A role for the *Speedy* gene family in the early stages of mammalian meiosis

by

Javier Lopez-Molina

B.A. Natural Sciences
Johns Hopkins University, 2003

Submitted to the Department of Biology in partial
fulfillment of the requirements for the degree of

Master of Science in Biology
at the
Massachusetts Institute of Technology
[June 2008]
May 2008

Signature of Author:_____
Department of Biology
May 22, 2008

Certified by:_____
David C. Page
Professor of Biology
Thesis Supervisor

Accepted by:_____
Stephen P. Bell
Professor of Biology
Graduate Committee Chairperson

A role for the *Speedy* gene family in the early stages of mammalian meiosis

by

Javier Lopez-Molina

Submitted to the Department of Biology
on May 22, 2008 in Partial Fulfillment of the
Requirements for the Degree of Master of Science in Biology

ABSTRACT

Meiosis is the process by which a diploid cell undergoes two sequential rounds of division without an intervening round of DNA replication. The result is the formation of haploid gametes. The genes and signals that regulate the decision to enter meiosis are not entirely elucidated in mammals. I hypothesize that the *Speedy/RINGO* gene family functions endogenously in the early stages of meiosis including: meiotic initiation, pre-meiotic DNA replication, or meiotic prophase. In order to validate this function for the *Speedy/RINGO* genes in vivo, I categorized *Speedy/RINGO* genes, chromosomal locations, sequences, expression patterns, and identified regulators. In mouse, I identified four *Speedy/RINGO* genes denoted: *SpeedyA*, *SpeedyB1*, *SpeedyB2*, and *SpeedyB3*. I detected mouse *SpeedyA*, *SpeedyB2*, and *SpeedyB3* mRNA in spermatocytes, the meiotic cells of the testis. Additionally, I found *SpeedyA* to be expressed in the embryonic ovary and its expression to be dependent on *Stra8*.

Thesis Supervisor: David C. Page

Title: Professor of Biology

INTRODUCTION

Speedy/RINGO (protein: SPY) has been shown to have a role in the maturation of oocytes and has also been hinted to be functional in the mitotic cell cycle of mammals. In this thesis, I will argue that the Speedy protein has a function at the early stages of the meiotic program. This argument is based on the ability of SPY to interact with known cell cycle regulators, the expression pattern of mouse *SpeedyA*, *SpeedyB2*, and *SpeedyB3*, and the regulation of *SpeedyA* mRNA by *Stra8* in the mouse embryonic ovary.

In the mammalian mitotic cell cycle, cyclins and their CDK (cyclin dependent kinase) counterparts regulate the correct timing and execution of mitotic events. A CDK is an enzyme that adds phosphate groups to its substrate proteins. In order to be active, a CDK needs to be bound by a cyclin and activated at the appropriate amino acid by phosphorylation. CDK substrate specificity depends on the cyclin that is bound to the CDK. The cell controls the amount of any given cyclin protein by altering the protein stability. This is often mediated by phosphorylation and ubiquitination (reviewed in Kuntzel et al., 1996). In this way, the cell cycle can be governed by a single CDK using multiple cyclins. It was recently discovered that a new class of proteins can bind and activate CDKs. The binding of these proteins was mediated through a domain called the *Speedy*-Box (Cheng et al., 2005b; Dinarina et al., 2004). The first of these class of proteins was identified in screen of a *Rad1* deficient strain of *Schizosaccharomyces pombe.* This *Xenopus laevis* gene, called *Speedy*, enabled resistance to UV or gamma irradiation (Lenormand et al., 1999). In a separate screen, a protein called RINGO (rapid inducer of G_2/M in oocytes) was identified that was necessary and sufficient to stimulate the resumption of the meiosis in G_2 arrested *Xenopus* oocytes (Ferby et al., 1999). These two genes are actually the same gene in *Xenopus*. It is now called *Speedy/RINGO*. I will, for simplicity and consistency, refer to this gene as *Xenopus Speedy*.

The in vitro functions of SPY proteins are currently being studied. One publication reports that human SPY/RINGO A2-CDK2 complex phosphorylates non-canonical CDK2 targets. [It should be noted that the gene studied in this referenced publication is *Speedy/RINGO A2*, the long-splice variant of the gene produced from the human *SpeedyA* locus (see appendix A). This is believed to be the closest human

homologue to *Xenopus Speedy*.] (Cheng et al., 2005a). Other studies have shown that the Speedy-box containing proteins appear to have a CDK preference (reviewed in Gastwirt et al., 2007). While SPYA appears to preferentially bind CDK2, it can also bind to CDK1, the main cyclin dependent kinase (Karaiskou et al., 2001). Thus, proteins of the *Speedy* family are able to modulate the kinase activity of the CDKs. The functional implications of this interaction suggest a role for the *Speedy* genes in the regulation of the mitotic cell cycle and the meiotic program.

The mitotic functions of *Speedy* are currently being explored in the context of mammalian cell culture and while they are not the focus of this study, they provide some clues as to possible roles for *Speedy* during early meiosis. In cell culture, human *SpeedyA* is upregulated during DNA damage and this upregulation promotes survival after DNA damage. Additionally, this activity is dependent on CDK2 activity (Barnes et al., 2003).

The ability of *Speedy* to regulate later events in meiosis has been well established in *Xenopus* oocytes and porcine oocytes, with most of the in vivo biological assays being performed in *Xenopus* oocytes. *Speedy* was initially found to be functional when added exogenously to these oocytes. However, knockdown of *Speedy* creates a delay in maturation, indicating that endogenous *Speedy* is required for the appropriate timing of maturation (Ferby et al., 1999).

In order to identify if *Speedy* genes are playing a role in meiosis, it is important to examine the expression of *Speedy* in mutants that arrest early in meiosis. One such mutant is the *Stra8* knockout mouse. *Stra8* was initially characterized as a testis-specific retinoic acid responsive gene (Oulad-Abdelghani et al., 1996) but was later also found to be expressed in the embryonic ovary (Menke et al., 2003). The germ cells in *Stra8*-/- mice arrest prior to premeiotic DNA replication. They express some early markers of meiosis, such as REC8 (a meiotic cohesin) and SCP3 (a structural protein of the synaptonemal complex), but these proteins are not loaded onto chromatin. Additionally, *Spo11* and *Dmc1*, two genes required for meiotic recombination, are absent in mutant germ cells (Baltus et al., 2006). This phenotype is the first meiotic initiation arrest identified outside of the yeast system.

In this thesis, I identify four mouse *Speedy* genes. I demonstrate a plausible connection between the *Speedy* genes and the early stages of meiosis by showing that all mouse *Speedy* genes are expressed in the adult testis. I show that *SpeedyA*, *SpeedyB2*, and *SpeedyB3* are expressed during early meiosis in spermatocytes and that *SpeedyA* is expressed in the embryonic ovary at the onset of meiosis. Finally, I show that *SpeedyA* expression in the embryonic ovary is downstream of *Stra8*, a gene required for meiotic initiation in mice.

RESULTS

The results of this thesis are divided into three objectives. The first objective is to identify *Speedy* genes, classify them based on conservation of the *Speedy*-box domain, and create a naming scheme based on this conservation to replace the existing scheme. The second objective is to determine the wildtype expression pattern of *Speedy* genes in the mouse at embryonic day 14.5 and in the adult mouse. The final objective is to study the regulation of *Speedy* by analyzing its expression in meiotic mutants available in our laboratory.

