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Computational identification of transcription frameworks of early committed spermatogenic cells

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Abstract

It is known that transcription factors (TFs) work in cooperation with each other to govern gene expression and thus single TF studies may not always reflect the underlying biology. Using microarray data obtained from two independent studies of the first wave of spermatogenesis, we tested the hypothesis that co-expressed spermatogenic genes in cells committed to differentiation are regulated by a set of distinct combinations of TF modules. A computational approach was designed to identify over-represented module combinations in the promoter regions of genes associated with transcripts that either increase or decrease in abundance between the first two major spermatogenic cell types: spermatogonia and spermatocytes. We identified five TFs constituting four module combinations that were correlated with expression and repression of similarly regulated genes. These modules were biologically assessed in the context that they represent the key transcriptional mediators in the developmental transition from the spermatogonia to spermatocyte.

Keywords

Bioinformatics; Spermatogenesis; Transcriptional regulation; Frameworks

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Introduction

Within the seminiferous tubule of the testis, spermatogenesis is marked by the succession of cellular events that lead to the production of viable and fertile spermatozoa (Holstein et al. 2003). In mouse the first wave of spermatogenesis from birth is complete in approximately 33 days and in man the first wave encompasses approximately 64 days. Spermatogenesis is initiated in the basal compartment of the germinal epithelium, with asymmetric division of spermatogonial stem cells A_{single} (As). This gives rise to daughter cells A_{paired} (Ap) that embark on the differentiative pathway (see de Rooij 2001). This first stage of spermatogenesis is characterized by the multiplication of spermatogonia through mitotic divisions. Each of the cells generated by a spermatogenic stem cell remains linked together by cytoplasmic bridges, until the later stages of spermiogenesis. Mitotic divisions appear to randomly occur between the different clusters of spermatogonia as Ap spermatogonia divide yielding $A_{aligned}$ (Aal) spermatogonia. With each division, the spermatogonia migrate further into the seminiferous tubule, toward the lumen, but they are unable to cross the tight junction between adjacent Sertoli cells that separate the basal and the luminal compartment. At any given moment Aal spermatogonia will differentiate into A1 spermatogonia which will then undergo a series of synchronized mitoses that give rise to type B spermatogonia. After the last mitotic division, the cells enter the meiotic phase of spermatogenesis that after two reduction divisions gives rise to a population of haploid round spermatids. Whereas the first meiotic division occurs over a long period and cells can be isolated in relatively pure form, the second reduction division giving rise to the round spermatid is compressed. The spermatids are then morphologically restructured shedding their cytoplasm as their chromatin condenses, the acrosome and flagellum form until they are released into the lumen of the seminiferous tubule as the terminally differentiated spermatozoa.

This complex differentiative process requires the induction of many genes regulated by mechanisms capable of restricting expression to specific stages of the spermatogenic pathway (Krawetz et al. 1999). For example, using a gain-of-function screen (Schulz et al. 2004) forced expression of numerous genes in *Drosophila* germ cells as well as in somatic progenitor cells, caused defects in early spermatogenesis.

Transcription factors (TFs) mediate gene expression by binding to their cognate sites within the promoter region. Many TFs belonging to major families such as CREB, heat-shock, Sox, zinc finger, homeo domain and basic helix-loop-helix, have been associated with the expression of spermatogenic genes (Maclean and Wilkinson 2005). One of the intensively studied TFs driving expression of spermatogenic genes is the testis-specific form of CREM, i.e., $CREM_T$. The importance of this TF was shown by the arrest of spermatogenic cells at the round spermatid stage in male mice when CREM was inactivated (Blendy et al. 1996; Nantel et al. 1996). $CREM_T$ is required for the transcription of postmeiotic genes including the protamines (P*rm*1, P*rm*2), the transition proteins (T*np*1, T*np*2), proacrosin and calspermin (Sassone-Corsi 1998). The importance of these and other TFs like Plzf (Buaas et al. 2004; Costoya et al. 2004), Hsf1 and Hsf2 (Zhang et al. 2002) is established.

TFs rarely operate in isolation. Complex patterns of regulation are cooperatively achieved through the action of n-element transcription modules or frameworks (Werner et al. 2003). These generate a binding structure sensitive to the states of potentially numerous regulatory pathways within the promoter region of a gene (Arnone and Davidson 1997). For example, it is likely that TFs work in concert as a transcription network to contextualize the expression of acrosin. Mutating the acrosin promoter SREBP2gc binding site was shown to decrease acrosin expression in spermatogenic cells (Wang et al. 2004). In vitro experiments have also suggested several other co-regulatory TFs of acrosin, including Tet-1 and YY1 (Nayernia et al. 1994; Schulten et al. 1999, 2001).

Two primary strategies for mapping regulatory networks have been developed. They are (1) analyzing the promoter regions of coordinately expressed genes for common TF binding sites and (2) identifying highly conserved sites in the promoter regions of orthologous genes. Both strategies rely on the postulate that transcription of similarly expressed genes when considered over sufficient genes can be statistically associated with sets of similar TFs.

The KSPMM database of spermatogenic promoter modules and motifs is a searchable web-based resource for the comparative analysis of promoter regions and their constituent transfactor elements in developing male germ cells (Lu et al. 2006b). The system is populated with promoter sequences from the database of transcription start sites (DBTSS) (Suzuki et al. 2004) and Transfac (Wingender et al. 1996) binding site matrix models to identify TF modules present in proximal promoter regions of genes coordinately expressed during spermatogenesis. This approach was adopted to assess whether other trans-acting factors may be involved in spermatogenic gene expression. A novel algorithm was used to determine over-representation of TF modules of co-expressed genes (Lu et al. 2006a; Naismith et al. 2008) from two independent microarray datasets for the first wave of murine spermatogenesis. Transcription frameworks governing the expression of spermatogenic genes from spermatogonia to spermatocytes were identified. The TFs were then confirmed using a proteomic strategy. The results of this study revealed a discrete set of TFs that are likely coordinated to govern gene expression during the first morphological progression of spermatogenesis.

