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# Insight Into the Molecular Program of Meiosis

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## 1. Introduction

“We estimate that >2,300 genes (~4% of the mouse genome) are dedicated to male germ cell-specific transcripts, 99% of which are first expressed during or after meiosis”. This quotation from a paper published by Schultz et al. (2003), reflects the tremendous complexity of gamete production, the essence of which is meiosis. Meiosis is a differentiative process in which seemingly contradicting molecular pathways are activated simultaneously. On one hand the regular components and checkpoints of the cell division machinery, which is complex enough by itself, are utilized, but on the other hand a whole array of genes are activated to enable the unique characteristics of the meiotic division, such as partition of homologous chromosomes, and not the sister chromatids, in meiosis I, or executing cell division without a prior DNA duplication in meiosis II. On one hand double strand breaks are deliberately formed to ensure pairing of homologous chromosomes and recombination, but on the other hand a whole array of genes involved in DNA repair and safeguarding genome integrity are alerted. The meiotic complexity is also exemplified by the extensive dependence on a cross-talk between germ cells themselves, and between the differentiating germ cells and their surrounding somatic cells, i.e. Sertoli cells in the testis or granulosa cells in the ovarian follicle. Finally, the complexity of the meiotic process is depicted by the differences between males and females, regarding both the outcome of the meiotic division (four basically similar post meiotic round spermatids in the male versus one functional egg and three polar body cells that degenerate in the female), and its kinetics (a continuous process in the male versus an in-continuous process in the female). It is, therefore, absolutely crucial that the very many different molecular pathways operating during meiosis be tightly concerted and regulated. However, Virginia Hughes, in a paper published in *Nature medicine* (2008), stated that: “So far, scientists have identified nearly 300 DNA mutations in man with reproductive defects”, implying that our understanding of the meiotic molecular network is still very limited, although significant progress has been made since 2008.

Various techniques were applied during the years to study the role of different genes during meiosis. These include: 1) developing spermatogenic cell culture systems and studying the effect of over-expressing / silencing specific genes on entry into and progressing through meiosis *in-vitro* (Farini et al. 2005; Feng et al. 2002; Nayernia et al. 2006); 2) developing genetically modified animal models, mainly mice, (including Knockout models) to study the effect of modification or deletion of a specific gene on the meiotic process *in-vivo* (review in Jamsai & O’Byran, 2011); 3) utilizing complementation approaches to detect genes with new

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meiotic functions, such as *Aym1* (Malcov et al. 2004); and 4) utilizing spermatogonial cell transplantation approaches in testicular repopulation studies (Brinster 2002; Brinster & Zimmermann 1994; McLean 2005). These studies contributed greatly to understanding the role of specific genes during meiosis, but it was not until the emergence of the microarray technology and the development of sophisticated bioinformatics tools that large scale studies on meiotic molecular networks and regulation could be executed. Indeed, several microarray studies on meiotic genes were performed (Chalmel et al. 2007; Schlecht et al. 2004; Schultz et al. 2003; Shima et al. 2004; Yu et al. 2003), yielding a huge amount of new information. However, the biological significance of the transcriptomic data obtained in these experiments, in terms of understanding the molecular program of meiosis, is still an ongoing challenge.

Using mouse spermatogenesis as a model system, we recently performed a comprehensive meiotic microarray study (Waldman Ben-Asher et al., 2010). This study was based on the known developmental schedule of the first spermatogenic wave (Bellve et al. 1977; Malkov et al. 1998). According to this developmental schedule, until post-natal age of 7 days (pn d7), the seminiferous tubules within the testis contain only pre-meiotic spermatogonia cells, along side with the somatic Sertoli cells. By pn d10, spermatocytes from the first spermatogenic wave enter prophase I of the meiotic division, and by pn d12 zygotene spermatocytes first appear. At pn d14 and 17, these cells reach the early and late pachytene stage, respectively. At pn d21, post meiotic round spermatids are found and at pn d24 and d27 elongating and elongated haploid spermatids are present in the testis, respectively. Testes from pn d35 mice are expected to contain the entire spermatogenic lineage. Thus, in our microarray study we compared the testicular transcriptomes of pups at: pn d7, pn d10, pn d12, pn d14, and pn d17. In these experiments we were able to clearly define six apparent patterns of gene expression throughout meiosis (Figure 1). Given this as a starting point, we will describe in this chapter the use of several bioinformatic approaches to ascribe biological significance to our results, thus getting new insights into the molecular program of meiosis.

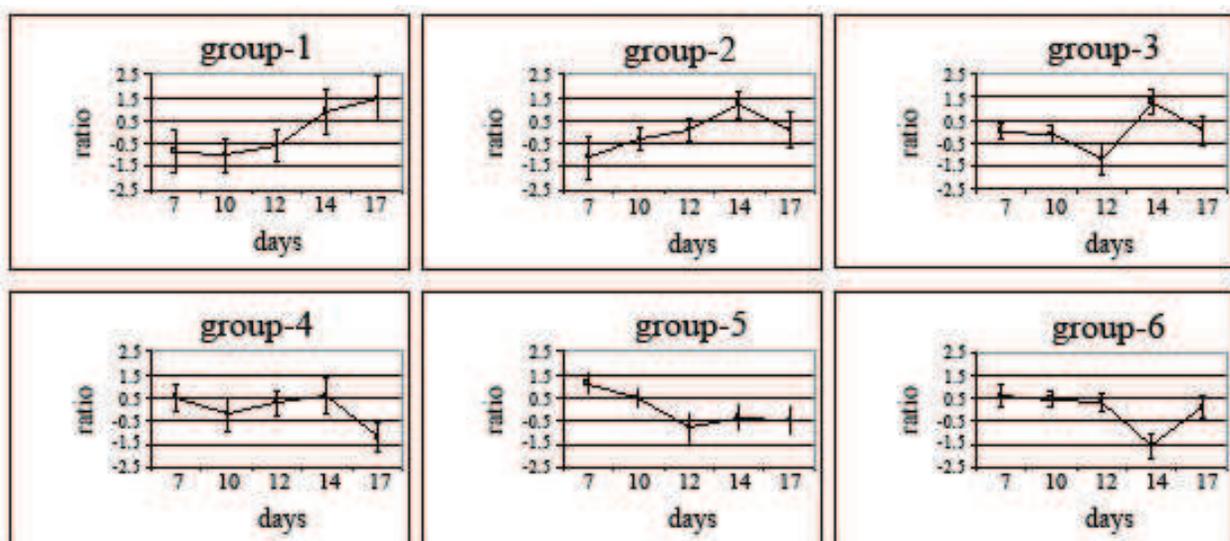


Fig. 1. Six main patterns of gene expression throughout meiosis. Mean expression level of the genes within each group is expressed as the mean ratio between the expression level and the geometric average for each developmental stage. (reproduced with permission from Waldman Ben-Asher et al., 2010).