Phylogeny of Speedy genes

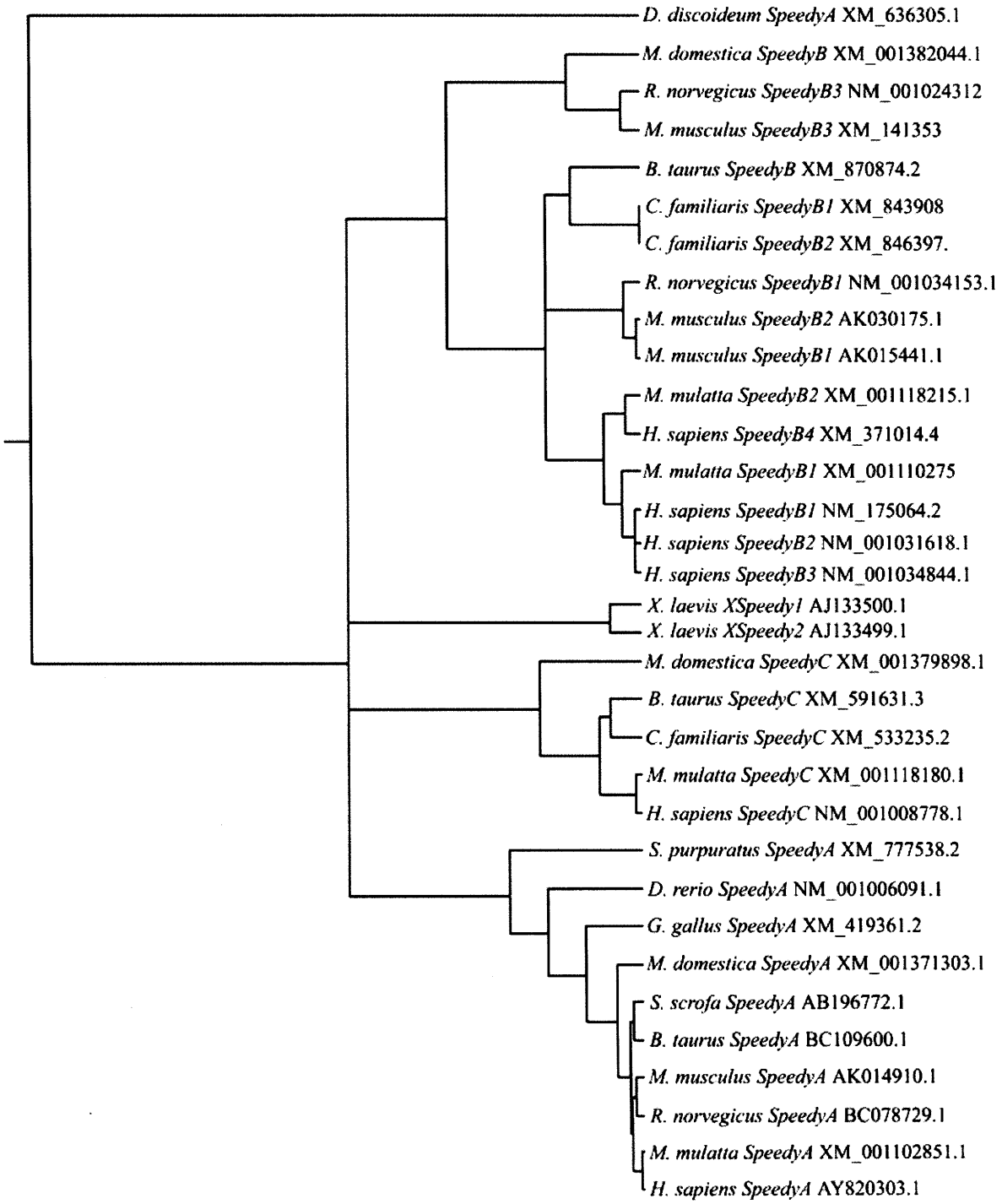
In order to classify the *Speedy* family genes in mouse, it was necessary to create an extended phylogeny of all known *Speedy*-box containing proteins. At the time of the writing of this thesis, the nomenclature of the *Speedy* gene family is inconsistent. A current review lists genes RINGO A through RINGO E, with alternate names ranging from SpyA1/RINGO1 to RINGO5, with no correlation between nomenclature and function (Gastwirt et al., 2007) (See also, Appendix C). I set out to create a nomenclature based on the relative conservation of the Speedy-box within subclasses of the *Speedy* gene family. I used the only *Speedy* gene found in *Dictyostelium discoidium* as an out-group.

Based on a phylogeny created from the alignments of available sequences from NCBI, there exist three branches of the *Speedy* family (Figure 1). The most conserved branch I refer to as the *SpeedyA* branch. This appears to be the most ancient and slowly evolving branch of *Speedy* family. *SpeedyA* genes appear in sea urchin, fish, chicken, and all sequenced mammals. There is only one *SpeedyA* gene per organism. (*Canis familiaris* did not have a *SpeedyA* by preliminary searches, however, the syntenic region to human *SpeedyA* does contain spliced ESTs: DN345999.) Interestingly, *Xenopus Speedy* genes do not clearly fall into any *Speedy* branch. Different algorithms for phylogenetic prediction all branched *Xenopus Speedy* genes outside of *SpeedyA*, *B*, and *C*. Because the *Xenopus Speedy*-box is unlike *SpeedyA*, *B*, or *C*, the interpretation of *Xenopus* protein experiments should be approached with caution.

The *SpeedyB* branch is only found in mammals. The *SpeedyB* genes are the least conserved class of *Speedy*-box containing genes. They appear to have expanded by duplication and subsequently diversified. For example, there are at least three *SpeedyB* genes in mouse: one on chromosome 2 (*SpeedyB3*) and two on chromosome 5 (*SpeedyB1* and *SpeedyB2*) in palindromic orientation. While the arms of this palindrome are not very well conserved, the coding regions of mouse *SpeedyB1* and *SpeedyB2* are well conserved at the nucleotide level: mRNAs from the opposite arms are 94% identical. Additionally, the duplication leading to this palindrome appears to have occurred in the mouse lineage, as rat lacks this palindrome, possessing only a single copy homologue of this gene, noted here as rat *SpeedyB1*. Rat also appears to have another *SpeedyB* that I denote as rat *SpeedyB3*. This gene is syntenic to mouse *SpeedyB3* and clusters with mouse *SpeedyB3* in the phylogeny as well. Another indication of the diversification of the *SpeedyB* branch is the WBSCR19 genes in *Homo sapiens*, *Macaca mulatta*, and *Canis familiaris* (Williams Beuren syndrome chromosome region 19) These genes appear in various copies in these organisms. Some of these genes in humans have been demoted to pseudogenes as there is no EST evidence for their transcription.

SpeedyC genes are only found in mammals and appear to be single-copy per organism. Interestingly, all eutherians with the exception of mouse and rat, have a copy of *SpeedyC*. Two possible explanations for the lack of this gene in rodents are as follows. *SpeedyC* was lost in mouse and rat or those sequences have yet to be obtained/assembled. A manual inspection revealed a putative *SpeedyC* region on mouse chromosome 19 that is syntenic to human *SpeedyC*. There are no ESTs from this region even though there are conserved stretches of nucleotides that likely correspond to exons of the mouse homologue of *SpeedyC* (positions are mouse chr19:6,023,167-6,024,551 of NCBI Build 37, July 2007). Since there are no ESTs, it is possible that *SpeedyC* is a pseudogene in mouse and rat.

Figure 1


D. discoideum SpeedyA XM_636305.1
M. domestica SpeedyB XM_001382044.1
R. norvegicus SpeedyB3 NM_001024312
M. musculus SpeedyB3 XM_141353
B. taurus SpeedyB XM_870874.2
C. familiaris SpeedyB1 XM_843908
C. familiaris SpeedyB2 XM_846397.
R. norvegicus SpeedyB1 NM_001034153.1
M. musculus SpeedyB2 AK030175.1
M. musculus SpeedyB1 AK015441.1
M. mulatta SpeedyB2 XM_001118215.1
H. sapiens SpeedyB4 XM_371014.4
M. mulatta SpeedyB1 XM_001110275
H. sapiens SpeedyB1 NM_175064.2
H. sapiens SpeedyB2 NM_001031618.1
H. sapiens SpeedyB3 NM_001034844.1
X. laevis XSpeedy1 AJ133500.1
X. laevis XSpeedy2 AJ133499.1
M. domestica SpeedyC XM_001379898.1
B. taurus SpeedyC XM_591631.3
C. familiaris SpeedyC XM_533235.2
M. mulatta SpeedyC XM_001118180.1
H. sapiens SpeedyC NM_001008778.1
S. purpuratus SpeedyA XM_777538.2
D. rerio SpeedyA NM_001006091.1
G. gallus SpeedyA XM_419361.2
M. domestica SpeedyA XM_001371303.1
S. scrofa SpeedyA AB196772.1
B. taurus SpeedyA BC109600.1
M. musculus SpeedyA AK014910.1
R. norvegicus SpeedyA BC078729.1
M. mulatta SpeedyA XM_001102851.1
H. sapiens SpeedyA AY820303.1

Expression pattern of Speedy genes

The hypothesis that *Speedy* genes are involved in early meiosis means that the mRNAs of these genes must be expressed early during meiosis. I am defining early meiotic events to include: meiotic initiation, meiotic DNA replication, or meiotic prophase. In the males, meiosis occurs in the postnatal and adult testes. In females, meiosis is initiated at around E14.5 (embryonic day 13.5) (McLaren 1981). The hypothesis predicts that *Speedy* genes should be expressed in the adult testis and the embryonic ovary.

To test this, I examined the expression pattern of the four mouse *Speedy* genes (*SpeedyA*, *SpeedyB1*, *SpeedyB2*, and *SpeedyB3*) in adult tissues using reverse transcription polymerase chain reaction (RT-PCR). The primer sequences used for this assay can be found in Appendix B. The tissue panel created consisted of RNA collected from heart, lung, kidney, testis, ovary, brain, spleen, and liver. In the adult mouse, expression of *SpeedyA, SpeedyB2,* and *SpeedyB3* was limited to the testis (Figure 2). All other organs did not have detectable levels of cDNA. Expression of *SpeedyB1* was undetermined due to PCR failure.

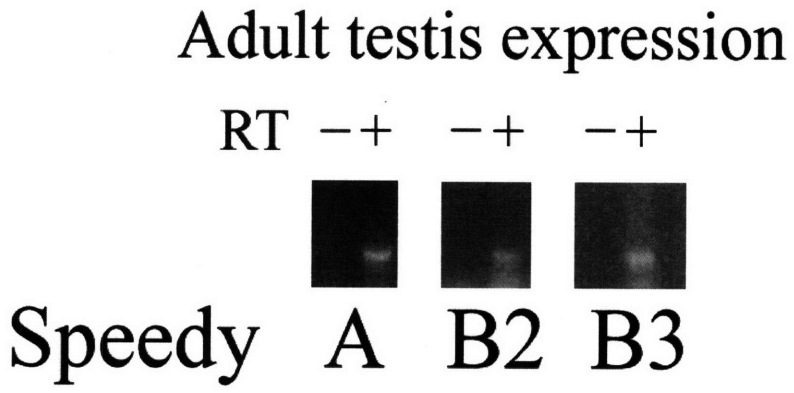


Figure 2: Presence of *Speedy* mRNAs in the testes of adult mice assayed by 35 cycles of PCR. Negative controls (RT -) are RNA samples that were not treated with reverse transcriptase enzyme.