Materials and methods

In-silico identification of transcription factor modules driving spermatogenic genes

Gene expression from two independent microarray time-course studies encompassing the first round of murine spermatogenesis were selected for analysis (Schultz et al. 2003a; Shima et al. 2004). The data from the three duplicate MG-U74 A, B and C microarrays for 11 time points between day 0 and day 56 (GEO Series GSE926) were employed as one dataset. A similar dataset covering ten time-points from day 1 to adult (GEO Series GSE640) was used as the validating dataset. Data from both sources were assigned to reflect the first two stages of development, spermatogonia (days 0–8), and spermatocytes (days 10–21) at which the germ cells are first observed. Comparisons between the median expression of genes across replicates and samples at these two different stages were undertaken. Those genes exhibiting stable expression within each stage and at least a twofold change in expression between stages ($P < 0.01$) were selected as exhibiting consistent stage linked modulated transcription.

Promoters for the genes of interest were obtained by querying the DBTSS on murine genome build 5 (May 2004). Analysis encompassed 1 kb 5′ of the transcription start site (TSS) and 200 bp 3′ of TSS. Where multiple TSSs were evidenced for a gene, the promoter sequences for all start sites were used as independent promoters. Candidate TF sites were identified using a threshold of a ≥0.96 match to the position weight matrices (Lu et al. 2006a). A single exception, the GATA family members bind essentially identical sequences, such that at ≥0.96 they are considered identical. Accordingly they were considered a single class the GATA-C (Class). To reduce complexity, transcription modules composed of binary elements were initially considered. This criterion enabled the identification of potentially functional hetero or homodimeric modules from a nonspecific separation model that permitted a distance range of no more than 200 bp and no less than 5 bp between two TFs (Frech et al. 1997; Klingenhoff et al. 1999). All possible module combinations were catalogued. The correlation between the expression changes, either positive, or negative, common to genes having a conserved subset of modules was then determined using a series of contingency tables (Lu et al. 2006a). The co-incidence matrices were highly biased. For

example, the absence of a module-combination with no significant change in expression was more likely to be observed. To reduce type I error a Liddell measure (Liddell 1976) was used to determine a *P* value, and threshold. This was set at $P < 0.005$. This test has been widely used for the analysis of clinical trial data and is based on the maximum likelihood estimate of a single parameter, in this case co-incidence, and provides greater power when compared to an exact test without randomization (Liddell 1976). The simplest module combinations capable of predicting expression were then considered further. Association of module combinations and genes changing in expression were visualized using the Osprey Network Visualization System, version 1.2.0 (Breitkreutz et al. 2003). SymAtlas (http://www.symatlas.gnf.org/SymAtlas) of the Genomic Institute of the Novartis Research Foundation was used to determine the tissue specificity of the transcripts retained in the analysis.

Isolation of spermatogenic stage-specific cells and nuclear protein extraction

Pachytene spermatocytes and round spermatids were isolated from adult CL/BL6 mice by unit density gravity sedimentation as described (Wykes and Krawetz 2003). The purity of the fractions was assessed through optical microscopy to ascertain the absence of contamination by testicular somatic cells (Sertoli cells, Leidyg cells). Fractions were typically of >90% pure. Spermatogonia were a gift from Dr. John McCarrey (University of Texas at San Antonio, USA). Nuclear protein extraction, used the Panomics Nuclear extraction kit and essentially as described by the manufacturer (Panomics Inc., Redwood City, CA). In brief, the cells were first washed in PBS then resuspended in Buffer A supplemented with 1 mM DTT, protease inhibitors and 0.4% IGEPAL to lyse the cells without affecting the nucleus. After centrifugation, the supernatant was removed and the nuclear pellet incubated in a high salt buffer supplemented with 1 mM DTT and protease inhibitors to extract the nuclear proteins comprising the TFs. These were then collected by centrifugation. The supernatant obtained after cell lysis was reserved for future analysis. Supernatant and nuclear protein extracts were stored at –20°C for subsequent use. Proteins were quantified using the Bradford assay.

Biological identification of spermatogenic transcription factors

Validation of spermatogenic TFs constituting the modules identified *in-silico* was carried out using the Panomic's TranSignal Protein/DNA Combo Arrays essentially as described by the manufacturer (Panomics Inc., Redwood City, CA). Briefly, nuclear proteins were incubated in the presence of a biotinylated DNA probe mix containing 345 TF binding site oligonucleotides. The protein/DNA complexes were purified away from unbound DNA probes, then the proteins released from the complex. The specifically bound DNA probes were then isolated, denatured then hybridized to an array containing 345 complementary TF binding sites. Subsequent to hybridization, the specifically hybridized sequences were detected by streptavidin–HRP chemiluminescence.

Western blot confirmation

Nuclear proteins (Panomics Inc., Redwood City, CA) were resolved on a 10% SDS-PAGE gel then transferred to Amersham Hybond ECL membranes (GE Healthcare Life Sciences, NJ). Detection of Stat1 and Stat3 employed the Stat Antibody Sampler according to the manufacturer's recommend protocol (Cell Signaling Technology, MA). Protein complexes were detected using the ECL Advance Western Blotting Detection (GE Healthcare Life Sciences, NJ).

