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Germ Cell Biology – from generation to generation

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ABSTRACT Germ cells hold a unique place in the life cycle of animal species in that they are the cells that will carry the genome on to the next generation. In order to do this they must retain their DNA in a state in which it can be used to recapitulate embryonic development. In the normal life cycle, the germ cells are the only cells that retain this ability to recapitulate development, referred to as developmental totipotency. The molecular mechanisms regulating developmental potency are poorly understood. Recently its has been shown that germ cells can be turned into pluripotent stem cells when cultured in specific polypeptide growth factors that affect their survival and proliferation. The ability to manipulate developmental potency in germ cells with growth factors allows the underlying mechanisms to be dissected. Germ cells are also the only cells that undergo the unique reductive division of meiosis. This too is essential for the ability of germ cells to form the gametes that will carry the genome into the next generation. Arguably meiosis is the most important division in the life of a nascent organism. Defects in meiosis can result in embryonic or fetal loss or, if the animal survives, in the birth of an individual with chromosomal abnormalities. Recent advances in our understanding of meiosis have come from knockout mice and studies on genes identified through studies of human infertility. This review will focus on these two key aspects of germ cell biology, developmental potency and meiosis.

KEY WORDS: *Germ cells, stem cells, developmental potency, meiosis, chromosome segretation, teratoma.*

Introduction

In her classic treatise "*Germ Cells and Soma: A New Look at an Old Problem*" published in 1981, Anne McLaren led the reader through the entire life cycle of a germ cell in the mouse (McLaren 1981). Starting with the period of oocyte growth the story unfolds through the fusion of the male and female germ cells at fertilization, through early embryogenesis up to the period of gastrulation; through the reappearance of definitive germ cells in the post-implantation embryo; through their migration to, and colonization of, the gonad anlagen; through sexual differentiation and back again to the formation of the gametes. It is a remarkable book because of the depth of knowledge displayed by a single individual about a complex cell lineage. At the very end of the book Anne restated some of the important, and then unanswered, questions about germ cell development and homeostasis:

> *"We are still almost totally ignorant of most aspects of the interactions between germ cells and soma. For example, we have no idea of how primordial germ cells are guided to the genital ridges; whether the somatic environment is necessary for the survival of germ cells at this stage, or whether they could be isolated and grown for an indefinite period in culture; how the X chromosome is switched on; how the signal for meiosis is transmitted and received; how meiotic arrest is achieved; what determines whether and when germ cells undergo atresia and degenerate; what determines when they leave the primordial pool and start to grow; what degenerative processes occur during the long period of arrest; what proteins are taken up from the follicle; or how much of the protein either taken up or synthesized is important for embryonic development." (McLaren 1981)*

While we now know much more about germ cell development and homeostasis, many of those questions are just as pertinent today as they were when the book was written some twenty years ago. Over the years, Anne has tackled many of those questions

Abbreviations used in this paper: GVBD, germinal vesicle breakdown; LIF, leukemia inhibiting factor; MPF, maturation promoting factor; PGC, primordial germ cell; SCF, stem cell factor; TNAP, tissue non-specific alkaline phosphatase, W, dominant white spotting; Sl, steel; SSEA-1, stage-specific embryonic antigen-1

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and some of them have become the focus of our own research. For many of us who begin to study one area of germ cell development, other features of the lineage soon become fascinating and we begin to investigate other questions about their development also. Two aspects of germ cells make them unique. First, they are the only cells in the normal life cycle of an organism that carry the genome on to the next generation. Second they are the only cells in an organism that are capable of undergoing the reductive meiotic division that is such a fundamental part of gametogenesis. These two subjects, developmental potency and meiotic regulation, have become the two areas that we have focused on over the last few years.

Germ cell survival and migration

Our own interest in PGC development began and was fostered in the laboratories of Chris Wylie and Janet Heasman at St. George's Hospital Medical School in London who used PGCs as a model with which to study cell migration. But how to study PGC migration? In the Wylie/Heasman lab the clawed toad, *Xenopus*, was the animal of choice and it indeed has many attractive features - at least as an experimental organism! But through a collaborative effort with Anne McLaren we realized that there were also many advantages to the use of the mouse as an experimental organism. Not least of these were the superior genetics of the laboratory mouse and the wide variety of sophisticated cell culture systems. With Anne's help, and the characteristically-enthusiastic support of Chris Wylie and Janet Heasman, we began to develop systems to study PGC development in mice. It was typical of Anne that she helped us even though she was working on the same question herself and which she had posed earlier: "*whether the somatic environment is necessary for the survival of germ cells at this stage, or whether they could be isolated and grown for an indefinite period in culture*". With various colleagues she had published several papers that formed the foundation for our own work. Importantly these papers helped us decide what to do and also what not to do. Studies carried out by Massimo de Felici with Anne at the Medical Research Council Mammalian Development Unit in London showed that mouse PGCs could be isolated from embryos and would survive if cultured in outgrowths of the genital ridge (De Felici and McLaren 1982); (De Felici and McLaren 1983). These cultures, which at the time we called Massimo Cultures, led us to think that PGCs needed something produced by somatic cells to survive and, therefore, perhaps they could be cultured on feeder layers of established cell lines. The use of STO cells (a generous gift of Dr. B.L.M. Hogan, ICRF, London), a mouse embryo-derived fibroblast cell line, led to the development of culture conditions in which PGCs could be maintained for up to 10 days and in which they would actively migrate (Donovan *et al.,*1986). These studies led in turn to the development of feeder-dependent culture systems in which it is possible to study many aspects of PGC behavior (Donovan *et al.,* 1987). We were able to study PGC behaviour before and after entry into the genital ridge and found that PGCs behave in culture much as they do in the embryo. In fact we also found that changes in the expression of cell surface antigens on PGCs also mirror that seen in the embryo. Together these data led us to suggest that PGCs may have an intrinsic clock that times their development (Donovan *et al.,* 1986). This idea was not new and was based in part on studies of oligodendrocyte and type II astrocyte progenitors carried out by Martin Raff and his colleagues (Raff *et al.,* 1985) (Temple and Raff 1986) (Raff *et al.,* 1988). At the time we believed that PGCs behaved *in vitro* as they do *in vivo* and stop migration and proliferation at about the same time as they would if they had been left in the embryo (Donovan *et al.,* 1986). If someone had asked us Anne McLarens' question "*whether (PGCs) could be isolated and grown for an indefinite period in culture"* our answer at that time would have been a resounding "No"!