Since RNA from all *Speedy* genes was detected in adult testis it was necessary to obtain a clearer profile of specifically which cells are expressing the *Speedy* genes. In the testis, meiosis is an ongoing process where spermatogonial stem cell populations divide to both maintain the stem cell population and to give rise to pre-meiotic, meiotic, and post-meiotic cells (McCarrey, 1993). These cell populations can be purified into: primitive type A spermatogonia, type A spermatogonia, type B spermatogonia, pre-leptotene, leptotene-zygotene, early pachytene, adult pachytene, and round spermatids. Additionally, residual bodies and Sertoli cells can also be purified (Wang et al., 2005). These cell populations were assayed by RT-PCR for *SpeedyA* and *SpeedyB2* gene expression (Figure 3). *SpeedyA*, *SpeedyB2*, and *SpeedyB3* is absent in Sertoli cells. *SpeedyA* appears very faintly in type-B spermatogonia and is maintained through round spermatids, where its expression decreases. To ensure that pre-leptotene spermatocytes were not contaminating the B-type spermatogonial fractions, I performed an assay for *Phosphoglycerate kinase 2* (*Pgk2*), which should not be expressed in B-spermatogonia (Wang et al., 2005). This confirmed that there is no detectable contamination of pre-leptotene RNA. If the expression pattern of the SPYA protein follows the expression of the RNA, then this protein may serve as a useful marker to distinguish A and B-type spermatogonia, since no such marker currently exists.

SpeedyB2 and *SpeedyB3* are expressed in pre-leptotene spermatocytes until adult pachytene, with SpeedyB3 expression persisting through round spermatids and residual bodies.

Figure 3

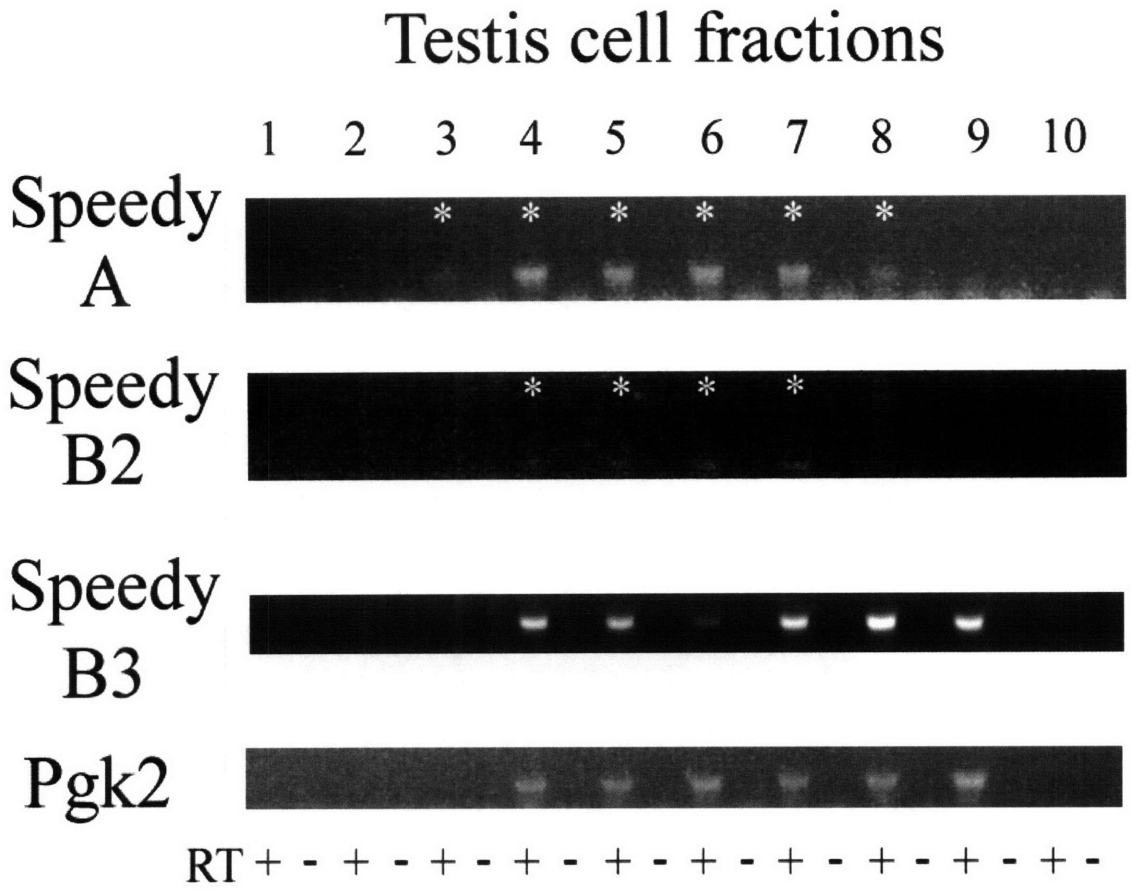


Figure 3: RT-PCR assay for *Speedy* genes and *Pgk2* in testis cell fractions. Cell types are labeled by number: (1) primitive type A spermatogonia, (2) type A spermatogonia, (3) type B spermatogonia, (4) pre-leptotene, (5) leptotene-zygotene, (6) early pachytene, (7) adult pachytene, (8) round spermatids, (9) residual bodies, and (10) Sertoli cells. Asterisks denote cell types that are positive for *Speedy* expression. Negative controls (RT -) are RNA samples that were not treated with reverse transcriptase enzyme.

I collected head, intestine, heart, bladder, lungs. mesonephrous, adrenals, and kidneys from E14.5 embryos. I then assayed for gene expression by RT-PCR. Only *SpeedyA* was expressed in the embryo at E14.5 (Figure 4); *SpeedyB2* and *SpeedyB3* were not detectable at this time in any tissues (data not shown). Interestingly, expression of *SpeedyA* appeared to be ubiquitous. A separate assay was performed using primers that would amplify specifically the long splice-variant (LSV) of *SpeedyA* to determine if the different spliceoforms had different expression patterns. The results of this assay were the same as before: ubiquitous embryonic expression of *SpeedyA* at E14.5.

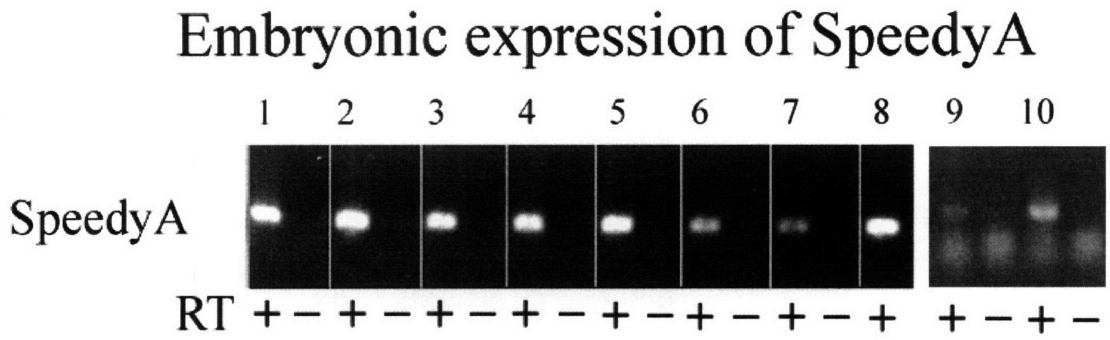


Figure 4: RT-PCR assay for *SpeedyA* in tissues from embryonic day 14.5. Tissues are labeled by number: (1) head, (2) intestine, (3) heart, (4) bladder, (5) lungs, (6) mesonephrous, (7) adrenals, (8) kidneys, (9) testis, and (10) ovary. SpeedyA is expressed in all tissues analyzed at this time-point. Negative controls (RT -) are RNA samples that were not treated with reverse transcriptase enzyme.

Regulation of SpeedyA

Several mouse mutants have been created to study the process of germ cell development and meiosis. Of these mutants, the *Stra8*-/- mouse is particularly useful in the elucidation of genes involved in meiosis since *Stra8* is required for the initiation of meiosis (Baltus et al., 2006). At E14.5, *SpeedyA* expression is no longer detectable in the ovaries of *Stra8*-/- mice (Figure 5). Additionally, neither *SpeedyB2* nor *SpeedyB3* are expressed, confirming the results obtained from the previous section. This implies that *SpeedyA* is not genetically upstream of *Stra8*, but rather downstream of this meiotic

initiator. Genes such as *Dmc1* and *Spo11*, that are essential for early meiosis, share the same regulation by *Stra8*.

