has also been recently suggested to play a role in the down-regulation of *Mis* expression after overt ovarian differentiation (Anttonen et al, 2003).

Role of GATA Phosphorylation in Hormonal Signaling in the Testis.—Testicular gene expression and function is tightly regulated by the pituitary trophic hormones LH

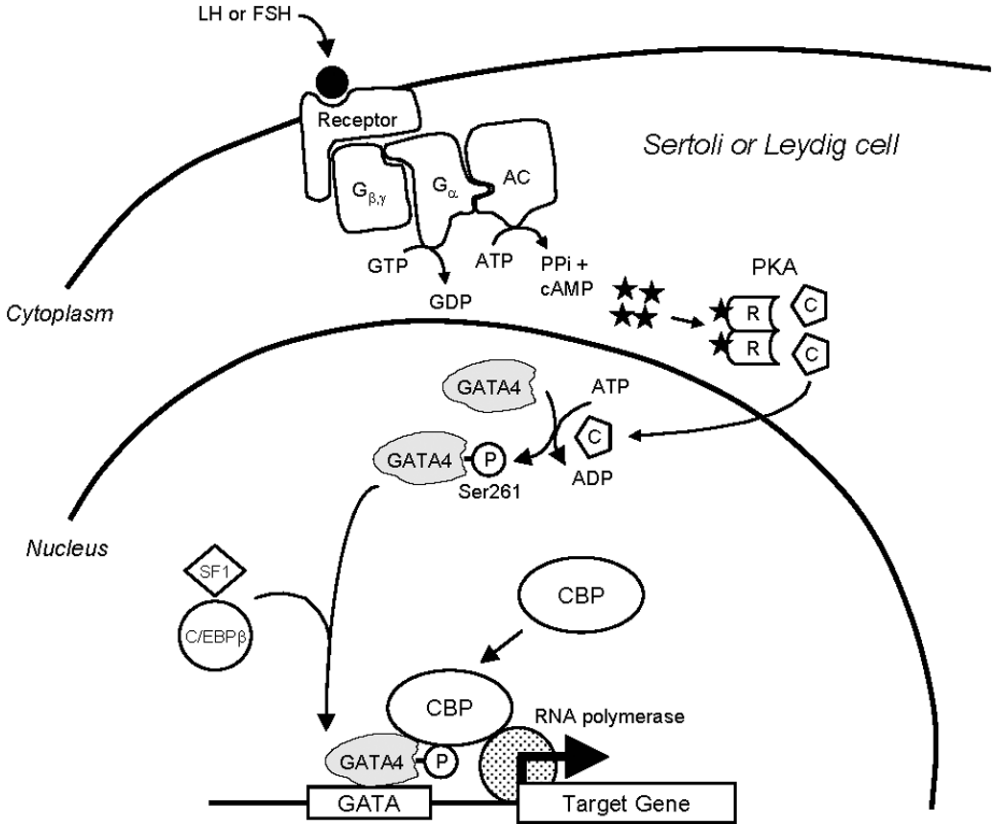

Figure 3. Proposed model for GATA factors as effectors of hormonal signaling in testicular cells. In the testis, Sertoli and Leydig cell gene expression and function are tightly regulated by the pituitary trophic hormones follicle-stimulating hormone (FSH) and leutinizing hormone (LH), respectively. Hormone binding to G-protein coupled cell surface receptors activates adenylate cyclase (AC), leading to increased intracellular cAMP levels. cAMP then binds the regulatory (R) subunit of protein kinase A (PKA), allowing dissociation of the PKA catalytic (C) subunit and its translocation to the nucleus, where it phosphorylates target proteins. In both Sertoli and Leydig cells, GATA factors are novel targets for PKA-mediated phosphorylation. As shown, phosphorylation of GATA4 at serine 261 allows for an enhanced cooperation with multiple transcriptional partners and the recruitment of the CBP coactivator. The end result is increased expression of hormonally regulated GATA-dependent target genes such as steroidogenic acute regulatory protein (*Star*), inhibin α (*Inha*), and P450 aromatase (*Cyp19*).