## 2. Regulating meiotic gene expression

### 2.1 Chromosomal localization of genes as an expressional regulatory factor

One of the intriguing findings we have noticed in analyzing our microarray results was that genes from the different expressional groups are not randomly distributed throughout the genome. There are specific chromosomes that preferentially harbor genes from specific expressional groups, whereas other chromosomes are preferentially depleted of genes from specific expressional groups (Waldman Ben-Asher et al., 2010). For this analysis, we first determined, bioinformatically, the chromosomal location of each of the genes within each of the six expression groups that were obtained, and calculated the percentage of genes within each group that appear on a specific chromosome. This, was then, compared to the percentage of annotated genes from the entire mouse genome that are located on each specific chromosome. The statistical significance of the differences between the distribution of the meiotic genes within each group and that of the genes of the entire genome on each chromosome was determined using the confidence interval test, with  $p < 0.05$  indicating statistically significant differences. To address the randomness of the chromosomal location of genes that are specifically up-regulated or suppressed during the various meiotic stages, 1000 random lists from the entire genome, consisting of the same number of genes as in each of our six expression groups (6,000 lists altogether), were created and the mean distribution of all 1000 randomly sampled lists in each group (expressed as percentage of genes on each chromosome),  $\pm$ SD, was calculated. We found that the obtained results were basically identical to those obtained with the whole genome distribution, with very small SDs. These results indicated that the distribution of annotated genes from the entire mouse genome along the chromosomes resembles random distribution, rendering the deviations in chromosomal distribution among meiotic genes, statistically and functionally significant. Our results, which are summarized in table 1, indicate that genes from group 1 are enriched on chromosome 11 and on chromosome 17, and are under represented on chromosome X. Genes from group 2 are enriched in chromosomes 3 and 15, and genes from group 3 are enriched in chromosome 11 whereas chromosome 13 is almost completely depleted of genes from this group. The distribution of genes from group 4, do not deviate significantly from the whole genome distribution. Genes from group 5 are under-represented in chromosome 4 and over-represented in chromosome 8, and group 6 genes are over-represented in chromosomes 1 and 6 and under represented in chromosome 15. Furthermore, an in-depth examination of the results in this analysis revealed a mirror-like patterns of expression of groups 2 and 6, with over and under representation on chromosome 15, respectively. This might point at chromosome 15 as containing genes that are especially required for the pachytene stage at day 14. A similar mirror-like patterns of expression exist also in groups 1 and 5 with over and under representation on chromosome 11, respectively (under representation of group 5 genes on chromosome 11 was just above the  $p < 0.05$  limit), suggesting that this chromosome contains meiotically-regulated genes that are not activated during the first steps of spermatogenesis, but only later, as cells enter and proceed through meiosis (p.n. days 12-17). The functional conclusion from this gene location analysis is that chromosomal location seems to be a factor in regulating gene expression during meiosis.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Chromo. 1						+
						10% vs. 6%
Chromo. 3		+				
		9% vs. 4%				
Chromo. 4					-	
					1% vs. 7%	
Chromo. 6						+
						9% vs. 5%
Chromo. 8					+	
					10% vs. 5%	
Chromo. 11	+		+			
	15% vs. 8%		17% vs. 8%			
Chromo. 13			-			
			0.1% vs. 4%			
Chromo. 15		+				-
		9% vs. 3%				1% vs. 3%
Chromo. 17	+					
	9% vs. 5%					
Chromo. X	-					
	0.5% vs. 4%					

Table 1. Summary of deviations in distribution of meiotic genes from the six expressional groups, along chromosomes, compared to whole genome/random distribution. All indicated deviations are statistically significant ( $p < 0.05$ ). Over-representation is denoted by (+), under-representation is denoted by (-). Percent of meiotic genes versus percent of whole genome genes are indicated. For example, 15% of group 1 genes are located on chromosome 11 versus 8% of whole genome/random distribution.

In an attempt to take the chromosomal location analysis one step forward, we asked, to what extent does genes from the same expressional group that are located on the same chromosome are clustered in the vicinity of each other. The rationale for this analysis was that such clustering might enable co-regulation of expression by sharing overall chromatin organization that favors either transcription or silencing. For this analysis we used the DAVID program, a program that identifies functional groups of genes that are enriched in a given dataset compared with their representation in the entire genome (Huang et al., 2009a; Huang et al., 2009b). We, therefore, looked for genes that were clustered to specific cytobands. As shown in table 2, in four of the six groups we found only small clusters (2-5 genes), within specific cytobands, that were statistically significant compared to random distribution along the specific chromosome ( $p < 0.05$ ). This suggests that clustering to specific chromosomal regions (cytobands) might, at the most, contribute to regulation of expression at the local level but does not contribute significantly (if at all) to the overall co-regulation of expression within each group.

Table 2. Summary of genes, from the different expressional groups, that are clustered at specific cytobands on the different chromosomes. Cytobands are denoted by the chromosome number followed by the specific cytoband location symbol. The p-value, as calculated by the DAVID program, represents the statistical significance of the clustering, compared to random distribution of the genes along the specific chromosome. Only clusters with  $p < 0.05$  were considered in this analysis.