Results

Microarray technology has enabled the identification of numerous genes associated with each stage of spermatogenesis. However, the transcriptional regulation of these genes remains poorly characterized. Using the initial microarray data describing the spermatogenic transcriptome (Schultz et al. 2003a; Shima et al. 2004), we selected the concordant group of transcripts that increase or decrease in abundance at least twofold after commitment to spermatogenesis, i.e., from spermatogonia to spermatocytes. Surprisingly only 160 transcripts were concordant between datasets and thus retained for analysis. TF modules, i.e., frameworks, within the promoter regions of these genes were then identified.

A summary of the number of promoters and module combinations that were identified in association with the change in expression between the two spermatogenic cell types is summarized in Table 1. Only one correlating framework was identified in the promoters of the genes encoding transcripts that increased from spermatogonia to spermatocytes. A greater number of correlating module combinations in the promoters of genes that decreased in expression from spermatogonia to spermatocytes was observed.

The modules represent the combination of five TFs, PAX2, GATA-C, STAT3, STAT1, and AREB6 defining the following modules PAX2-GATA-C + STAT3-GATAC; STAT1-GATA-C module along with either a GATA-CPAX2, STAT3-STAT3, or AREB6-GATA-C. While STAT1 and AREB6 were solely associated with a decrease in transcript levels, PAX2, GATA-C and STAT3 were associated with both the increase and decrease in expression. This may reflect that these factors work together in both positive and negative combinations to limit expression within a specified range.

Increasing levels of transcripts from spermatogonia to spermatocytes

As shown in Fig. 1, as meiosis begins, a single transcription framework, PAX2-GATA-C + STAT3-GATA-C, was common among the promoters of the 70 genes that exhibited an increase in transcript abundance from spermatogonia to spermatocytes. A subset of genes, i.e., Bad, Nphp1, Lrrc28, Tsga8, Sumo, Pde1c, Capbpip1 were DBTSS classified as containing two promoters and both were considered. A list of the genes containing this framework, their SymAtlas level of expression in testis and ontology is summarized in Table 2. These genes were representative of a broad range of ontologies including transport, cell cycle, metabolism, protein biosynthesis, protein phosphorylation, RNA processing, signal transduction, transcription, cell organization, biogenesis, and protein degradation.

Decreasing levels of transcripts from spermatogonia to spermatocytes

As summarized in Fig. 2 and Table 3, three transcription frameworks were common among the promoters of 83 genes that presented a decrease in transcript abundance from spermatogonia to spermatocytes. A subset of genes, i.e., Ddx3, Notch2, Ric8b, Otud5, Cugbp2, Tcf12 was attributed in DBTSS as containing two promoter regions. As above, both were considered. Interestingly all frameworks contained the STAT1-GATA-C module accompanied with either a GATA-C-PAX2, STAT3-STAT3, or AREB6-GATA-C module. Fourteen genes were associated with all module combinations. Ontology groups associated with each framework included signal transduction, transport, transcription, metabolism, RNA processing, protein biosynthesis, and protein degradation.

Identification of transcription factors present in spermatogenic cell nuclei

To ascertain whether the TFs were present in spermatogenic cell nuclei, spermatogenic cells were isolated from testis and interrogated with Panomic's TranSignal Protein/DNA Combo Arrays. The results are shown in Fig. 3. All of the TFs identified by the computational

approach were represented on the 345-element array. Patterns of TFs present in type A spermatogonia and spermatocytes were very similar. TFs previously shown to be present in spermatogenic cells, such as SP1 (J3), YY1 (J2), E2F (J1) and Ahr/Arnt (J4) were detected (Persengiev et al. 1996; Schulten et al. 2001; Schultz et al. 2003b; El-Darwish et al. 2006). TFs including PAX2 (G21), GATA1 (G6), GATA2 (I6), and STAT1/STAT3 (M22) were detected in both cell types, while AREB6 (B10) was not. It is of note that both STAT1 and STAT3 were detected using a binding site common to both TFs, whereas STAT1 and STAT3 specific sequences appeared absent. STAT proteins are primarily cytosolic and translocate to the nucleus upon activation through phosphorylation and dimerization (Desrivieres et al. 2006). The presence of STATs was verified by Western analysis, using antibodies specific for STAT1 and STAT3. Both cytosolic and nuclear fractions for each cell type were resolved by SDS-PAGE, transferred to nitrocellulose membranes and then processed for immunodetection of STAT proteins. As shown in Fig. 4, both STAT1 and STAT3 are present in spermatogenic cells.

Discussion

An in silico strategy was developed to mine microarray data for common transcriptional control elements. The utility of this strategy was previously validated shown using a yeast cell cycle dataset where known TFs were indentified (Lu et al. 2006a). Having validated this strategy, the question becomes, can transcriptional frameworks that demarcate differentiation be identified? To directly address this question, two studies of the first wave of murine spermatogenesis were identified and the changes in the transcriptional profiles from spermatogonia to spermatocyte compared. This resolved several combinations of TF modules embedded within promoters, i.e., frameworks that were strikingly correlated with their coordinate change in expression from the spermatogonial to spermatocyte stage of spermatogenesis. When taken individually, the TFs that comprise the frameworks identified, i.e., PAX2, GATA-C, STAT1, STAT3, and AREB6, have many binding sites in the promoters analyzed. However, the modules they constitute and identified using our computational approach are generally present once or twice in the promoters. Thus, considering modules instead of the individual binding sites eliminated the possibility that the transcription factors were identified because their binding sites were present many times in the promoter regions analyzed. A total of 160 transcripts changed expression in a concordant manner. This somewhat low level of concordance was unexpected since both studies used the same approach for RNA extraction through the first wave of spermatogenesis and the same microarray platform. This likely reflects the high stringency of the statistical bounds employed in this study to minimize type 1 error.