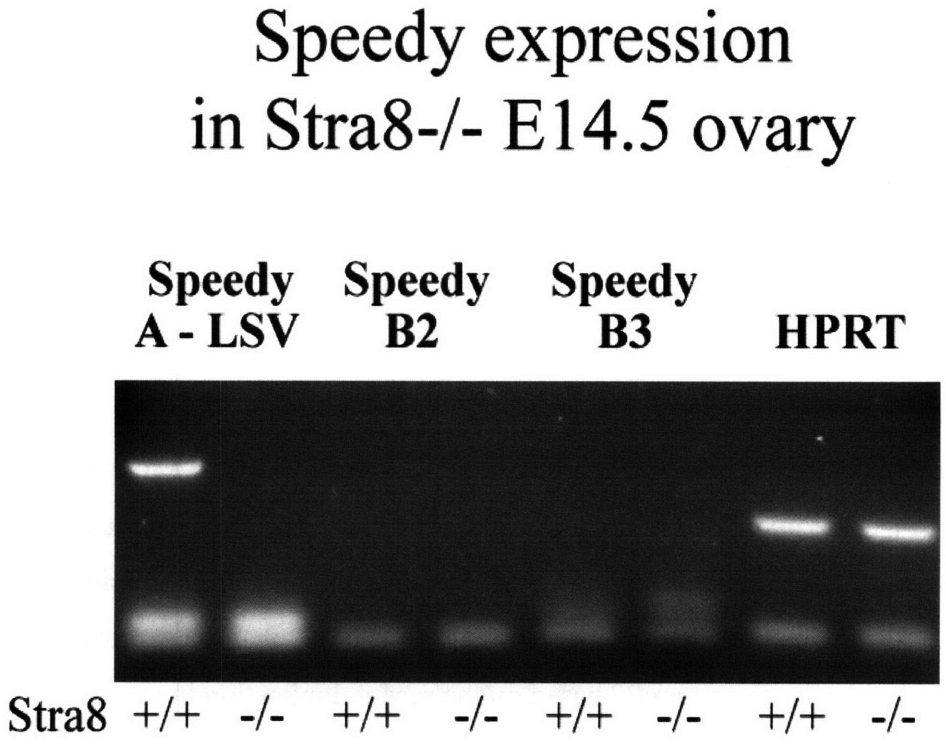


Figure 5: RT-PCR assay for *Speedy* genes in wild-type and *Stra8*$^{-/-}$ E14.5 ovaries.

CONCLUSIONS

Phylogenetic analysis of *Speedy*-box containing genes identified three branches of genes: *SpeedyA*, *SpeedyB*, and *SpeedyC*. A *Speedy* gene was found in the organisms *Dictyostelium discoidium*, *Danio rero*, and *Strongylocentrotus purpuratus*. There were no *Speedy*-box genes detected in *Caenorhabditis elegans* or *Drosophila melanogaster*. This suggests that the Speedy family is ancient and was lost in these lineages. *SpeedyB* genes appear to be evolving quickly, as evidenced by the variability of the *WBSCR19* genes in primates and dogs. *SpeedyB* and *SpeedyC* branches appear to be more recent than *SpeedyA*, with genes present only in mammals. The diversity of the *Speedy* genes is similar to that of the cyclin genes; there exist multiple family members, and they appear to have diversified in the mammalian lineage.

The expression in mouse of the *Speedy* genes is consistent with a role in early meiosis: the RNAs are expressed in the testis, and they are expressed in germ cells at pre-leptotene stage and maintained through round spermatids. *SpeedyB1* expression was unable to be assayed due to PCR failures. However, it is likely that the expression of *SpeedyB1* will be similar to *SpeedyB2* based on the recent duplication in mouse – there is no such palindrome in rat or opossum – and the fact that these two mRNAs are 94% identical on the nucleotide level. However, this is speculation from limited information and no promoter analysis.

SpeedyB2 and *SpeedyB3* are not expressed in the embryonic ovary at E14.5. Given their expression in pachytene spermatocytes, one might assume an equivalent expression during female meiosis in the embryonic ovary. Surprisingly, this was not the case. At E14.5, there are a number of female germ cells in pachytene, yet *SpeedyB2* and *SpeedyB3* were not expressed. It may be that these *Speedy* genes are expressed at a later time in female meiosis or that they are not expressed at all. A possible explanation for the male-specific expression is that the *SpeedyB* family is not involved in meiosis, but rather lays the groundwork for spermiogenesis. Another possible explanation is that they are simply not employed or needed during female meiosis. Male germ cells spend approximately ten days in pachytene whereas female germ cells spend only a few hours

to a day (McLaren 1981). Perhaps this difference in meiotic prophase length is tied to the function of *SpeedyB2* and *SpeedyB3*.

Mouse *SpeedyA* RNA is the only *Speedy* gene assayed that is expressed in the ovary at E14.5. Additionally, *SpeedyA* is present in many other tissues at E14.5. It is possible that *SpeedyA* serves a role in the mitotic cell cycle during embryogenesis, since its expression seems ubiquitous. This role of *SpeedyA* may not be shared with the other *Speedy* family members based on expression. Its expression in the ovary at this time point, however, is dependent on *Stra8*: *Stra8*-/- ovaries fail to express *SpeedyA*. This implies a role in the meiotic program.

While the *Speedy* genes are interesting regulators, there are a number of potential challenges that face future researchers. Studying the mouse *Speedy* genes genetically requires some caution. For example, *SpeedyA*'s ubiquitous expression in the embryo may necessitate the use of a conditional null allele to bypass a potential embryonic lethal phenotype. More expression data should be collected regarding how early *SpeedyA* is expressed embryonically and when its expression decreases. This may be necessary in order to study both the potential mitotic as well as meiotic defects of a *SpeedyA*-/-. In order to investigate the meiotic role, an efficient germ-line promoter to drive the excision of the allele in the correct lineage must be obtained. If the expression of the driver is too early such as Tissue Nonspecific Alkaline Phosphatase (TNAP), the phenotype may be premature cell death unrelated to the role of *SpeedyA* in meiosis. If the driver is too late, the cell may accumulate enough *SpeedyA* to drive the meiotic program and a phenotype may not be observed. The stability of SPYA protein at the different stages of the cell cycle should also be characterized. Given that most cell cycle machinery is controlled post-translationally, it would be surprising if *Speedy* genes were not regulated in that manner. Different difficulties accompany the study of the *SpeedyB* genes in mouse. Since *SpeedyB1* and *SpeedyB2* are located in a young palindrome in mouse, the locus will likely have to be targeted together in order to observe a phenotype. Additionally, the similar expression and *Speedy*-box of *SpeedyB3* suggests that it could compensate for a loss of *SpeedyB1* or *SpeedyB2*. A triple-knockout may be necessary to study this branch in mouse.

If *Speedy* genes are involved in meiotic initiation, it will be necessary to study the endogenous biochemical function of these genes. What is it about *Speedy* that makes the germ cell initiate meiosis? The likely answer is Speedy/CDK substrates are different than Cyclin/CDK substrates. Since *Speedy* is known to interact with CDKs, existing tools can be used to determine CDK substrate differences between Speedy/CDK complexes and Cyclin/CDK complexes. CDKs generated from chemical genetic screens (Shah and Shokat, 2002) can be used to track the phosphorylated protein substrates of the Speedy/CDK complexes. In this way, a list of unique targets could be assembled to shed light on the biochemistry of meiotic initiation.

These results implicate the *Speedy* family as playing a role in the early steps of meiosis. This conclusion is based on the expression pattern of the *Speedy* genes during spermatogenesis and the embryonic regulation of *SpeedyA* in the ovary by *Stra8*.

MATERIALS AND METHODS

Phylogeny

In order to identify Speedy-box containing genes, the Speedy-box from *Xenopus Speedy* was BLASTed on NCBI against a translated non-redundant nucleotide database (tBLASTn). Identified sequences were manually curated to obtain just the Speedy-box sequences. The Speedy-boxes were then aligned using the default settings of ClustalW (Chenna et al., 2003). Multiple phylogenies were then created using the PHYLIP software package (Felsenstein, 2007). The maximum likelihood molecular clock algorithm was used for Figure 1. The parsimony method, compatibility, maximum likelihood, and neighbor-joining algorithms provided similar results.

Speedy Expression Profiles

Adult tissue samples were obtained from three month old C57Bl/6 male and female mice. Embryonic tissue samples were obtained from males and females at E14.5 from C57Bl/6 wildtype and *Stra8*-/- mice. Tissues were frozen, thawed, and then homogenized in TRIzol reagent (Invitrogen). RNA from testis cell fractions were obtained as described previously (Wang et al., 2005).

Nucleic acids were resuspended and DNAse treated using the TurboDNAse kit (Ambion/Applied Biosystems) according to manufacturer's protocol. RNA was reverse transcribed using the RETROscript kit (Ambion/Applied Biosystems) and the provided oligo(dT) primers. Polymerase chain reactions were performed as described previously (Wang et al., 2005) using the primers in Appendix B. Annealing temperatures were 55 degrees for all primers used. Hypoxanthine guanine phosphoribosyl transferase (HPRT) was used as a positive control in all assays. Phosphoglycerate kinase 2 was assayed as described previously (Wang et al., 2005).

ACKNOWLEDGEMENTS

This thesis was made possible by the generous contribution of materials from various individuals. I would like to thank Ericka L. Anderson for contributing *Stra8*-/- cDNA and John McCarrey for contributing RNA from testis cell fractions. I would like to thank Daniel Winston Bellott and Helen Skaletsky for their crucial assistance in creating the phylogenies and in primer design for the various mouse *Speedy* genes. A deep thanks goes to Mark E. Gill from whom I inherited the task of untangling the *Speedy* gene family. Additionally, Mark performed preliminary analyses on the regulation and embryonic expression of *SpeedyA* (long splice-variant). This information proved valuable in stimulating our understanding of the *Speedy* genes. I would like to thank my mentor, David C. Page, for his assistance and guidance, and the entire Page Lab for their intellectual stimulation and support. I would like to finally thank my family and my roommate, Robin Stevens.