But soon our attention shifted from studying cell migration to understanding growth control in PGCs. A major breakthrough in understanding PGC growth and survival came from the cloning of the genes encoded at the *W* or *Dominant White Spotting* and the *Sl* or *Steel* loci. Mice carrying mutations at either of these loci can have severe defects in three lineages, germ cells, haemopoietic stem cells and melanocytes. Importantly, mice carrying mutations at the *W* or *Sl* loci can be completely sterile. Our first adventures with these animals began with the help of Anne McLaren and her colleague at the MRC MDU, Mia Buehr as well as Dot Bennett at St. George's Hospital Medical School in London. Histological studies had demonstrated that the defect in germline development in these animals occurred as early as 9.5 days post coitum (dpc) (Mintz 1957) (Mintz and Russell 1957). With the demonstration that *W* encodes the c-Kit receptor and *Sl* encodes its' ligand Stem Cell Factor, the stage was set for the characterization of the role of these gene products in germline development. We have argued that Stem Cell Factor (SCF), acting through the c-Kit receptor, is required for PGC survival (Dolci *et al.,* 1991). That conclusion is based on studies showing that PGCs do not survive in culture on cells that do not produce SCF and on studies showing that a neutralizing c-Kit antibody has the same effect (Dolci *et al.,* 1991) (Godin *et al.,* 1991) (Matsui *et al.,* 1991). Moreover, long term survival of PGCs seems to require a membrane-bound form of SCF (Dolci *et al.,* 1991). This requirement imposes certain constraints on PGC growth and survival and in part helps answer the question: "*whether the somatic environment is necessary for the survival of germ cells at this stage*". Our answer is "Yes". PGCs require production of membrane-bound SCF by somatic cells in order to be able to survive. Without SCF they will die through programmed cell death. This fact in turn places a constraint on where PGCs can migrate in the embryo. It follows that if PGCs migrate away from cells producing SCF they will die. Because PGCs expressing c-Kit have a requirement for membrane-bound forms of SCF it also seemed possible that the c-Kit/SCF interaction could form part of an adhesion mechanism. In this way, SCF could act not only as a survival factor but also be part of the pathway on which PGCs migrate. Such a conclusion could be drawn from earlier studies on PGC migration in *Sl* mutant mice that predated the molecular cloning of the *Sl* locus (McCoshen and McCallion 1975). These studies demonstrated that in mice expressing only soluble forms of SCF, many PGCs get lost and fail to colonize the embryonic gonad (McCoshen and McCallion 1975). Subsequent studies by Anne and Mia Buehr and their colleagues demonstrated that in *W* mutant mice PGCs clump together and fail to undergo the characteristic changes in morphology that presage the onset of migration (Buehr *et al.,* 1993).

One of the challenges for the future will be to understand the role of the c-Kit signaling pathway in regulating both PGC survival and to dissect its' possible role in cell migration. Some of these questions can be addressed by site-directed mutagenesis of the c-