Analysis of microarray data from isolated adult spermatogenic cells (Lee et al. 2006) and the first wave of spermatogenesis (Schultz et al. 2003a; Shima et al. 2004) corroborates the presence of the majority of these TFs. Interestingly, GATA1 and GATA2 proteins were present in accordance with transcriptome data. Of the five TFs computationally predicted, four were validated as present in spermatogonia and spermatocyte nuclei using a protein/DNA array. Recently the presence of GATA binding sites in spermatocyte-specific genes was reported (Lee et al. 2006), and mRNAs corresponding to the GATA family members have been identified (Schultz et al. 2003a; Shima et al. 2004). Their over-representation in module combinations regulating spermatogenic cell expression is novel. Other TFs that were identified have been associated with spermatogenesis. For example PAX2 was detected in a testis-specific manner in the rainbow trout (Baron et al. 2005) and shown to be important in the formation and maintenance of the male reproductive tract in mammals (Kobayashi and Behringer 2003). STAT3 has been detected in adult mouse testis (Murphy et al. 2005) and is suggested to be involved in the self-renewal of spermatogenic cells in *Drosophila* (Tulina and Matunis 2001). STAT1 was detected in mature spermatozoa (D'Cruz et al. 2001) and as

summarized by Western analysis in Fig. 4, both STAT1 and STAT3 are present in cells of the spermatogenic lineage.

The protein/DNA array enabled the validation of the majority of the modules identified as changing in gene expression from spermatogonia to spermatocytes, in addition to identifying other TFs present in murine spermatogenic cells. This method has been successfully applied in other studies to identify pathways by which IL-13 down-regulates the inducible nitric oxide synthase gene (Shao et al. 2007), the TFs downstream of the protease activated receptor in mouse urinary bladder during inflammation (Saban et al. 2007), and the cis-elements regulated by toxic nitric oxide (NO) concentrations in neuroblastoma cells (Dhakshinamoorthy et al. 2007). In general, the module combinations identified *in-silico* in the promoters of genes that change between spermatogonia and spermatocytes were validated. The sole exception was the AREB6-GATA-C + STAT1-GATA-C combination. AREB6 was not detected in either of the spermatogenic cell types using the protein/DNA array method. Perhaps another currently uncharacterized TF binds to this location. Irrespective, within this framework the STAT1-GATA-C module was validated. Furthermore, the majority of the genes associated with this module were associated with either one or both of the other two modules to form functional frameworks. It is possible that the STAT1-GATA-C module in itself is sufficient to down-regulate those genes. Whether all module combinations are required to down-regulate the 14 genes associated with all three frameworks remains to be determined.

Other spermatogenic-specific TFs have been identified, but their consensus binding site sequences largely remain unknown and the computational identification of transcription frameworks must be afforded this consideration. For example, Sohlh1, a basic helix-loop-helix TF detected in oocytes and spermatogonia, was recently suggested to be involved in differentiation of spermatogonia to spermatocytes (Ballow et al. 2006a). Similarly, Sohlh2, is only detected in spermatogonia in the male (Ballow et al. 2006b). At present, the binding sites for these TFs remains to be fully elucidated.

Extending this approach to determine transcription frameworks governing the expression of coregulated genes in adult spermatogenic cells is the clear next step. To date only one microarray dataset from isolated male germ cells has become publicly available (Namekawa et al. 2006) and caution must be exercised as recent studies suggest differences in the “behavior” of spermatogenic cells during pubertal and adult spermatogenesis (Jahnukainen et al. 2004; Yoshida et al. 2006; Ebata et al. 2007). This will require careful consideration.

Analysis of combinations of corelated modules in the promoters of coregulated genes enables the determination of potential frameworks involved in gene expression in a tissue/cell specific context. The multi-factor composition of all the significantly detected module combinations has highlighted the extent to which the conjunction of TFs permit tissue-specific contextualization of regulation to be achieved with even a relatively limited Transcription Factor vocabulary. The crosstalk between multiple transcription networks may permit relatively ubiquitous binding sequences such as those targeted by GATA TFs to exert a highly stage-specific influence to fine tune and specify gene expression.

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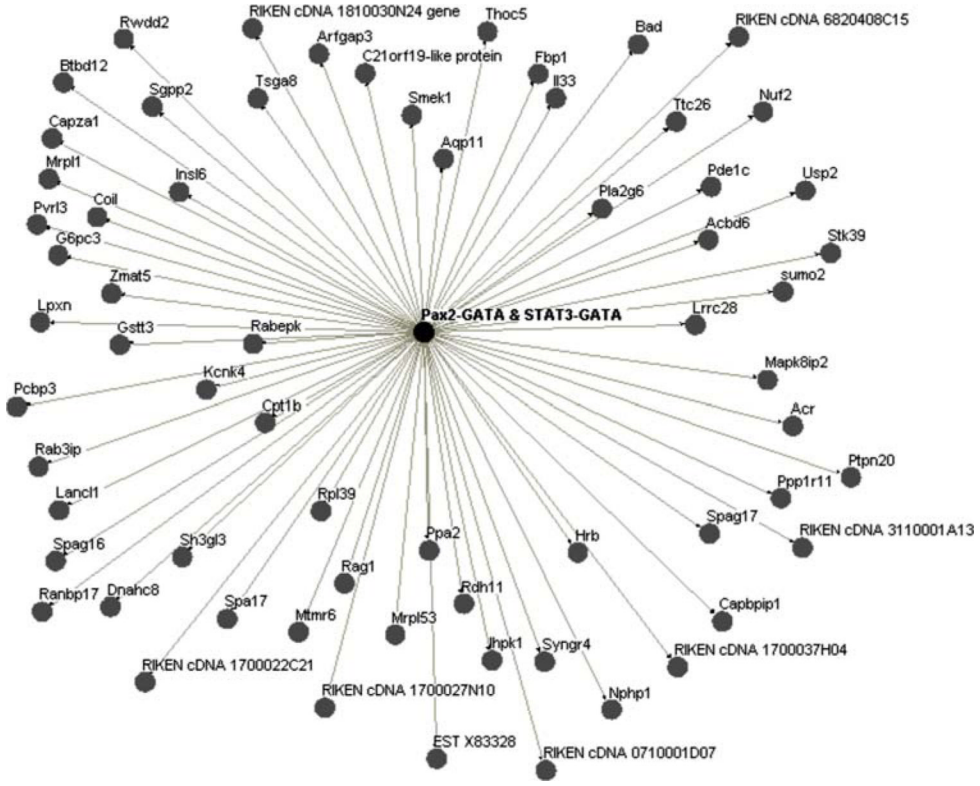

Fig. 1.