Group	Cytoband	No. of genes	p-value	Gene symbol	Gene name
1	10 D3	3	0.04	DDIT3	DNA-damage-inducible transcript 3
				slc16a7	solute carrier family 16 member 7
				USP15	ubiquitin-specific protease 15
2	17 B1	2	0.01	Ppt2	palmitoyl thioesterase 2
				MSH5	mutS homolog 5
2	15 A1	2	0.01	GHR	growth hormone receptor
				Lifr	leukemia inhibitory factor receptor
2	3 F2.1	3	0.02	PEX11B	peroxisomal membrane protein 11B
				TCHH	trichohyalin
				ANP32E	acidic (leucine-rich repeat) protein 32 family class E member 3
2	15 D3	3	0.04	Gml	GPI anchor
				Hemt1	hematopoietic protein 1
				LRRC6	leucine-rich repeat-containing protein 6
5	5 C3.1	2	0.01	pgm1	phosphoglucomutase 1
				UGDH	UDP-glucose 4-epimerase
6	4 C3	2	0.01	TYRP1	tyrosinase-related protein 1
				Ptprd	protein tyrosine phosphatase, transmembrane type 10
6	11 D	5	0.02	PPY	pancreatic polypeptide
				utp18	UTP18, small subunit of RNA polymerase III
				NT5C3L	5'-nucleotidase cytosolic 3-like
				copz2	coatamer protein 2
				igfbp4	insulin-like growth factor binding protein 4
6	13 A3.3	3	0.02	LOC636537	Signal sequence
				dsp	desmoplakin
				TXNDC5	thioredoxin domain-containing protein 5
6	7 F5	2	0.03	Lsp1	lymphocyte stromal protein 1
				TSPAN3	tetraspanin 3
6	3 G3	3	0.04	Gm11295 or ELOVL6	ELOVL6 family class 1 member 6
				TMEM56	transmembrane protein 56
				NPNT	nephron protein

## 2.2 Common cis-regulatory sequence elements within each expressional group

Unique cis-regulatory elements common to genes within a transcriptional group, if found, may explain co-regulation and similar expression patterns. To address this issue, regarding our six expression groups, we first created a file for each expression group, containing all of the gene promoter sequences in Fasta format. The length of the promoter region was defined as 1200 bp consisting of 1000 bp upstream to the transcription Initiation Site (TIS) and 200 bp downstream the TIS. The promoter region was extracted from the UCSC database, using the table application (NCBI37/mm9 assembly). Next, we used these lists as input in the Genomatix-MatInspector application (Cartharius et al., 2005) to search for matches against transcription factor (TF) recognition motifs. MatInspector is a software tool that utilizes a large library of matrix descriptions for transcription factor binding sites to locate matches in DNA sequences. The output of this application was a list of transcription factor families whose DNA recognition motifs are common to the promoters of the different expression groups. We defined a common TF family binding site as a motif which is

TF	Group1	Group2	Group3	Group4	Group5	Group6
NKXH			*	*	*	*
ETSF	*	*	*	*	*	*
HOXF	*		*	*	*	*
CREB	*	*	*	*	*	*
TBPF			*			
GATA			*	*		
FKHD	*		*		*	*
NR2F	*	*	*	*	*	*
EVI1	*		*	*	*	
MZF1			*			
PAX6		*	*			
CLOX			*	*		
SORY	*					
ZBPF		*			*	*
MYT1				*		
RXRF	*	*		*	*	*
SP1F	*	*		*	*	*
EGRF	*	*		*		*
MYBL	*			*		*
MAZF				*		*
E2FF	*	*		*		*

Table 3. Common TF binding motifs in promoter sequences of genes within each expression group. An asterisk represents the presence of the specific transcription factor target sequence in at least 90% of the genes in that group. In yellow - TF common to all groups. In red - TF common to only one group.

represented in at least 90% of the promoter sequences of each specific group (Table 3). We noticed that three transcription factor families, ETSF (Human and murine ETS1 factors), CREB (cAMP-responsive Element Binding proteins) and NR2F (nuclear receptor subfamily 2 factor), were common to all groups, suggesting they are unlikely to be responsible for the differential expression pattern of any individual group. Of special interest were four TF families which appeared only in one group: TBP (TATA-binding protein factors) in group 3, MZF1 (Myeloid Zinc Finger 1 factors) also in group 3, SORY (Sox/Sry-sex/testis determining and related HMG box factors) in group 1 and MYT1 (MYT1 C2HC zinc finger protein) in group 4. TATA box binding protein (TBP) is a general transcription factor that plays an important role in transcription initiation of many genes. Various members of the TBP family have been identified, such as the TBP-related factors (TRFs) as well as numerous tissue-specific homologs of TBP-associated factors (TAFs) (Hochheimer & Tjian, 2003). TRF2 (known also as TLP or TRP) has a testis-specific form which is first detectable at pn d14 mouse testis and its level is increased at later stages of testicular development (Sugiura et al., 2003). Our microarray results showed a rather similar pattern of expression for TRF2 (Figure 2 – green line). Interestingly, four other genes of the TBP family (TBP, TAF1b, TAF9( 2 probes) ,MED20) were present in our microarray list of meiotic regulated genes, all having the same pattern of increased level of expression from pn d12 (Figure 2). The similar expression pattern of these transcription factors through pn days 12-17 may suggest that they work together through the meiotic phase, and might account, at least in part, for the expression pattern of group 3.

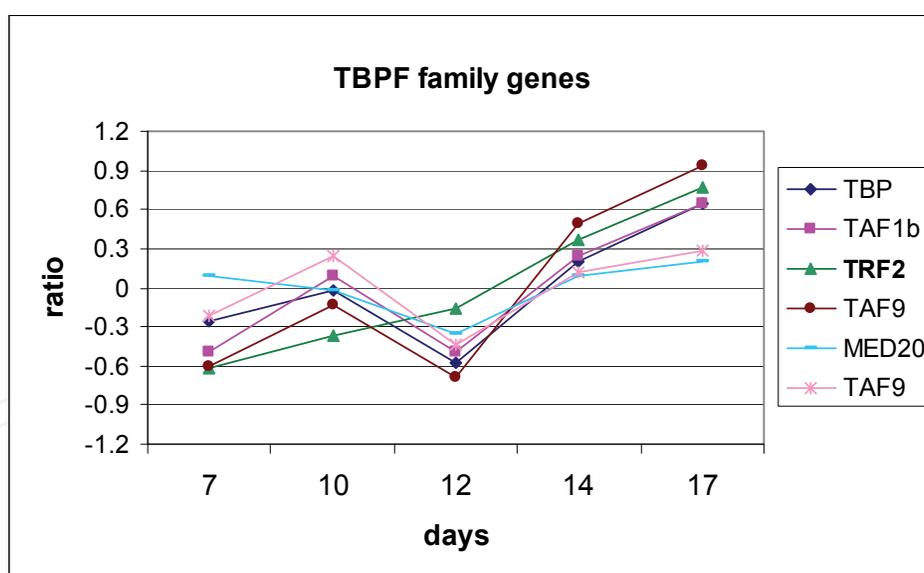


Fig. 2. Expression pattern of members of the TBP family of transcription factors during meiosis, as obtained in our microarray analysis.