APPENDICES

Appendix A:

All identified Speedy homologues, sequences provided are the Speedy-box domains that were aligned in ClustalW.

>gi|56791750|gb|AY820303.1| Homo sapiens speedy A isoform 1 mRNA, complete cds, alternatively spliced_202_600

GTCAGGATATGACTGCTTTCTTTAAATTATTTGATGACGATTTAATTCAAGAT
TTCTTGTGGATGGACTGCTGCTGTAAAATTGCAGACAAGTATCTTTTGGCTAT
GACCTTTGTTTATTTCAAGAGGGCTAAATTTACTATAAGTGAGCATACCAGGA
TAAATTTCTTTATTGCTTTGTATCTGGCTAATACAGTTGAAGAAGATGAAGAA
GAAACCAAGTACGAAATTTTTCCATGGGCTTTAGGGAAAAACTGGAGAAAAT
TGTTCCCTAATTTCTTAAAGTTAAGGGACCAGCTCTGGGATAGAATTGACTAT
AGGGCTATTGTAAGCAGGCGATGTTGTGAGGAGGTTATGGCCATTGCACCAA
CCCATTATATCTGGCAAAGAGAACGTTCT

>gi|56912201|ref|NM_001008778.1| Homo sapiens speedy homolog C (Drosophila) (SPDYC), mRNA_169_561

AGGAGGTCCAGGCCTTCCTCAGCCTTCTGGAGGACAGTTTTGTCCAGGAATTC
CTCTCCAAAGACCCCTGCTTCCAGATTTCAGATAAGTATCTCCTGGCCATGGT
GCTGGTCTACTTCCAGCGCGCCCACCTGAAGCTCAGCGAGTATACCCACAGC
AGCCTGTTCTTGGCCCTGTACCTTGCAAACGACATGGAGGAGGACCTGGAGG
GCCCCAAATGTGAGATTTTTCCATGGGCCCTGGGAAAAGATTGGTGTTTACG
AGTGGGGAAATTCCTGCACCAGAGGGATAAGCTTTGGGCACGGATGGGTTTC
CGGGCTGTTGTGAGCCGCCAGTGCTGTGAGGAGGTCATGGCAAAGGAGCCAT
TCCACTGGGCTTGGACTCGGGACCGG

>gi|54400683|ref|NM_001006091.1| Danio rerio zgc:101624 (zgc:101624), mRNA_266_664

GACAAGAAATGGCCGCCTTCTTCAGACTGTTCGATGATGATCTAATACAGGA
CTTTCTGTGGATGGACTGCTGCTGCAAACTTACTGACAAGTATTTGTTGGCAA
TGACATTTGTGTACTTCAGGAGGGCTCGCTTCAGTATCGGTGAACACAGCAG
AATAAACTTCTTTCTTGCTCTCTATCTGGCAAACACTATGGAAGAGGATGAGG

AGGAGACCAAATATGAGATCTTCCCCTGGGCTTTGGGAAAGAGCTGGAGGAA
ACATTTCCCACGATTCCTGAAGCAAAGAGACCAACTGTGGGCACGTATTGAG
TACAGAGCTGCTGTCAGCAGACGATGCTGTGAGGAGGTGATGGCTATTGTGC
CGTCTCACTTCGTCTGGCAGCGGGAGCGTGCA

>gi|66730297:129-1151 Rattus norvegicus similar to hypothetical protein 4933411G11 (LOC499886), mRNA_613_864

AGGACCTCGAAGCATTCTACCGACTCCTGGAGGATCCTGTGGTCCAGAACTT
CTTGGCAGCTGACATCTTCTTCAGGGTGACCGACAAGTATCTGCTGTCTATGG
TGGTGGAGTACTTTGGTCGCGTTGGGCTTCCTGGACATCTCTACAACAGGATC
CACTTCTTCCTGGCCCTTTACATCGCCTGCGACATGGAGGAAGACGACCCCAT
ATCCAAGAGGAGCATCTTCCAATTCCTGCTGGGCAGGGAC

>gi|77798178|ref|NM_001034844.1| Homo sapiens similar to Williams Beuren syndrome chromosome region 19 (LOC441251), mRNA_304_540

CCTTCAACAGGCTGCTTGAGGATCCTGTCATTAAAAGATTCCTGGCCTGGGAC
AAAGGTCTGAGGGTGTCGGACAAGTATCTCCTGGCTATGGTCATAGTGTATTT
CAGCCGGGCCGGCCTCCCCTCCTGGCAATACCAATGCATTCATTTCTTCCTGG
CTCTCTACCTGGCCAATGACATGGAGGAGGACGACGAGGACCCCAAACAAA
ACATCTTCTACTTCCTGTATGGGAAG

>gi|73958149|ref|XM_846397.1| PREDICTED: Canis familiaris hypothetical protein LOC609183 (LOC609183), mRNA_274_513

CGTTCAACAGGCTGCTCGAGGATCCTGTCATTAAAAGATTTCTGGCCTGGGAC
AAAGATCTGAGAGTATCCGACAAGTATCTCTTGTCCATGGTCATCGCCTATTT
TAGCCGCGCTGGTCTCTTCTCCTGGCAGTACCAGCGAATTCATTTCTTCCTGG
CACTCTACCTGGCCAATGATATGGAAGAGGACAACCAGGCCCCCAAACAAGC
CATCTTTTCATTCCTTTATGGGAAGAAC

>gi|4995926|emb|AJ133500.1| Xenopus laevis mRNA for p33 ringo (ls27)_346_738

AGGAGCGCCAGGCCTTTTACAGGCTCCTAGAAAATGAGCTGATTCAGGAATT
TCTTTCTATGGACTCCTGTCTAAAGATTTCAGACAAGTATCTCATAGCAATGG
TTCTAGCATATTTTAAGCGGGCGGGCCTCTACACCAGCGAGTACACAACCAT
GAATTTCTTTGTTGCTCTGTATCTGGCTAATGACATGGAGGAAGATGAAGAA
GACTATAAATATGAAATCTTCCCCTGGGCACTAGGAGATTCATGGCGTGAGT

TTTTCCCACAATTTTTACGTCTCCGGGACGACTTCTGGGCTAAAATGAACTAC
CGAGCAGTTGTTAGCCGAAGATGTTGTGATGAGGTAATGGCGAAAGATCCCA
CTCATTGGGCCTGGCTCAGAGATCGT

>gi|4995924|emb|AJ133499.1| Xenopus laevis mRNA for p33 ringo (ls26)

AGGAGCGCCAGGCCTTCTACAGGCTCCTAGAAAATGAGCAGATTCAGGAATT
CCTTTCTATGGACTCCTGTCTAAGGATTTCCGACAAGTATCTCATAGCAATGG
TTCTAGCATATTTTAAGCGGGCAGCGGGCCTCTACACCAGCGAGTACACAAC
CATGAATTTCTTTGTTGCCCTGTATCTGGCTAATGACATGGAGGAAGATGAAG
AAGACTATAAATATGAAATCTTCCCCTGGGCACTAGGAGACTCGTGGCGTGA
GCTTTTCCCACAATTTTTGCGTCTCCGGGACGACTTCTGGGCTAAAATGAACT
ACCGAGCAGTTGTTAGTCGAAGGTGCTGTGATGAGGTAATGTCCAAAGATCC
CACTCATTGGGCCTGGCTGAGAGATCG

>gi|26326158|dbj|AK030175.1| Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4933411G11 product:hypothetical protein, full insert sequence_811_1050

TCACCAAGTTGCTCGAGGATCCCGTGGTGAAAAAATTCCTGACCTGGGACAA
GATGCTGCGGGTGTCAGACAAGTACCTCCTGTCTATGGTCATAGCTTATTTCA
GCCGCGCTGGGCTCTTCTCCTGGCAGTACAGGCCCATCCACTTCTTCCTGGCT
CTCTACCTGGCCAATGACATGGAGGAGGACAACCAGGCCCCTAAGCAAGAC
ATTTTTTACTTCCTCTATGGGAAGAGCTAT

>gi|113418686:1-1107 PREDICTED: Homo sapiens similar to Williams Beuren syndrome chromosome region 19 (LOC729511), mRNA_640_876

CCTTCAACAGGCTGCTTGAGGATCCTGTCATTAAAAGATTCCTGGCCTGGGAC
AAAGATCTGAGGGTGTCGGACAAGTATCTCCTGGCTATGGTCATAGCGTATTT
CAGCCGGGCCGGCCTCCCCTCCTGGCAATACCAACGCATTCATTTCTTCCTGG
CTCTCTACCTGGCCAATGACATGGAGGAGGACGACGAGGACCCCAAACAAA
ACATCTTCTACTTCCTGTATGGGAAG

>gi|109113324|ref|XM_001118215.1| PREDICTED: Macaca mulatta hypothetical protein LOC722015 (LOC722015), mRNA_453_695