Transcription factor (TF) module combinations of encoded transcripts that increase in abundance. TF module combinations overrepresented in the promoters of genes that increase in expression from spermatogonia to spermatocytes were determined. They are represented as an interaction map

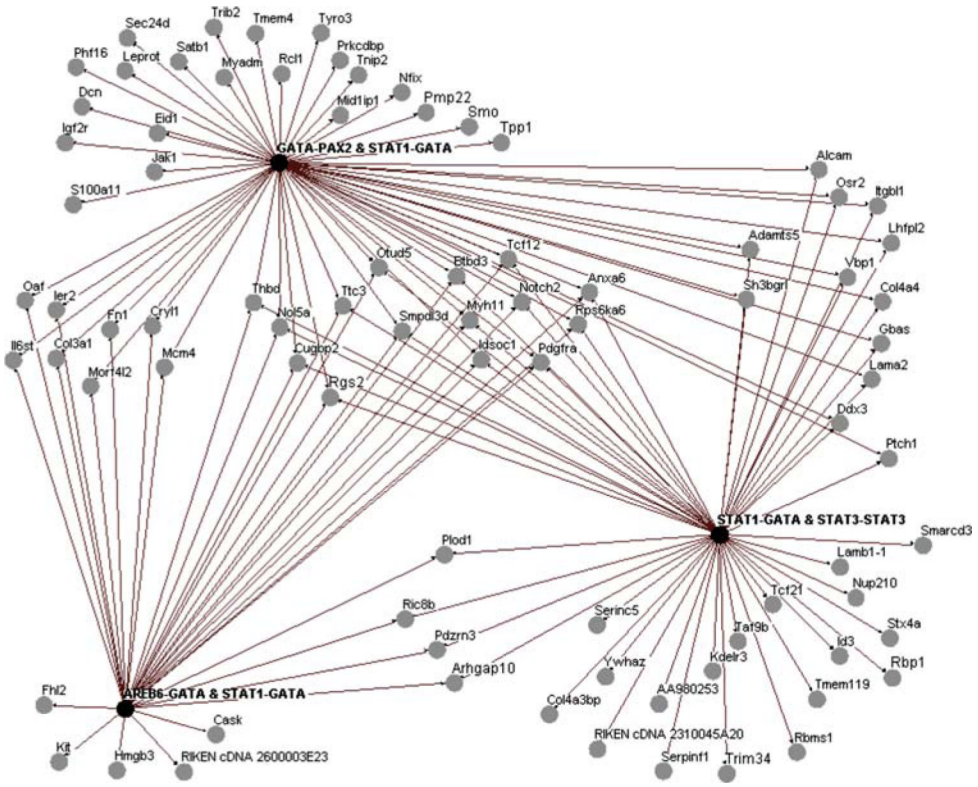

Fig. 2.

TF module combinations of encoded transcripts that decrease in abundance. TF module combinations overrepresented in the promoters of genes that decrease in expression from spermatogonia to spermatocytes were determined. They are represented as an interaction map. While the majority of genes are represented by a single unique combination, multiple combinations of modules are observed for others as revealed by a series of interconnecting nodes

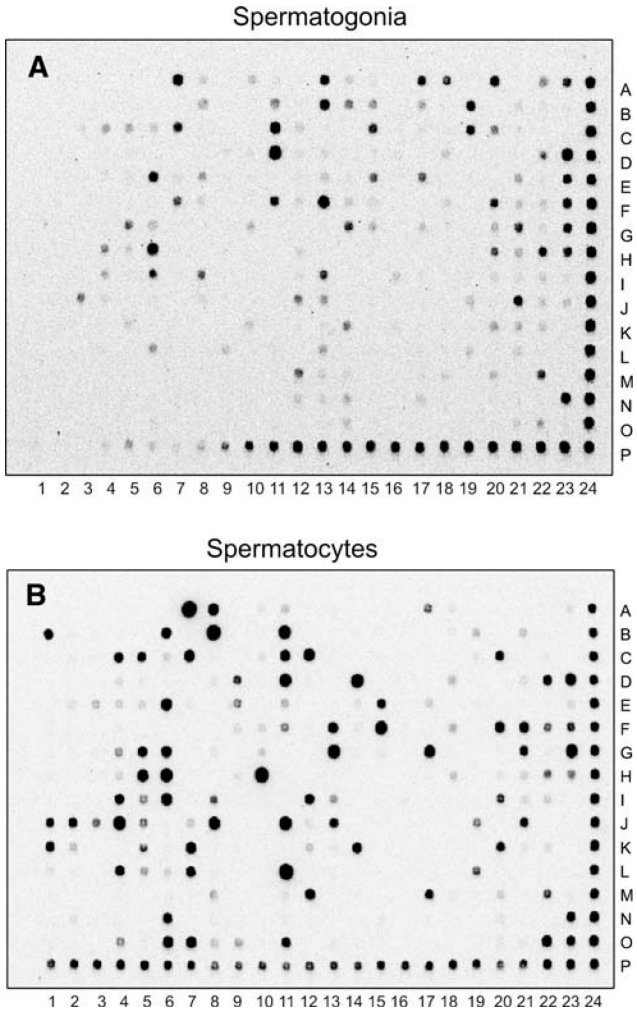

Fig. 3.