The specific binding motif for the myelin transcription factor 1 (Myt1) family appeared in the promoter sequences of more than 90% of genes of group 4. This family of transcription factors is comprised of three zinc finger genes: Myt1 (known also as Nzf2), Myt1L (known also as Png1), and Myt3 (known also as Nzf3 or St18). These transcription factors belong to the structurally unique CCHHC class, that are expressed predominantly in the developing Central Nervous System, CNS (Romm et al., 2005). Nonetheless, in rat cells, Myt1 was

reported to interact with Sin3b, a protein that mediates transcriptional repression by binding to histone deacetylases (HDACs) (Romm et al., 2005). In our microarray results, the second member of the family, Myt1L, showed an increase in its expression between days 12 to 14, suggesting the potential involvement in regulating the characteristic transcriptional repression seen in group 4 between d14 and d17.

The SORY TF family consists of high mobility group (HMG) genes from two subfamilies: HMGA and HMGB. This family includes the SRY gene as well as various SOX genes, all of which function as transcriptional activators. Some of these genes were reported to play a role during spermatogenesis. For example, Sox3 expression was shown to be restricted to type A spermatogonia and to be required for spermatogenesis through a pathway that involves Ngn3 (Raverot et al., 2005). Sox7 and Sox17 were reported to function synergistically in the transcription of the Mouse laminin- $\alpha$ 1 gene during differentiation of mouse F9 embryonal carcinoma cells into parietal endoderm cells (Niimi et al., 2004), and Hager et al. (2005) showed that laminin- $\alpha$  chains are vital for spermatogenesis. Notwithstanding, the expression pattern of some members of this family of transcription factors during meiosis, as depicted in our microarray analysis, do not intuitively favor specific involvement in transcriptional regulation of group 1 genes. Nevertheless, further analysis is required before definite conclusions are drawn.

The MZF1 family represents the Myeloid zinc finger protein 1 (also known as Znf42, Mzf2, Zfp98, or Zfp121). It belongs to the Krüppel family of zinc finger proteins, and it was found to play a key role in regulating transcription during differentiation along the myeloid lineage (Yan et al., 2006). These authors also demonstrated that over-expression of MZF1 repressed the ERCC1 promoter activity upon cisplatin exposure, suggesting that MZF1 might be a repressor of ERCC1 transcription. ERCC1 is a critical gene within the nucleotide excision repair pathway and only recently it was shown to play an essential role in DNA damage repair during spermatogenesis related recombination. Deficiency of this gene results in the production of abnormal sperm (Hsia et al., 2003; Paul et al., 2007). Our analysis revealed that only group 3 promoters met the limit of 90% representation of the Mzf1 TF binding site. The Mzf1 expression pattern itself was not revealed by our microarray analysis since it did not pass the stringent selection for genes exhibiting at least two-fold change in expression, compared to the geometric average, at any of the meiotic stages that were tested (Waldman Ben-Asher et al., 2010). However, it is still very well possible that Mzf1 indeed plays a role in repressing expression of meiosis-related genes, such as those of group 3.

Finally, it is, of-course, possible that the differential pattern of expression in each group is a result of a combinatorial co-regulation by several transcription factors. In this context it is noteworthy that none of the expressional groups share the same distribution of common TF motifs in their promoters (Table 3).

### 3. Functional analysis of gene networks – Apoptosis as a test case

Following the expression kinetics of genes, within specific gene networks, throughout meiosis, enables an insight as to how specific processes are operated and regulated during meiosis. In this study, we used apoptosis as a test case for such an analysis. Apoptosis plays a crucial role during spermatogenesis in general and meiosis in particular. It determines overall testicular cell load, balancing the proportion of the different cell types within the seminiferous tubules, and it plays a role in the removal of aberrantly differentiated meiotic spermatocytes and spermatids during and after meiosis (review in Print & Loveland, 2000).