CCTTCAACAGGCTGCTTGAGGATCCTGTCATTCAAAAATTCCTGGCCTGGGAC
AAAGATCTGAGGGTGTCAGACAAGTATCTCCTGGCTATGGTCATAGCGTACT

TTAGCCGTGCCGGCCTCTTCTCATGGCAATACCAACGCATTCATTTCTTCCTG
GCTCTCTACCTGGCCAGTGACATGGAGGAGGACAACCAGGCCCCCAAACAAG
ACATCTTCTCCTTCCTCTACGGCAAGGACTAC
>gi|109102514|ref|XM_001102851.1| PREDICTED: Macaca mulatta similar to speedy homolog 1 isoform 2, transcript variant 3 (LOC703530), mRNA_349_747
GTCAGGATATGACTGCTTTCTTTAAATTATTTGATGACGATTTAATTCAAGAT
TTCTTGTGGATGGACTGCTGCTGTAAAATTGCAGACAAGTATCTTTTGGCTAT
GACCTTTGTTTATTTCAAGAGGGCTAAATTTACTATAAGTGAGCATACCAGGA
TAAATTTCTTTATTGCTCTGTATCTGGCTAATACAGTTGAAGAAGATGAAGAA
GAAACCAAGTATGAAATTTTTCCATGGGCTTTAGGGAAAAACTGGAGAAAAT
TGTTCCCTAATTTCTTAAAGTTAAGGGACCAGCTCTGGGATAGAATTGACTAT
AGGGCTATTGTAAGCAGGCGATGTTGTGAGGAGGTTATGGCCATTGCACCAA
CCCATTATATCTGGCAAAGAGAACGCTCT
>gi|77627751|ref|NM_001034153.1| Rattus norvegicus similar to speedy B isoform (MGC125213), mRNA_445_684
TCACCAGGCTGCTCGAGGATCCCGTGGTGAAAAAATTCCTGAACTGGGACAA
GATGCTGAGGGTGTCAGACAAGTATCTCCTGTCTATGGTCATAGCTTATTTCA
GCCGCGCTGGGCTTTTCTCCTGGCAGTATAGGCCAATCCACTTCTTCCTGGCT
CTGTACCTGGCCAATGACATGGAGGAGGACAACCAGGCCCCTAAACAAGAC
ATCTTTTACTTCCTCTACGGGAAGAGCTAC
>gi|12853025|dbj|AK014910.1| Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4921517J08 product:hypothetical protein, full insert sequence_288_686
GCCAGGAAATGACTGCTTTCTTTAAATTATTTGATGATGATTTAATTCAAGAT
TTCTTGTGGATGGACTGCTGCTGCAAGATTGCAGACAAGTATCTTTTGGCTAT
GACCTTTGTTTATTTCAAGAGAGCTAAATTTACTATAAATGAGCATACCAGGA
TAAATTTCTTTATTGCTCTGTATCTGGCTAATACGGTTGAAGAAGATGAAGAA
GAAGCCAAGTATGAAATTTTTCCATGGGCTTTAGGGAAAAACTGGAGAAAAC
TGTTCCCTAATTTCTTAAAGTTAAGGGACCAACTCTGGGACAGAATTGACTAT
AGGGCTATTGTAAGCAGGCGATGCTGTGAAGAGGTCATGGCCATTGCGCCAA
CCCATTACATCTGGCAACGAGAGCGGTCT

>gi|81674637|gb|BC109600.1| Bos taurus cDNA clone IMAGE:8051100, partial cds_293_691

GTCAGGAAATGACTGCTTTCTTTAAATTATTTGATGACAATTTAATTCAGGAT
TTCTTGTGGATGGACTGCTGCTGTAAAATTGCAGACAAGTATCTTTTGGCTAT
GACCTTTGTTTATTTCAAGAGAGCTAAATTTACTGTAAATGAGCATACCAGGA
TAAATTTCTTTATTGCTCTGTATCTCGCTAATACAGTTGAAGAAGATGAAGAA
GAATCCAAATATGAAATTTTTCCATGGGCTTTAGGGAAAAACTGGAGAAAAT
TATTCCCTGACTTCTTAAAGTTAAGGGACCAACTCTGGGATAGAATTGACTAT
AGGGCTATTGTAAGCAGGCGATGCTGTGAGGAGGTTATGGCCATTGCTCCAA
CCCATTATATATGGCAACGAGAACGCTCT

>gi|109105826|ref|XM_001118180.1| PREDICTED: Macaca mulatta similar to speedy C (LOC721978), mRNA_904_1296

AGGAGGTCCAGGCCTTCCTCAGCCTTCTGGAGGACAGTTTTGTCCAGGAATTC
CTCTCCAAAGACCCCTGCTTCCAGATTTCAGATAAGTATCTCCTGGCCATGGT
GCTGGTCTACTTCCAGCGCGCCCACCTGAAGCTCAGCGAGTACACCCACAGC
AGCCTGTTCTTGGCCCTGTACCTTGCAAACGACATGGAGGAGGACCTAGAGG
GGCCCAAATGTGAGATTTTTCCATGGGCCTTGGGAAAAGATTGGTGTTTACG
AGTGGGGAAATTCCTGCACCAGAGGGATAAGCTTTGGGCACGGATGGGTTTC
CGGGCTGTTGTGAGCCGCCAGTGCTGTGAGGAGGTCATGGCAAAGGAGCCAT
TCCACTGGGCTTGGACTCGGGACCGG

>gi|67906119|dbj|AB196772.1| Sus scrofa RINGO mRNA for rapid inducer of G2/M progression in oocytes, complete cds_202_600

GTCAGGAAATGACTGCTTTCTTTAAATTATTTGATGATGATTTAATTCAAGAT
TTCTTGTGGATGGACTGCTGCTGTAAAATTGCAGACAAGTATCTTTTAGCTAT
GACCTTTGTTTATTTCAAGAGAGCCAAATTTACTATAAACGAGCATACCAGG
ATAAATTTCTTTATTGCTCTGTATCTGGCTAATACAGTTGAAGAAGATGAAGA
AGAATCCAAATATGAAATTTTTCCATGGGCTTTAGGGAAAAACTGGAGAAAA
TTATTCCCTGATTTCTTAAAGTTAAGGGACCAACTCTGGGATAGAATTGACTA
TAGAGCTATTGTAAGCAGGCGATGCTGTGAGGAGGTTATGGCCATTGCTCCA
ACACATTATATATGGCAACGAGAACGCTCT

>gi|73983703|ref|XM_533235.2| PREDICTED: Canis familiaris similar to speedy C (LOC476026), mRNA_184_546

AGCACAGTTTCCTCCAGGAATTCCTTTCCAGAGATCCCTGTTTCCAGATTTCG
GATAAGTATCTCCTGGCCATGGTGCTGGTCTACTTTCGGCGTGCCAACCTGAA
GCTCAGCGAGTACACCCATAGCAACTTGTTCTTGGCCCTGTTTCTTGCAAACG
ACATGGAGGAGGATCTTGAGGACCCTAAATGTGTGATTTTCCTGTGGGCCCT
GGGAAAAGATTGGCGTTGTCGGGTAGCAGACTTCCTACATCAAAGGGATAAG
CTGTGGGCCCGGATGGGCTTCCGGGCCATGGTGAGCCGCCAGTGCTGTGAGG
AGGTCATGGCCAAGGAGCCGTCCCACTGGGCCTGGACTCGAGAGCGG

>gi|51261149|gb|BC078729.1| Rattus norvegicus speedy homolog 1 (Drosophila), mRNA (cDNA clone MGC:93178 IMAGE:7134092), complete cds_209_607

GTCAGGAAATGACTGCTTTCTTTAAATTATTTGATGATGATTTAATTCAAGAT
TTCTTGTGGATGGACTGCTGCTGCAAAATCGCAGACAAGTATCTTTTGGCTAT
GACCTTTGTTTATTTCAAGAGAGCTAAATTTACTATAAGTGAACATACCAGGA
TAAATTTCTTTATTGCTCTATATCTGGCTAATACAGTTGAAGAAGATGAAGAA
GAAGCCAAGTACGAAATTTTTCCATGGGCTTTAGGGAAAAACTGGAGAAAAT
TGTTCCCTAATTTCTTAAAGTTACGGGACCAGCTGTGGGACAGAATTGACTAT
AGGGCTATTGTAAGCCGGCGCTGCTGTGAAGAGGTTATGGCCATTGCACCAA
GCCATTACATCTGGCAACGCGAGCGGTCT

>gi|118087626|ref|XM_419361.2| PREDICTED: Gallus gallus similar to rapid inducer of G2/M progression in oocytes (LOC421293), mRNA_297_695

GCCAGGAAATGACAGCTTTCTTTAAACTCTTTGATGACGATCTAATTCAAGAC
TTCCTAT
GGATGGACTGTTGCTGTAAAATTGCAGACAAGTATCTTTTGGCAATGACATTC
GTTTACTTCAAGAGGGCAAACTTCACAGTAGATGAGCATACCAGACTCAATT
TCTTCGTTGCTCTGTATCTGGCAAACACAGTGGAAGAAGACAATGAAGAATC
AAAGTATGAGATTTTCCCATGGGCTCTGGGAAAAAACTGGAGAAAGCTCTTT
CCTGATTTCTTAAAGTTAAGAGATCATCTATGGAGTAGAATTGACTACAGGG
CTATTGTAAGCAGACGTTGCTGTGAAGAGGTAATGGCTATTGCTCCAACACA
TTACATATGGCAGCGAGAACGTTCT