Identification of TFs present in adult mouse spermatogenic cells. Nuclear protein fractions from (**a**) spermatogonia and (**b**) spermatocytes were interrogated using a protein/DNAbinding assay. The interaction of 345 elements spotted on the array was assessed by hybridization to a corresponding set of biotinylated probes

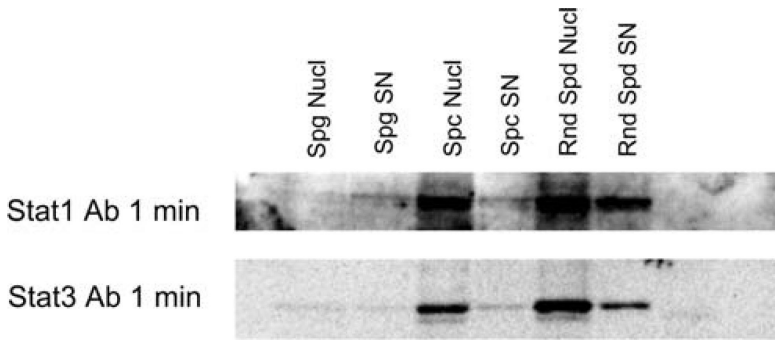

Fig. 4.

Immunodetection of STAT1 and STAT3 in nuclear extracts from adult mouse spermatogenic cells. *Spg* spermatogonia, *Spc* spermatocytes, *Rnd Spd* round spermatids were isolated by unit gravity sedimentation. Nuclear extracts, Nucl, were then prepared from each cell type. Equivalent quantities of protein supernatant SN and Nucl extracts obtained were separated on SDS-PAGE, transferred to Nitrocellulose, then the presence of STAT1 and STAT3 assessed by immunodetection. The hybridizing portion is shown

Table 1

Data summary of module combinations that correlate with changes in expression from spermatogonia to spermatocytes

Stage and direction of >twofold expression	Promoters in category	Module combinations
Spermatogonia–spermatocytes: increase	77	1
Spermatogonia–spermatocytes: decrease	83	3

Table 2

Summary of the properties of transcripts increasing in expression from spermatogonia to spermatocytes

AffyID	Gene name	Gene ID	Testis expression (SymAtlas)	Function
104346_at	Acyl-coenzyme A binding domain containing 6	Acbd6	Testis-specific	
97811_at	ADP-ribosylation factor GTPase activating protein 3	Arfgap3	Median	Vesicle-mediated transport
109772_at	Aquaporin 11	Aqp11	Testis-specific	Transport
104328_at	Aquaporin 9	Aqp9	Overexpressed testis	Water transport
99670_at	Bcl-associated death promoter	Bad	Median	Induction of apoptosis
133861_at	BTB (POZ) domain containing 12	Btbd12	Overexpressed testis	
98959_at	C21orf19-like protein	C21orf19-like protein		
104029_at	Calmegin	Clgn	Overexpressed testis	Proteolysis and peptidolysis
109455_at	CAP-binding protein complex interacting protein 1	Capbpip1		
93499_at	Capping protein (actin filament) muscle Z-line, alpha 1	Capza1	Overexpressed testis	Actin cytoskeleton organization and biogenesis
113828_at	Carnitine palmitoyltransferase 1b	Cpt1b	Median	Fatty acid beta-oxidation
116319_at	CDP-diacylglycerol synthase 1	Cds1	Median	Phospholipid biosynthesis
97377_at	Coilin	Coil	Overexpressed testis	Regulation of transcription
95309_at	Dynein, axonemal, heavy chain 8	Dnahc8	Testis-specific	
95662_at	EST X83328	EST X83328		
96918_at	Fructose bisphosphatase 1	Fbp1	–	Gluconeogenesis
104310_at	Glucose 6 phosphatase	G6pc3	Overexpressed testis	
106663_at	Glutathione S-transferase, theta 3	Gstt3	Overexpressed testis	
103397_at	HIV-1 Rev binding protein AU045498	Hrb	Overexpressed testis	mRNA-nucleus export
130609_at	Inositol hexaphosphate kinase 1	Ihpk1	Median	Myo-inositol metabolism
113957_at	Insulin-like 6	Insl6	Testis-specific	Regulation of transcription
113122_i_at	Interleukin 33	Il33	Median	
103656_at	LanC (bacterial lantibiotic synthetase component C)-like 1	Lancl1	Median	G-protein coupled receptor protein signaling pathway
116115_at	Leucine rich repeat containing 28	Lrrc28	Median	
110229_at	Leupaxin	Lpxn	Overexpressed testis	Signal transduction
93675_at	Male germ cell-associated kinase	Mak	Testis-specific	Protein amino acid phosphorylation
111893_at	Mitochondrial ribosomal protein L1	Mrpl1	Median	Protein biosynthesis
99153_at	Mitochondrial ribosomal protein L53	Mrpl53	Median	
116680_at	Mitogen-activated protein kinase 8 interacting protein 2	Mapk8ip2	Overexpressed testis	Vesicle-mediated transport
106572_at	Myotubularin related protein 6	Mtmr6	Overexpressed testis	Protein amino acid dephosphorylation
106256_at	NDC80 kinetochore complex component	Nuf2	–	
98614_at	Nephronophthisis 1 (juvenile) homolog	Nphp1	Testis-specific	Actin cytoskeleton organization and biogenesis
109727_at	PHD finger protein 2	Phf2	Median	Regulation of transcription
105240_at	Phosphodiesterase 1C	Pde1c	Overexpressed testis	Signal transduction
97965_at	Phospholipase A2, group VI	Pla2g6	Overexpressed testis	Phospholipid metabolism