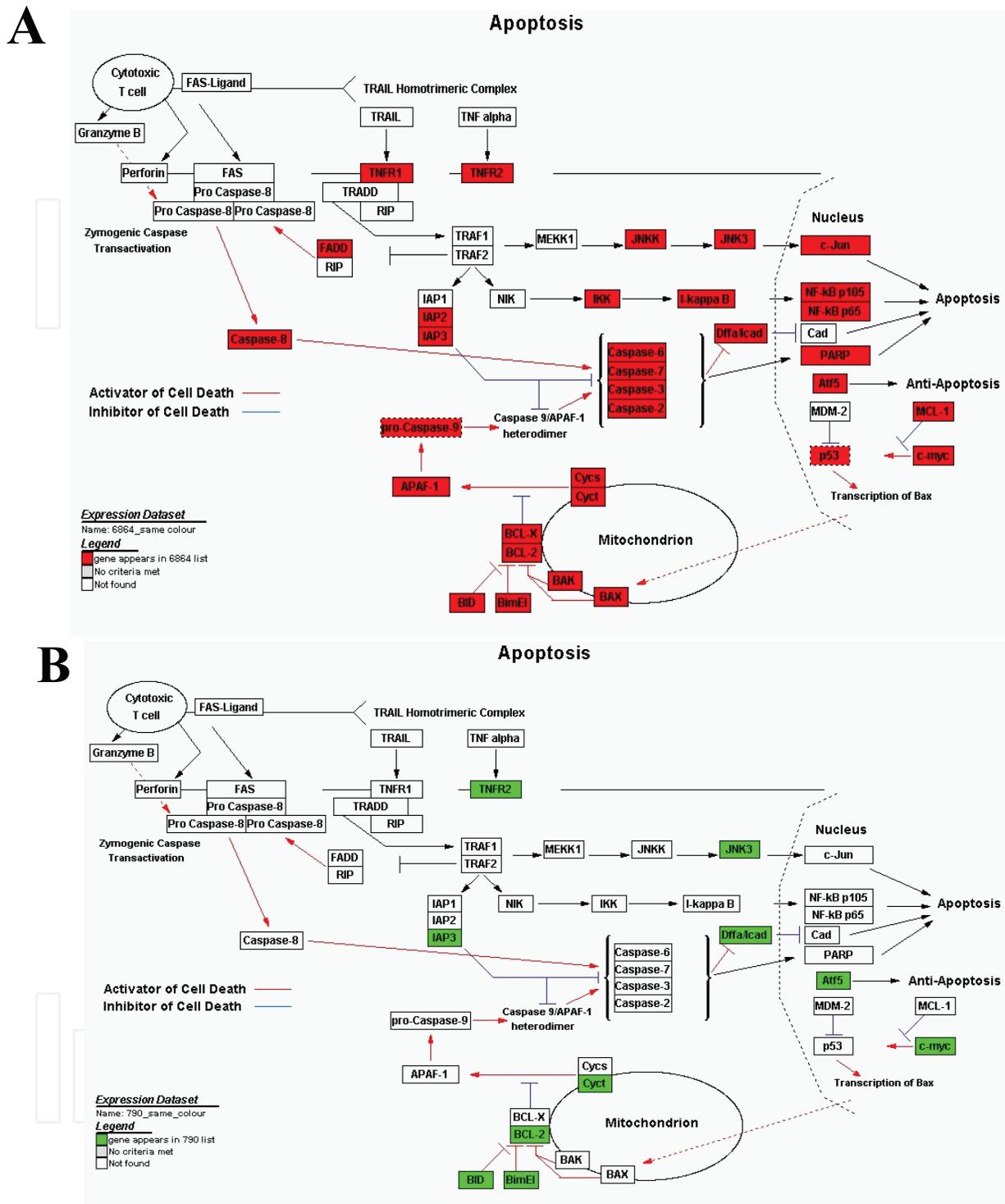


Fig. 3. Apoptotic expression maps highlighting in red genes that appear in our 6864 present sequences (A), and in green genes that appear in our 790 regulated sequences (B). These maps were obtained by applying the "Gene Map Annotator and Pathway Profiler" program to our microarray results.

Moreover, spermatocytes are unique in the sense that they "voluntarily" give up DNA integrity and undergo massive DNA breaks to enable synapsis of homologous chromosomes and crossing-over between them during meiosis. This puts conflicting

requirements on the cell. On the one hand, a situation in which each and every chromosome harbors several double strand breaks (DSB) favors activation of the apoptotic pathway. On the other hand, these breaks are physiologically induced and the cells must not be sentenced to death unless breaks are not properly repaired or chromatin is not properly organized. To get an insight as to how these conflicting requirements are balanced, we applied the "Gene Map Annotator and Pathway Profiler" program to our microarray results to characterize the apoptotic pathway during meiosis. Two expression maps were used: one representing the 6864 present sequences (genes whose expression was detected in our microarray analysis but did not pass the two-fold change selection), and the other representing the 790 regulated sequences (Figure 3). 33 genes from our "present" sequences, and 10 genes from the regulated list, lighted-up using this program (Figure 3A-B). These 10 genes included TNFR2, Bid, BimEL, c-Myc and CytCt (a testis specific isoform of cytochrome C), which have a generally accepted pro-apoptotic function, and IAP3, Bcl-2, Dffa and ATF5 generally known as anti-apoptotic genes. The tenth gene, JNK3, is part of the more general MAP kinase signal transduction pathway that can either promote apoptosis or survival through activation of c-Jun (Ham et al., 2000; Kennedy & Davis, 2003). Following the specific expression pattern of these 10 regulated genes (Figure 4), it is apparent that towards the zygotene stage (pn d12), the caspase inhibitor IAP3 is up-regulated, whereas CytCt level is low, a pattern that restricts apoptosis. It is also apparent that at early pachytene (pn d14) the anti-apoptotic gene, Bcl-2, is up-regulated together with the anti apoptotic factor Dffa (Inhibitor of Caspase Activated DNase - ICAD), and BimEL, a mild negative regulator of Bcl-2. In contrast, as a mirror image, the pro-apoptotic genes Bid, which negatively regulates Bcl-2, and TNFR2, together with the anti-apoptotic transcription factor ATF5 (known also as ATFx), JNK3, and the caspase inhibitor IAP3 are down-regulated. This pattern is reversed by day 17. Thus, Bcl-2, Dffa and BimEL are down-regulated whereas Bid, TNFR2, IAP3, JNK3 and ATF5 are up-regulated. Note also that CytCt is up-regulated between pn d12 and pn d17.