>gi|113426636|ref|XM_371014.4| PREDICTED: Homo sapiens hypothetical LOC388333 (LOC388333), mRNA_453_695

CCTTCAACAGGCTCCTTGGGGATCCTGTCGTTCAAAAATTCCTGGCCTGGGAC
AAAGACCTGAGGGTGTCAGACAAGTATCTCCTGGCTATGGTCATAGCGTATT
TTAGCCGTGCCGGCCTCTTCTCGTGGCAATACCAACGCATTCATTTCTTCCTG
GCTCTCTACCTGGCCAGTGACATGGAGGAGGACAACCAGGCCCCGAAACAA
GACATCTTCTCCTTCCTCTACGGGAAGAACTAC

>gi|126338939|ref|XM_001379898.1| PREDICTED: Monodelphis domestica similar to speedy C (LOC100030426), mRNA_115_507

AGGAGGTTCGAGCCTTCCTCAACCTTCTAGAGGACAGCTACCTGCAGCAATT
CTTTGCCACAGATCTCTGCTTTCAGATCTCAGATAAGTATCTCTTGGCAATGG
CTCTGATTTATTTCCAAAGAGCTGGCCTGCAGCTCAGTGAATATACCCACAGC
AACCTCTTCTTAGCCCTGTATCTAGCGAATGACATGGAGGAAGACAGAGAGG
ACCCCAAGTATGAGATTTTGCCCTGGGCCCTGGGCCAGGGCTGGCGGAAACT
TGTGCCCAGCTTCTTGCGCCAGAGAGACCAGCTGTGGGCACGTATGGGCTAT
AGGGCAGCTGTAAGCCGACACTGCTGTGAAGAGGTCATGGCAAAGGAACCA
TCCCATTGGGCTTGGACAAGGGAGCGA

>gi|119917282|ref|XM_870874.2| PREDICTED: Bos taurus similar to speedy B (LOC618542), mRNA_295_531

TCAACAAGGTGCTTGAGGATCATGTCGTTAAGAGATTCCTGGCCTGGGACAG
AAACCTGAAGGTATCCGACAAGTATCTCCTGGTGATGGTGATCGCTTATTTCA
GCCGAGCCAGCCTCCTCTTCTGGCAATACCGGAGGATCCATTTCTTCATAGCT
CTCTACCTGGCCAGTAAGATGGAAGAGGACAACCAGGCCCACAAACAGATC
ATCTTTTCGTTCCTCTATGGAAGGAAC

>gi|126303115|ref|XM_001371303.1| PREDICTED: Monodelphis domestica similar to rapid inducer of G2/M progression in oocytes (LOC100026500), mRNA_202_600

GTCAAGAAATGACTGCTTTCTTTAAATTATTTGATGATGACTTAATTCAAGAT
TTCTTGTGGATGGATTGCTGCTGTAAAATTGCAGATAAGTATCTTCTGGCTAT
GACCTTTGTTTACTTCAAGAGGGCTAAGTTTAGTATAAATGAACATACCAGA
ATAAATTTCTTTATTGCTCTGTATCTGGCTAATACGGTTGAAGAAGATGAAGA
AGAATCAAAGTATGAAATTTTTCCATGGGCTCTGGGAAAAAACTGGAGGAAA

TTATTTCCTAATTTCTTAAAGTTAAGGGATCAGCTTTGGGATAGGATTGACTA
TAGGGCTATTGTAAGCAGACGGTGCTGTGAAGAGGTTATGGCTATTGCTCCA
ACACATTATATATGGCAACGAGAGCGATCC

>gi|30795187|ref|NM_175064.2| Homo sapiens Williams Beuren syndrome chromosome region 19 (WBSCR19), mRNA_605_844

CCTTCAACAGGCTGCTTGAGGATCCTGTCATTAAAAGATTCCTGGCCTGGGAC
AAAGATCTGAGGGTGTCGGACAAGTATCTCCTTGCTATGGTCATAGCGTATTT
CAGCCGAGCCGGCTTCCCCTCCTGGCAATACCAACGCCTTCATTTCTTCCTGG
CTCTCTACCTGGCCAATGACATGGAGGAGGACGACGAGGACTCCAAACAAA
ACATCTTCCACTTCCTGTATGGGAAGAAC

>gi|71979926|ref|NM_001031618.1| Homo sapiens similar to Williams-Beuren syndrome critical region protein 19 (MGC119295), mRNA_304_543

CCTTCAACAGGCTGCTTGAGGATCCTGTCATTAAAAGATTCTTGGCCTGGGAC
AAAGATCTGAGGGTGTCGGACAAGTATCTCCTGGCTATGGTCATAGCGTATTT
CAGCCGGGCCGGCTTCCCCTCCTGGCAATACCAACGCATTCATTTCTTCCTGG
CTCTCTACCTGGCCAATGACATGGAGGAGGACGACGAGGACTCCAAACAAA
ACATCTTCCACTTCCTGTATAGGAAGAAC

>gi|109065955:1-1296 PREDICTED: Macaca mulatta similar to Williams Beuren syndrome chromosome region 19 (LOC718058), mRNA_805_1044

CCTTCAACAGGCTGCTTGAGGATCCTGTCATTAAAAGATTCCTGGCCTGGGAC
AAAGATCTGAGGGTGTCGGACAAGTATCTCCTGGCTATGGTCATAGCATATTT
TAGCCGGGCCGGCCTCTTCTCCTGGCAATACCAACGCATTCATTTCTTCCTGG
CTCTCTACCTGGCCAATGACATGGAGGAGGACAGCGAGACCCCCAAACAAA
ACATCATCTACTTCCTGTACAGGAAGAAC

>gi|115699935|ref|XM_777538.2| PREDICTED: Strongylocentrotus purpuratus similar to speedy A (LOC577302), mRNA_160_552

AAGAAATAACTGCGTTTTTCCGGCTTTTTGATGATGATGTTATTCAAGATTTT
CTCTGGATGGACTGCTGTGCCAAGACAGCAGACAAGTATCTCTTGGCCATGG
TCTTTGCTTACTTCAAGAGAGCACAGTACACCATCAAGCTATATACAAGAAT
GAACTTCTTTGTAGCACTGTATTTGGCAAGCGACATGGAAGAAGATGAAGAG
GATGACAAGTACAACATTTTCCCGTGGGCACTGGGGAGAAACTGGAGAAATA

CTTATCCTGGCCTTCTTCGCAAGCGGGACAGGCTGCTAAGGACTATCCAATAC
AGAGCTGCAGTCAGTAGGAAGTGCTGTGAAGAGGTCATGAGCTTGGTTCCAG
ACCACATCATCTGGAAGCGTGACCGT

>gi|126304256|ref|XM_001382044.1| PREDICTED: Monodelphis domestica hypothetical protein LOC100033219 (LOC100033219), mRNA_229_477

AGGATCAGGAAGCCTTCTACAGACTTCTTGAGGATCCTGTCATCCAGAGTTTC
TTGGAGGCTGATGTTTTCCTAAGAGTGTCTGATAAGTATCTGCTCTCTATGGT
GGTTGAATATTTTGGCCGAGTGGGACTCCCAGGAGATTGCTATAACAGGATC
CACTTCTTTCTGGCCCTCTATATTGCCTGTGACATGGAGGAAGACAACCCTGT
CTCTAAGTGGAGCATCTTTCCTTTTGTGCTTGGAAAG

>gi|119919313|ref|XM_591631.3| PREDICTED: Bos taurus similar to speedy C (LOC513874), mRNA_112_504

AGGAGCTCCGGGCCTTCCTCCACCTTCTGGAGCACAGTTTCCTCCAGGATTTC
CTCTCCAAAGATCCCTGTTTCCAGATTTCAGACAAGTATCTCCTGGCCATGGT
GCTGGTCTACTTCCGGCGTGCCAACCTGCAGCTCAGCGAGTACACCCACAGC
AATCTGTTCCTGGCACTGTACCTTGCAAATGACATGGAGGAAGACCTGGAGG
ACCCCAAAAGCGTGATTTTTCTGTGGGCCCTGGGCCAAGATTGGCATCATCA
AGTGTCAGACTTCCTGCATCAGAGGGACAAGCTGTGGGCACGGATGGGCTTC
AGGGCTGTTGTGAGACGCCAGAGCTGCGAGGAGGTCATGGCCAAGGAGCCG
ACCCACTGGGCCTGGACTCGGGAGCGG

>gi|12853787|dbj|AK015441.1| Mus musculus adult male testis cDNA, RIKEN full-length enriched library, clone:4930451F05 product:hypothetical protein, full insert sequence_494_733

TCACCAAGCTGCTCGAGGATCCTGTGGTGAAAAAATTCCTGACCTGGGACAA
GATGCTGAGGGTGTCAGACAAGTACCTCCTGTCTATGGTCATAGCTTATTTCA
GCCGCGCTGGGCTCTTCTCCTGGCAGTACAGGCCCATCCACTTCTTCCTGGCT
CTCTACCTGGCCAATGACATGGAGGAGGACAACCAGGCCCCTAAGCAAGAC
ATTTTTTACTTCCTCTATGGGAAGAGCTAT