and FSH. In response to hormonal signals, gene expression is modulated predominantly through the cAMP-dependent intracellular signaling pathway, leading to activation of protein kinase A (PKA). PKA then translocates to the nucleus and phosphorylates target proteins (Montminy, 1997; Daniel et al, 1998). The best studied target of cAMP/PKA signaling is the transcription factor CREB (cAMP response element [CRE]-binding protein), which binds as a dimer to the 8-bp palindromic sequence CRE found in the regulatory regions of some cAMP-regulated genes (Mayr and Montminy, 2001). In the testis and other steroidogenic tissues, however, the promoters of several cAMP-regulated genes, such as steroidogenic acute regulatory protein (*Star*), inhibin a (*Inha*), stem cell factor (*Sl*), P450c17 (*Cyp17*), P450scc (*Cyp11a1*), and aromatase (*Cyp19*), lack ''classical'' CRE elements. This suggests that transcription factors, besides CREB, must also be acting as downstream effectors of cAMP signaling. Indeed, GATA4 has recently been identified as a novel target of hormone-induced cAMP/PKA signaling in gonadal cells. In response to cAMP, GATA4 is directly phosphorylated by PKA on a specific serine residue (Ser261) in the zinc finger region of the protein. This amino acid is perfectly conserved between the rat, mouse, human, bovine, rabbit, frog, fish, and chick GATA4 proteins. Phosphorylation of GATA4 allows for synergistic interactions with the C/EBPβ transcription factor (Tremblay et al, 2002) and permits recruitment of the CBP transcriptional co-activator (Tremblay and Viger, 2003c). This leads to robust stimulation of transcription from target gonadal promoters such as *StAR* (Tremblay and Viger, 2003c). To date, two independent phosphorylation sites have been mapped in the GATA4 protein: Ser105, which is a target of the MAP kinase (MAPK) signaling pathway in the heart (Liang et al, 2001), and Ser261 in gonadal cells (Tremblay and Viger, 2003c). Since the GATA4 protein contains two distinct phosphoacceptor sites, differential phosphorylation of GATA4 in response to various signals might constitute an important regulatory mechanism. However, the interrelationship between these two

phosphorylation sites for hormone-regulated gonadal gene expression has not yet been documented. Initial in vitro experiments suggest that Ser261, but not Ser105, is essential for the PKA-mediated enhancement of GATA4 transcriptional activity, at least on the *StAR* promoter (Viger et al, unpublished data). The fact that the Ser261 site alone is crucial for the PKA enhancement of GATA4 transactivation on the *StAR* promoter does not formally exclude a role for Ser105 phosphorylation on other targets genes or in response to other signals. In fact, gonadal cells do respond to certain stimuli that activate the cAMP pathway but without the involvement of PKA (Richards, 2001). In these cases, activation of MAPK appears to play a predominant role. Therefore, different signaling pathways and kinases might converge on GATA4 to regulate gonadal gene expression and function. Since the recruitment of CBP is a direct consequence of PKA-mediated phosphorylation of GATA4 (Tremblay and Viger, 2003c), and since CBP-mediated acetylation is known to modulate transcription factor activity, the GATA4 protein itself may be acetylated by CBP in response to hormonal stimulation. The fact that a deacetylase inhibitor (trichostatin A) can enhance the transcriptional activity of GATA4 alone or when used in combination with PKA/CBP (Figure 2) indicates that the GATA4 protein is indeed acetylated under basal conditions and that acetylation likely increases in response to hormonal stimulation. Thus, as depicted in Figure 3, GATA factors could very well represent the foundation of a large transcriptional complex (involving other transcription partners, such as SF1, C/EBPβ, and the co-activator CBP/p300) that is required for the activation of different sets of genes in response to differing signals, such as hormones, growth factors, and stress signals in the testis and other steroidogenic tissues.

Conclusion

Five years ago, little was known about the role of GATA factors in the reproductive system. Initial studies showed that certain GATA family members were indeed expressed in both the testis and ovary, and our thinking at that time was that they might be key regulators of a number of gonadal target genes. Little did we know that GATA factors would turn out to be important regulators of gonadal gene transcription throughout development. In the testis, this is particularly true for GATA4, which appears to function as a ''master regulator'' of multiple genes involved in both Sertoli and Leydig cell function. Indeed, in the past few years, the scope of GATA action in the male has broadened to include early testis development (Tevosian et al, 2002), male sex differentiation (Tremblay and Viger, 2003a), and steroidogenesis (Tremblay and Viger, 2003b). To date most of our insights have come from the identification of novel GATA-dependent promoters. In the years to come, further light will undoubtedly be shed on the role of GATA factors in andrology as selective in vivo GATA knockout or knockdown techniques are eventually applied to the testis and other tissues of the male reproductive tract.

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