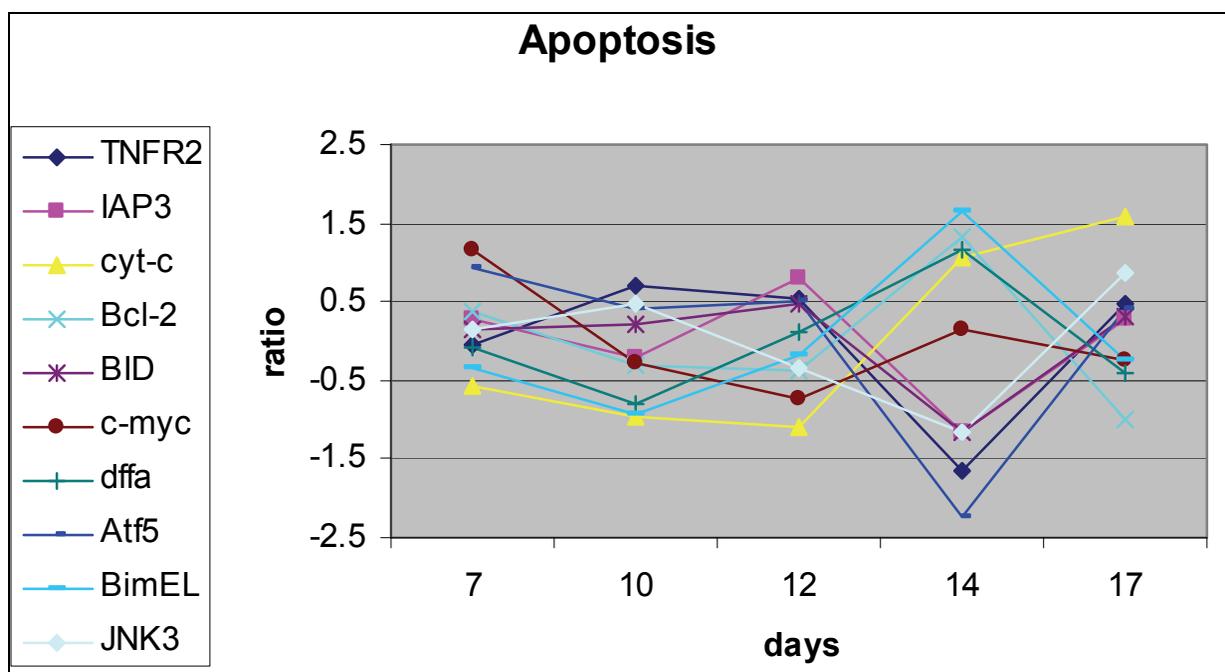


Fig. 4. Specific expression patterns of the ten genes that were highlighted in the apoptotic expression map of the 790 regulated sequences.

By drawing two maps, one for the 6864 present sequences and one for the 790 regulated sequences, we could determine two groups of apoptotic genes, the operational background genes and the actively regulated genes. The operational background genes are those whose transcript level does not change much as meiosis proceeds, but if needed, are available to execute apoptosis. The actively regulated genes are those pro and anti apoptotic genes whose transcript levels fluctuate significantly during the various meiotic stages and create a delicate balance between apoptosis and survival. DSB first appear just before zygotene, between pn d10 and pn d12, to enable synapsis of homologous chromosomes and crossing-over (review in Hochwagen & Amon, 2006). At this stage, elevated levels of the caspase inhibitor, IAP3, and low levels of CytCt seem to restrict apoptosis. As cells progress to early pachytene, high levels of Bcl2 and Dffa, together with low levels of the negative regulator of Bcl2, Bid, seem to protect cells from apoptosis and facilitate crossing-over, subsequent repair and chromatin organization. At this stage CytCt increases, IAP3 decreases, and elevated c-myc might put cells on stand-by to execute apoptosis if something goes wrong. By pn d17, representing late pachytene, DSB are repaired, and any un-repaired cell must undergo apoptosis. This is reflected by the mirror image where high CytCt, elevated Bid, TNFR2 and ATF5 together with down regulation of Bcl2 and Dffa are apparent. These results suggest that during meiosis, a delicate interplay between anti and pro-apoptotic genes and their relative abundance in a given cell determine its fate to life or death.

#### **4. Comparing transcriptomes - A lesson to be learned**

One way to ascribe biological significance to microarray results is to compare data obtained in parallel experiments on different differentiative systems sharing common molecular processes. Given that B-cell differentiation and meiosis both share DNA rearrangement processes (V(D)J recombination and meiotic recombination, respectively) we reasoned that novel insights could be obtained by comparing our meiosis microarray results to a B-cell differentiation database. Hoffmann et al. (2003) have classified the differentially expressed genes during murine B cell development into 20 clusters according to their expression pattern along the 5 differentiative stages: Pre-BI, Large Pre-BII, Small Pre-BII, Immature B and Mature B cells, and used this cluster classification to compare gene expression between parallel developmental stages of B cells and T cells. We focused our attention on genes that were highly expressed in either Pre BI cells (clusters 1, 2, 3 and 5, in Hoffmann et al, 2003), which undergo V(D)J recombination of the heavy chain (especially V to DJ rearrangement), or in small Pre BII and immature B cells (clusters 9, 10, 11, 12, 16 and 17, in Hoffmann et al, 2003) undergoing a second wave of rearrangement of the light chain ( $V_L$  to  $J_L$ ). These genes were compared to meiotic genes up-regulated towards early pachytene (d14) when meiotic recombination occurs (groups 1, 2 and 3, in this study). For the comparison, the accession numbers of the 390 sequences contained within the three relevant meiotic clusters, as well as of the 955 sequences consisting of the relevant B-cell differentiation clusters (obtained from supplementary data provided by the authors in Hoffmann et al, 2003), were all translated to new Affymetrix accession numbers to form a common identification base. Following this analysis, 11 genes emerged from the cross between the meiotic genes and the Pre BI specific genes, and additional 10 genes from the comparison between the meiotic genes and the genes up-regulated in small Pre BII and immature B cells (Figure 5). A more in-depth

observation at some of these genes raises interesting insights as to some of the molecular pathways operating in these processes.

#### 4.1 Rad54I and HOP2

Up-regulation of Rad54 and Hop2 (genes characteristic of the homologous recombination DNA repair pathway) during meiosis was not unexpected since the heterodimer Hop2-Mnd1, as well as Rad54, are known to physically interact with the recombinases Rad51 or Dmc1 during meiotic recombination and to stimulate their activity by facilitating the DNA-strand-invasion step, a key step in the homologous recombination process (Petukhova et al, 2005; Sung & Klein, 2006). On the other hand, V(D)J recombination during B-lymphocyte differentiation is thought to occur through the Non-Homologous End Joining (NHEJ) pathway, and hence up-regulation of these genes was less expected. Moreover, Essers et al., (1997) showed that RAD54<sup>-/-</sup> mice are viable and exhibit apparently normal V(D)J and immunoglobulin class-switch recombination. Nevertheless, up-regulation of these genes specifically during V(D)J recombination suggests that they might indeed play some role in NHEJ processes, and that in the absence of Rad 54 there might be compensating genes that function. If this is the case, Rad54 joins other homologous recombination DNA repair genes, such as the MRN complex (Mre11, Rad50 and Nbs1) and BRCA1, that were found to play a role in the NHEJ pathway as well (Durant & Nickoloff, 2005; Sancar et al, 2004).