AffyID	Gene name	Gene ID	Testis expression (SymAtlas)	Function
111239_at	Poliovirus receptor-related 3	Pvrl3	Testis-specific	
111288_at	Poly(rC) binding protein 3 AlphaCP-3	Pcbp3	Overexpressed testis	mRNA metabolism
113953_at	Potassium channel, subfamily K, member 4	Kcnk4	Median	Potassium ion transport
93207_at	Preproacrosin	Acr	Testis-specific	Proteolysis and peptidolysis
113964_at	Protein phosphatase 1, regulatory (inhibitor) subunit 11	Ppp1r11	–	
93658_at	Protein tyrosine phosphatase, non-receptor type 20	Ptpn20	Testis-specific	
103881_at	Pyrophosphatase (inorganic) 2	Ppa2	Overexpressed testis	
95539_at	RAB3A interacting protein	Rab3ip	Median	
109997_at	Rab9 effector protein with kelch motifs	Rabepk	Median	
113638_at	Rag1/Nwc fusion	Rag1	Median	
140878_at	RAN binding protein 17	Ranbp17	Testis-specific	Protein-nucleus import
99591_i_at	Retinol dehydrogenase 11	Rdh11	Overexpressed testis	Retinol metabolism
104544_at	Ribosomal protein L39-like protein	Rpl39	Low	Protein biosynthesis
107508_at	RIKEN cDNA 0710001D07	RIKEN cDNA 0710001D07	Testis-specific	
111023_at	RIKEN cDNA 1700022C21	RIKEN cDNA 1700022C21	Testis-specific	
115373_at	RIKEN cDNA 1700027N10	RIKEN cDNA 1700027N10	Overexpressed testis	
97210_at	RIKEN cDNA 1700037H04	RIKEN cDNA 1700037H04	Overexpressed testis	
108060_at	RIKEN cDNA 1810030N24 gene	RIKEN cDNA 1810030N24	Overexpressed testis	
96640_at	RIKEN cDNA 3110001A13	RIKEN cDNA 3110001A13	Median	
129854_at	RIKEN cDNA 6820408C15	RIKEN cDNA 6820408C15	Testis-specific	
117204_at	RWD domain containing 2	Rwdd2	Overexpressed testis	
113152_at	Serine/threonine kinase 39	Stk39	Overexpressed testis	Protein amino acid phosphorylation
101412_at	SH3-domain GRB2-like 3	Sh3gl3	Overexpressed testis	Signal transduction
112181_at	SMEK homolog 1	Smek1	–	
105805_at	Sperm associated antigen 16	Spag16	Overexpressed testis	
133455_at	Sperm associated antigen 17	Spag17	Median	
101850_at	Sperm autoantigenic protein 17 Sp17	Spa17	Testis-specific	Signal transduction
136288_at	Sphingosine-1-phosphate phosphotase 2	Sgpp2	Median	
110159_at	SUMO/sentrin specific peptidase 2	Sumo		
99531_at	Synaptogyrin 4	Syngr4	Testis-specific	Transport
112945_at	Testis specific gene a8	Tsga8	Testis-specific	
113799_at	Tetratricopeptide repeat domain 26	Ttc26	Overexpressed testis	
109128_at	THO complex 5	Thoc5	–	
92821_at	Ubiquitin specific peptidase 2	Usp2	Overexpressed testis	Ubiquitin-dependent protein catabolism
113166_at	Zinc finger, matrin type 5	Zmat5	Overexpressed testis	

Affymetrix probe ID, gene name and gene ID are presented for each gene analyzed. Expression in testis compared to other mouse tissues from GNF SymAtlas database and the cellular function, when available from NIH David are summarized

Table 3

Summary of the properties of transcripts decreasing in expression from spermatogonia to spermatocytes

AffyID	Gene name	Gene ID	Testis expression (SymAtlas)	Function
116914_at	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 5	Adamts5	Median	Proteolysis and peptidolysis
107969_at	Activated leukocyte cell adhesion molecule	Alcam	Low	Signal transduction
94304_at	Annexin A6	Anxa6	Low	Blood coagulation
111383_at	BTB (POZ) domain containing 3	Btbd3	Median	
102248_f_at	Calcium/calmodulin-dependent serine protein kinase (MAGUK family) DXPri1	Cask	Median	Protein amino acid phosphorylation
101542_f_at	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3	Ddx3	Low	
93534_at	Decorin	Dcn	Low	Organogenesis
99191_at	EP300 interacting inhibitor of differentiation 1	Eid1	Low	
92852_at	Fibronectin 1	Fn1	Low	Signal transduction
100986_at	Four and a half LIM domains 2	Fhl2	Median	Transcription
102402_at	Glioblastoma amplified sequence AV006093	Gbas	Low	Metabolism
99109_at	Immediate early response 2	Ier2	Median	
92614_at	Inhibitor of DNA binding 3	Id3	Low	Development
135201_at	Integrin, beta-like 1	Itgbl1	Median	Integrin-mediated signaling pathway
94345_at	Interleukin 6 signal transducer	Il6st	Low	Signal transduction
106222_at	Ipoma HMGIC fusion partner-like 2	Lhfpl2	Low	
97247_at	Isochorismatase domain containing 1	Idsoc1	Low	
98911_at	Janus kinase 1	Jak1	Median	Intprotein amino acid phosphorylation
104464_s_at	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor	Kdelr3	Median	Intracellular protein transport
99577_at	Kit ligand	Kitl		
99956_at	Kit oncogene	Kit	Median	Protein amino acid phosphorylation
92366_at	Lamin alpha 2	Lama2	n.a	Cell adhesion
101948_at	Laminin B1 subunit 1	Lamb1-1	Median	
95135_at	Mid1 interacting protein 1 [gastrulation specific g12-like (zebrafish)]	Mid1ip1	Low	
93041_at	Minichromosome maintenance deficient 4 homolog	Mcm4	Low	Regulation of transcription
97990_at	Myosin, heavy polypeptide 11	Myh11	Median	Cytoskeleton organization and biogenesis
101930_at	Nuclear factor I/X	Nfix	Low	Regulation of transcription
95109_at	Nucleolar protein 5A	Nol5a	Low	Regulation of transcription
108076_at	Nucleoporin 210	Nup210	Low	
106557_at	OAF homolog	Oaf	Median	
96813_f_at	OTU domain containing 5	Otud5	Low	
113047_at	PDZ domain containing RING finger 3	Pdzrn3	Low	
102395_at	Peripheral myelin protein	Pmp22	Low	Negative regulation of cell proliferation
114557_at	PHD finger protein 16	Phf16	n.a	