>gi|66814435|ref|XM_636305.1| Dictyostelium discoideum AX4 hypothetical protein (DDBDRAFT_0206540) mRNA, complete cds

GGAGAATGAGATTAAGACAGTTCCATACTGGAAAAGATATTTTATGGAGAGC
ATTAGATTATAAAACAGTTGTCGATTTTCCACATATTCAAAAAACAATTAAAG
CTTTTCCAAATCACGATATTTTAAAAAGAGAAAGAACT

>gi|94367229|ref|XM_141353.7| PREDICTED: Mus musculus gene model 355, (NCBI) (Gm355), mRNA

AGGACCTCGAAGCCTTCTACCGACTTCTGGAGGATCCTGTGGTCCAGAATTTC
TTGGCAGCGGACATTTTCTTCAGGGTGACTGACAAGTATCTGCTGTCTATGGT
GGTGGAGTATTTTGGTCGAGTTGGGCTTCCTGGACATCTCTACAACAGGATCT
ACTTCTTCCTGGCCCTCTATATCGCCTGTGACATGGAAGAGGATGACCCCATA
TCCAAGAGGAGCATCTTCCAATTCTTGCTGGGCAGAGAC

>gi|74013188|ref|XM_843908.1| PREDICTED: Canis familiaris similar to Williams Beuren syndrome chromosome region 19 (LOC607286), mRNA

AGGATCCTGTCATTAAAAGATTTCTGGCCTGGGACAAAGATCTGAGAGTATC
CGACAAGTATCTCTTGTCCATGGTCATCGCCTATTTTAGCCGCGCTGGTCTCTT
CTCCTGGCAGTACCAGCGAATTCATTTCTTCCTGGCACTCTACCTGGCCAATG
ATATGGAAGAGGACAACCAGGCCCCCAAACAAGCCATCTTTTCATTCCTTTAT
GGGAAGAAC

Appendix B:

Table of primers used

	SpeedyA
forward primer	TCTTTTGGCTATGACCTTTGTT
reverse primer	TTCTGTCCCAGAGTTGGTCC
RT-PCR product size	215
genomic product size	6643
	SpeedyA-long splice variant
forward primer	GGACATGGTCTGTGTGGAAA
reverse primer	CAGGCTTCTCCGTTTGGTTA
RT-PCR product size	248
genomic size	248
	SpeedyB2
forward primer	ACCATGCCGTCTAAATCTGC
reverse primer	TCTGCAGCGTCATCCTGA
RT-PCR product size	193
genomic size	2676
	SpeedyB3
forward primer	CGGACATTTTCTTCAGGGTG
reverse primer	ACCCAGGCTTGGTAATCCAT
RT-PCR product size	268
genomic size	939

Appendix C:

Table of names used for sequences, existing nomenclature obtained from NCBI, and Genbank accession numbers

Nomenclature used here	Other names	Genbank Accession
Speedy Branch A		
D. discoideum SpeedyA		XM_636305
S. purpuratus SpeedyA	similar to SpeedyA	XM_777538.2
D. rerio SpeedyA	zgc:101624, Speedy1	NM_001006091.1
G. gallus SpeedyA	Speedy homolog A (Drosophila)	XM_419361.2
M. domestica SpeedyA	Similar to RINGO	XM_001371303.1
S. scrofa SpeedyA	RINGO, Speedy homolog 1, SpeedyA2, RINGOA2	AB196772
B. taurus SpeedyA	Speedy homolog 1, Speedy homolog A	BC109600.1
M. musculus SpeedyA	Spdy1, SpeedyA, SpeedyA1, SpeedyA2, RINGOA2, GS4	AK014910.1
R. norvegicus SpeedyA	Speedy homolog A (Drosophila)	BC078729.1
M. mulatta SpeedyA	Speedy homolog 1	XM_001102851.1
H. sapiens SpeedyA	Spy1, Spdy1, SpyA2, SpeedyA, Speedy homolog A (Drosophila), RINGO3, RINGOA2	AY820303.1
Speedy Branch B		
M. domestica SpeedyB		XM_001382044.1
M. musculus SpeedyB1	SpeedyB, RINGOB, RINGO4, Speedy homolog 2	AK015441.1
M. musculus SpeedyB2	4933411G11Rik	AK030175.1
M. musculus SpeedyB3	GM355	XM_141353
R. norvegicus SpeedyB1	Similar to SpeedyB isoform	NM_001034153
R. norvegicus SpeedyB3	Similar to 4933411G11	NM_001024312
B. taurus SpeedyB	Similar to SpdyB	XM_870874.2
C. familiaris SpeedyB1	Similar to WBSCR19	XM_843908
C. familiaris SpeedyB2		XM_846397.1
M. mulatta SpeedyB1	Similar to WBSCR19	XM_001110275
M. mulatta SpeedyB2		XM_001118215.1
H. sapiens SpeedyB1	WBSCR19, RINGO1, RINGOE	NM_175064.2
H. sapiens SpeedyB2	Similar to WBSCR19	NM_001031618.1
H. sapiens SpeedyB3	Similar to WBSCR19	NM_001034844.1
H. sapiens SpeedyB4		XM_371014.4
Speedy Branch C		
M. domestica SpeedyC	Similar to SpeedyC	XM_001379898.1
B. taurus SpeedyC	Speedy homolog C (Drosophila)	XM_591631.3
C. familiaris SpeedyC	Speedy homolog C (Drosophila)	XM_533235.2
M. mulatta SpeedyC	Similar to SpeedyC	XM_001118180.1
H. sapiens SpeedyC	Speedy homolog C (Drosophila) RINGO2	NM_001008778
Xenopus Speedy genes		
X. laevis XSpeedy1	P33 RINGO, X-RINGOB, ls27, X-Spy1	AJ133500.1
X. laevis XSpeedy2	P33 RINGO, X-RINGOA, ls26, X-RINGO	AJ133499.1

BIBLIOGRAPHY

Baltus, A. E., Menke D.B., Hu, Y.C., Goodheart, M.L., Carpenter, A.E., de Rooij, D.G., Page, D.C. (2006) In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. Nat Genet *38*, 1430-1434

Barnes, E.A., Porter, L.A., Lenormand, J.L., Dellinger, R.W., Donoghue, D.J. (2003) Human Spy1 promotes survival of mammalian cells following DNA damage. Cancer Res *63*, 3701-3707

Cheng, A., Gerry, S., Kaldis, P., Solomon, M.J. (2005a) Biochemical characterization of Cdk2-Speedy/Ringo A2. BMC Biochem *6*:19

Cheng, A., Xiong, W., Ferrell, J.E. Jr., Solomon, M.J. (2005b) Identification and comparative analysis of multiple mammalian Speedy/Ringo proteins. Cell Cycle 4, 155-165

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G., Thompson, J.D. (2003). Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res *31*, 3497-3500

Dinarina, A., Perez, L.H., Davila, A., Schwab, M., Hunt, T., Nebreda, A.R. (2005) Characterization of a new family of cyclin-dependent kinase activators. Biochem J *386*, 349-355.

Felsenstein, J. (2007) PHYLIP (Phylogeny Inference Package), version 3.67. Department of Genetics, University of Washington, Seattle.

Ferby, I., Blazquez, M., Palmer, A., Eritja, R., Nebreda, A.R. (1999) A novel p34(cdc2)-binding and activating protein that is necessary and sufficient to trigger G(2)/M progression in Xenopus oocytes. Genes Dev *13*, 2177-2189

Gastwirt, R.F., McAndrew, C.W., Donoghue, D.J. (2007) Speedy/RINGO regulation of CDKs in cell cycle, checkpoint activation and apoptosis. Cell Cycle *6*, 1188-1193

Karaiskou, A., Perez, L.H., Ferby, I., Ozon, R., Jessus, C., Nebreda, A.R. (2001) Differential regulation of Cdc2 and Cdk2 by RINGO and cyclins. J Biol Chem *276*, 36028-36034

Küntzel, H., Schulz, A., Ehbrecht, I.M. (1996) Cell cycle control and initiation of DNA replication in *Saccharomyces cerevisiae*. Biol Chem *377*, 481-487

Lenormand, J.L., Dellinger, R.W., Knudsen, K.E., Subramani, S., Donoghue, D.J. (1999) Speedy: a novel cell cycle regulator of the G2/M transition. EMBO J *18*, 1869-1877

McCarrey, J.R. (1993) Development of the germ cell. In Desjardins, C. and Ewing, L. (eds), Cell and Molecular Biology of the Testis, Vol. V. Oxford University Press, New York, 58–89

McLaren, A. (1981) "Germ Cells and Soma: A new look at an old problem." Yale University Press, New Haven, CT.

Oulad-Abdelghani, M., Bouillet, P., Décimo, D., Gansmuller, A., Heyberger, S., Dollé, P., Bronner, S., Lutz, Y., Chambon, P. (1996) Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J Cell Biol *135*, 469-477

Shah, K., Shokat, K.M. (2002) A Chemical Genetic Screen for Direct v-Src Substrates Reveals Ordered Assembly of a Retrograde Signaling Pathway. Chem Biol *9*, 35-47

Wang, P.J., Page, D.C., McCarrey, J.R. (2005) Differential expression of sex-linked and autosomal germ-cell-specific genes during spermatogenesis in the mouse. Hum Mol Genet *14*, 2911-2918