#### 4.2 Mog1 and Ranbp5

These two genes are involved with Ran-GTP-dependent nuclear / cytoplasmic transport of proteins. Mog1 is a nuclear protein that stimulates the release of GTP from Ran, forming a Mog1-Ran complex which stabilizes Ran in a nucleotide-free form thereby modulating nuclear levels of RanGTP (Steggerda & Paschal, 2000; Baker et al, 2001). Ranbp5 is an importin  $\beta$  related protein (also known as importin  $\beta$ 3) that acts in a nucleocytoplasmic transport pathway that is distinct from the importin-alpha-dependent import of proteins (Deane et al, 1997). Both genes were previously reported to be expressed during spermatogenesis (Li et al, 2005; Loveland et al, 2006) but the fact that both are up-regulated during DNA rearrangement processes might hint that their target proteins for nucleocytoplasmic transport are involved with DNA rearrangement.

#### 4.3 p107

One process that a cell undergoing DNA rearrangement must avoid is cell division. It is, therefore, expected that during physiological rearrangement processes cells would repress cell cycle promoting genes. p107, a member of the Rb pocket protein family of cell cycle regulators, forms repressive complexes with either E2F4 or E2F5 (Iaquinta & Jacqueline, 2007). Such complexes have been detected by ChIP analyses in many E2F-responsive promoters of G0 cells, ensuring they do not divide. Moreover, recruitment of HDACs (histone deacetylases) to these complexes further ensures that these important cell cycle genes stay silent (Cobrinik, 2005). Up-regulation of p107 in differentiating meiotic and B-cells, might, therefore, play a role in silencing cell division genes until DNA rearrangement processes has been successfully completed.

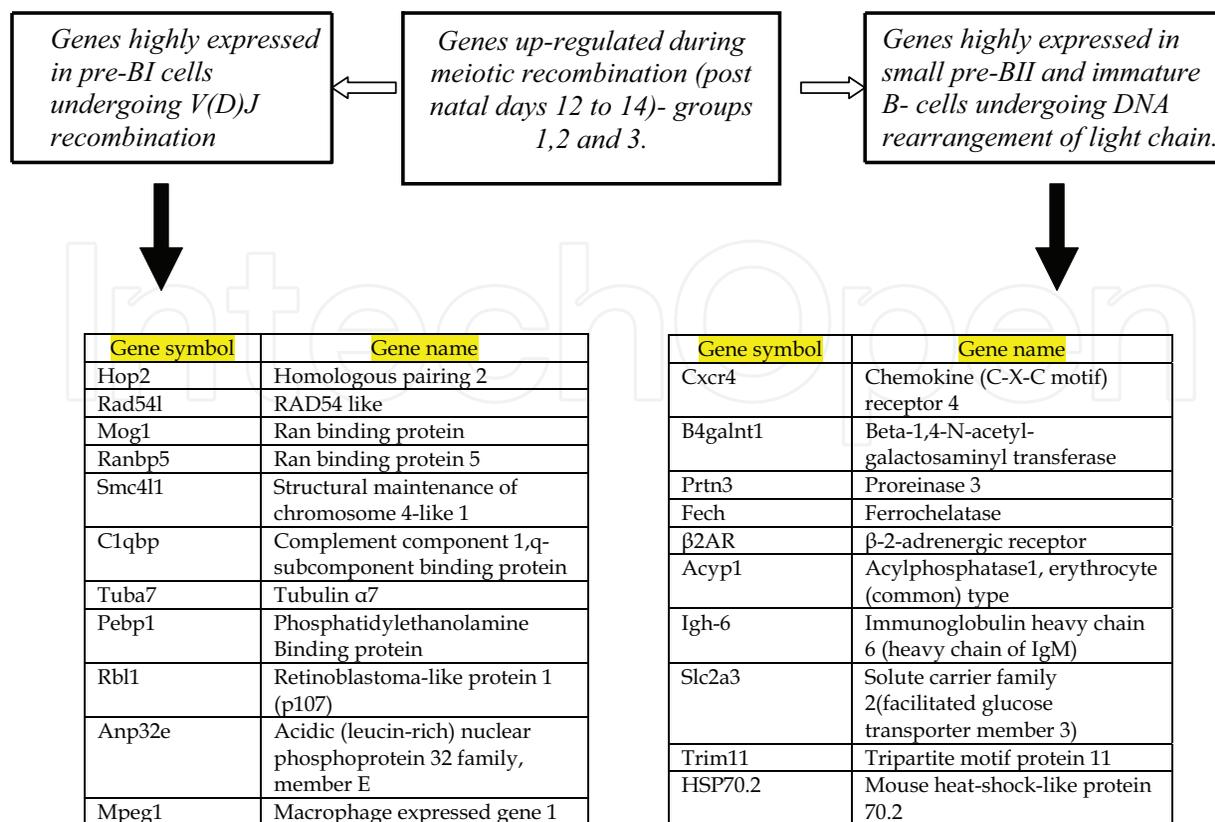


Fig. 5. Comparison between genes up-regulated towards the early pachytene stage (pn d14) where spermatocytes undergo meiotic recombination (groups 1, 2 and 3 in this study), and genes up-regulated in pre-BI or in small pre-BII and immature B-cells, undergoing V to DJ rearrangement of the heavy chain and  $V_L$  to  $J_L$  rearrangement of the light chain, respectively, during B-cell differentiation. The “B-cell differentiation” data was obtained from microarray data sets and clustering as reported by Hoffmann et al. (2003).

#### 4.4 SMC4

In eukaryotes, the Structural Maintenance of Chromosome (SMC) proteins constitute a family of six highly conserved members of chromosomal ATPases, involved in chromosomal structural dynamics (review in Hirano, 2006). These SMC proteins form different complexes based on three SMC heterodimers. SMC1 and SMC3 form a heterodimer that, together with two other non-SMC subunits, form the cohesion complex which keeps sister chromatids together from S-phase until anaphase, when they are separated into two daughter cells. SMC2 and SMC4, form a heterodimer that together with three other non-SMC subunits compose the condensin complex, which plays an important role in mitotic/meiotic chromosomes condensation, as well as in non-mitotic chromatin condensation processes. A third pair of SMC subunits, SMC5 and SMC6, is thought to be essential for genomic integrity and DNA damage response (Hirano, 2006; De Piccoli et al., 2009). These latter SMC sub-units were reported to be highly expressed in the testes of mammals, together with a recently identified meiosis-specific SMC1 related protein (SMC1 $\beta$ ) that was suggested to be crucial for completion of meiosis in mammals (Revenkova et al, 2004; Hirano, 2006). It is therefore interesting that among all SMC proteins, it is the SMC4 related protein that was identified in our comparison. This might

suggest that SMC4, as part of the condensin complex is important for the DNA rearrangement processes. Indeed, some DNA repair roles have recently been attributed to cohesins and condensins, in addition to their traditional function, with condensins being involved specifically with single-strand break repair (Coldecott 2008; De Piccoli, 2009). This might imply that although DNA rearrangement consists basically of double strand breaks, single-strand break repair processes might also take place during rearrangement. Alternatively, condensins might play a role in DNA repair processes other than that of single-strand breaks. It is also possible, of-course, that SMC4 plays an as yet unknown role that has not been characterized to date.