AffyID	Gene name	Gene ID	Testis expression (SymAtlas)	Function
95079_at	Platelet derived growth factor receptor, alpha polypeptide	Pdgfra	Low	Protein amino acid phosphorylation
102990_at	Procollagen, type III, alpha 1	Col3a1	Median	Histogenesis and organogenesis;organogenesis
105660_at	Procollagen, type IV, alpha 3	Col4aebp		
136277_at	Procollagen, type IV, alpha 4	Col4a4	Median	Regulation of transcription
112304_at	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1	Plod1	Low	
97496_f_at	Protein Kinase C delta-binding protein	Prkcdbp	Median	
97844_at	Regulator of g-protein signaling 2	Rgs2	Median	Cell cycle
115426_at	Resistance to inhibitors of cholinesterase 8 homolog B	Ric8b	Median	
104716_at	Retinol binding protein 1	Rbp1	Median	Retinoid metabolism
133065_at	Rho GTPase activating protein 10	Arhgap10	Median	
116381_at	Ribosomal protein S6 kinase polypeptide 6	Rps6ka6	Overexpressed testis	Signal transduction
109975_at	RIKEN cDNA 2310045A20	RIKEN cDNA 2310045A20	Median	
116890_at	RIKEN cDNA 2600003E23	RIKEN cDNA 2600003E23	Median	
96207_at	RNA binding motif, single stranded interacting protein 1	Rbms1	Low	Regulation of translation
98923_at	RNA terminal phosphate cyclase-like 1	Rcl1	Low	
98600_at	S100 calcium binding protein A11	S100a11	Low	Negative regulation of cell
109669_at	SEC24 related gene family, member D	Sec24d	Low	Intracellular protein transport
93574_at	Serine (or cysteine) peptidase inhibitor, clade F, member 1	Serpinf1	Median	Cell proliferation
106281_f_at	Serine incorporator 5	Serinc5	Low	
96812_at	Smoothened homolog	Smo	Low	G-protein coupled receptor protein signaling pathway
111448_f_at	Special AT-rich sequence binding protein 1	Satb1	Median	Regulation of transcription
115354_at	Sphingomyelin phosphodiesterase, acid-like 3B	Smpdl3d	Median	
108488_at	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	Smarcd3	Low	Regulation of transcription
103781_at	Syntaxin 4A	Stx4a	Low	Intracellular protein transport
115703_at	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor	Taf9b	Overexpressed testis	
98555_at	Tetratricopeptide repeat domain 3	Ttc3	Low	
104601_at	Thrombomodulin	Thbd	Low	Blood coagulation
112472_at	TNFAIP3 interacting protein 2	Tnip2	Median	Negative regulation of viral genome replication
98981_s_at	Transcription factor 12	Tcf12	Median	Regulation of transcription
103050_at	Transcription factor 21	Tcf21	Median	Regulation of transcription
113196_at	Transmembrane protein 119	Tmem119	Low	
100039_at	Transmembrane protein 4	Tmem4	Low	
113139_at	Tribbles homolog 2	Trib2	Low	
115520_at	Tripartite motif protein 34	Trim34	n.a	
93595_at	Tripeptidyl peptidase I Cln2	Tpp1	Low	

AffyID	Gene name	Gene ID	Testis expression (SymAtlas)	Function
96766_s_at	TYRO3 protein tyrosine kinase 3	Tyro3	Median	Signal transduction
97544_at	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	Ywhaz	Low	RAS protein signal transduction
103955_at	Crystallin, lambda 1	Cryl1	n.a	Fatty acid metabolism
104030_at	Patched homolog 1 (*Drosophila*)	Ptch1	Median	
104188_at	Notch homolog 2 (*Drosophila*)	Notch2	Median	Regulation of transcription
113982_at	Odd-skipped related 2 (*Drosophila*)	Osr2	Overexpressed testis	
93600_at	Leptin receptor overlapping transcript	Leprot	Median	
93806_at	SH3-binding domain glutamic acid-rich protein like	Sh3grbl	Median	Protein complex assembly
95117_at	Insulin-like growth factor 2 receptor	Igf2r	Low	Signal transduction
96285_at	Myeloid-associated differentiation marker	Myadm	Low	
96632_at	Mortality factor 4 like 2	Morf4l2	n.a	
97255_at	CUG triplet repeat, RNA binding protein 2	Cugbp2	Median	mRNA splice site selection
98038_at	High mobility group box 3	Hmg3	Median	Regulation of transcription
101509_at	Von Hippel-Lindau binding protein 1	Vbp1	Low	Protein folding

Affymetrix probe ID, gene name and gene ID are presented for each gene analyzed. Expression in testis compared to other mouse tissues from the GNF SymAtlas database and the cellular function, where available from NIH David are summarized