#### 4.5 Cxcr4

This gene encodes the chemokine receptor 4, a G-protein-coupled receptor for the CXCL12 chemokine (known also as SDF-1). Upon activation, this receptor mediates several biological activities, among which are the migration of primordial germ cells to the gonads (Molyneaux et al, 2003; Stebler et al, 2004), retention of primordial follicles in an un-activated state in the neonatal mouse ovary (Holt et al, 2006), and the retention of differentiating B cells in the bone marrow until maturation (Palmesino et al, 2006). Upon stimulation, CXCR4 has also been reported to induce the MAP-kinase cascade and the PI3/PKB pathway, which may elicit an anti-apoptotic response (Palmesino et al, 2006). The activated expression of Cxcr4 in both differentiating B lymphocytes in the bone marrow and during meiosis in the testis might suggest the intriguing possibility that it plays a somewhat similar role in the testis, i.e. retention of spermatocytes within the seminiferous epithelium until maturation (completion of meiosis and spermiogenesis) has been completed. Alternatively, it is also possible that it acts as a survival factor during meiosis.

#### 4.6 $\beta$ -2-adrenergic receptor ( $\beta$ 2AR)

A disturbing unresolved discrepancy exists between the important role ascribed to the follicle-stimulating hormone (FSH) during spermatogenesis and the apparent lack of phenotype seen in FSH KO mice (review in Huhtaniemi, 2006). FSH binds to and activates the FSH receptor (FSHR) on Sertoli cells, which in turn utilize the cAMP / PKA signaling pathway to activate the CREB transcription factor. CREB activation is crucial for the ability of Sertoli cells to nurture primary spermatocytes and to support their survival. Expression of a dominant negative form of CREB in Sertoli cells resulted in apoptosis of spermatocytes (review in Don & Stelzer, 2002). Our results regarding the expression of  $\beta$ 2AR, might provide at least a partial explanation to this discrepancy. On the one hand the  $\beta$ 2AR was shown to activate the cAMP- PKA- CREB pathway in B-cells (Kin & Sanders, 2006). On the other hand, it was reported to be expressed in Sertoli cells of immature rats (Jacobus et al, 2005), although there are no data available as to its expression in Sertoli cells of mature animals. Our results demonstrating up-regulation of  $\beta$ 2AR during meiosis suggest it might activate the cAMP- PKA- CREB pathway in Sertoli cells and hence compensate, at least partially, for the absence of FSH in the KO models. This hypothesis must, however, be experimentally tested.

In conclusion, the comparison between genes activated during B-cell differentiation and meiotic differentiation has focused our attention on several common genes, some of which shed light on novel molecular aspects of spermatogenesis in general, and of meiosis in particular.

## 5. Conclusions

The microarray technology has revolutionized the area of gene expression research by providing enormous amounts of transcriptome / proteome / phosphoproteome data, and enabling comparison between data sets representing the same tissue in different organisms, different tissues within the same organism or different treatments or conditions within a specific tissue or cell-type. The challenge in analyzing such experiments is to put these data in order and to extract the biological significance of it. In this study we used various bioinformatics tools in an attempt to ascribe biological significance to our microarray results, comparing the transcriptome of the mouse testis at five post-natal developmental ages representing different meiotic stages of the first spermatogenic wave. We found that chromosomal location of genes (but not clustering within a specific chromosome) could be a factor in determining specific patterns of gene expression during meiosis. Furthermore, we determined the distribution of common TF binding motifs in promoter sequences of genes within each of the six expressional groups that were determined (representing six major patterns of expression), pointing at specific transcription factors (or combination of transcription factors) that might contribute to the co-regulation of gene expression within each group. Expression kinetic analysis of gene networks is an important way of ascribing biological significance to microarray results. Using apoptosis as a test case we demonstrated herein how by a timely interplay between pro and anti apoptotic genes the delicate balance between the need to enable DNA breaks for pairing and recombination and the need to discard cells that their DNA has not been properly repaired, is kept. Finally, by comparing genes that are up-regulated during meiotic recombination, to genes up-regulated during DNA rearrangement in differentiating B-cells, we were able to get some new ideas regarding genes and molecular pathways operating during meiosis. Nevertheless, we have described only the tip of the iceberg of what could be concluded from our data, as well as from data obtained in corresponding studies executed by other groups, and most importantly, by the combined analyses of all these data sets. Further analysis and interpretations must await further studies.

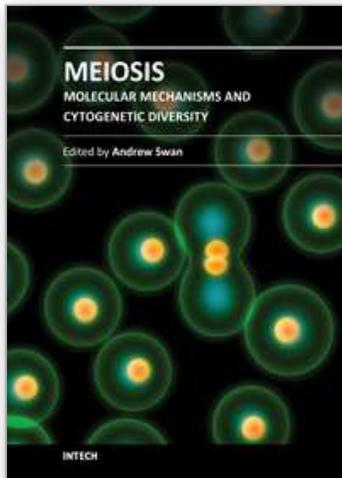
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Meiosis, the process of forming gametes in preparation for sexual reproduction, has long been a focus of intense study. Meiosis has been studied at the cytological, genetic, molecular and cellular levels. Studies in model systems have revealed common underlying mechanisms while in parallel, studies in diverse organisms have revealed the incredible variation in meiotic mechanisms. This book brings together many of the diverse strands of investigation into this fascinating and challenging field of biology.

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