Kit receptor by homologous recombination in embryonic stem (ES). Our own approach to dissecting the role of the c-Kit pathway has been to develop systems to manipulate gene expression in PGCs using retroviral-mediated gene delivery. This system, pioneered by Harold Varmus and his colleagues for use in glial cells, allows for delivery of multiple genes in a directed fashion to a specific cell lineage or tissue (Holland *et al.,* 1998a; Holland and Varmus 1998a). We have adapted this system to deliver genes in a combinatorial way to PGCs and to begin to determine which molecules act downstream of the activated c-Kit receptor. In other cell types SCF-induced dimerization of the c-Kit receptor leads to activation of a variety of signaling molecules, including JAK2, Src, Shc, Grb2, PLCγ, Ras and PI3K (for review, see Blume-Jensen *et al*., 1998). The PI3K pathway has been demonstrated to be of special importance in male spermatogonial stem cells (Blume-Jensen *et al.,* 2000) (Kissel *et al.,* 2000) (Feng *et al.,* 2000). Once activated, PI3K produces phosphatidylinositol 3,4 phosphates (PtdIns3,4Ps), which lead to activation of different signaling molecules, including PDK1, PKCδ, PLCγ, Ras and AKT (for review, see (Fruman *et al.,* 1999) (Chan *et al.,* 1999) (Rameh and Cantley 1999). PI3K activation is sufficient to stimulate AKT (Franke *et al.,* 1997). In addition, other molecules that are distinct from PI3K have been demonstrated to activate AKT, such as PDK 1, PKCδ, Src and G-protein-coupled receptors (Downward 1998). In turn, AKT activation by phosphorylation and translocation to the plasma membrane promotes a variety of events, including cell survival, proliferation, differentiation (for review see (Downward 1998) (Chan *et al.,* 1999)) and oncogenic transformation (Aoki *et al.,* 1998) (Holland and Varmus 1998b). Using retroviral-mediated gene delivery we have discovered an important role for AKT in regulating PGC survival downstream of the c-Kit receptor. Such an approach is widely applicable for dissecting any signaling pathway known to act in PGCs. Moreover, this system can also be used for functional studies in PGCs on many of the new genes that have been discovered as a result of the human and mouse genome projects. In this way the function of multiple genes can be examined rapidly and without the need for transgenesis. This gene transfer system can be used to study many aspects of gene regulation of germ cell behaviour and growth. Germ cell migration is a complex problem and understanding how germ cells get to the gonad will likely require the use of a variety of techniques including gene transfer, targeted gene modification and cell marking. Perhaps SCF is part of the migratory pathway but other molecules, such as laminin and fibronectin are likely to be required also (Anderson *et al.,* 1999). Then there is the possibility that PGC migration is guided by chemotaxis. In fact, as Anne says in *Germ Cells and Soma* "*Route finding - how the germ cells find their way - remains deeply mysterious*" (McLaren, 1981).

Germ cells, stem cells and developmental potency

Determining that SCF is a survival factor for PGCs was an important observation because it led to the realization that it is not a powerful mitogen. By observing PGC proliferation on different feeder layers we noticed that PGC proliferation was not closely related to the level of SCF produced (Dolci *et al.,* 1991). In fact conditioned medium from STO cells stimulated proliferation of PGCs which were cultured on NIH-3T3 cells. Since in our assays NIH-3T3 cells produced more SCF than STO cells, these data suggested that STO cells produce a soluble factor (distinct from SCF) that was a PGC mitogen (Dolci *et al.,* 1991). They also suggested that SCF acted in concert with other factors to stimulate PGC proliferation. Much of this work was carried out in our lab by Susanna Dolci, a student of Massimo de Felici and one of Anne's scientific grandchildren so to speak! Rapidly some of the PGC mitogenic factors were identified. One such factor was Leukemia Inhibitory Factor (LIF) (Matsui *et al.,* 1991) (Dolci *et al.,* 1993). In our lab, Linzhao Cheng found that LIF and a related cytokine, Oncostatin M, act directly on PGCs to promote their growth (Cheng *et al.,* 1994). LIF also seems to play some role in effecting PGC survival and together with SCF can stimulate PGC proliferation. The importance of the LIF pathway in regulating PGC survival is demonstrated by the finding that mice lacking the signaling component of the LIF receptor, gp130, are severely deficient in PGCs ((Yoshida *et al.,* 1996) and Tetsuya Taga, personal communication). That fact was also nicely demonstrated by another of Anne's one-time colleagues at the MRC MDU, Norio Nakatsuji who showed that antibodies to gp130 could block PGC survival in culture (Koshimizu *et al.,* 1996). Another PGC mitogen that was identified by Jim Resnick in our lab and by Yasuhisa Matsui in Brigid Hogan's lab was basic Fibroblast Growth Factor (bFGF) (Matsui *et al.,* 1992) (Resnick *et al.,* 1992). Together SCF, LIF and bFGF have a remarkable effect on PGC proliferation in culture. Instead of ceasing proliferation in culture, as described earlier, we found that PGCs would form large colonies of cells that could be passaged indefinitely (Matsui *et al.,* 1992) (Resnick *et al.,* 1992). These cells, which we termed embryonic germ or EG cells, continued to express markers of germ cells such as tissue non-specific alkaline phosphatase (TNAP) and the stage-specific embryonic antigen-1 (SSEA-1). Both these markers are also shared with embryonic stem (ES) cells and embryonal carcinoma (EC) cells. EC cells are the stem cells of testicular tumors and are thought to arise from PGCs that fail to undergo mitotic arrest in the embryonic gonad (Stevens 1967a). Thus, somehow we had created conditions *in vitro* that mirrored those that occur during the formation of testicular tumors (Matsui *et al.,* 1992) (Resnick *et al.,* 1992). During embryogenesis PGCs enter the genital ridge and proliferate to establish the population of cells that will form the gametes. In the normal course of events, male PGCs will enter mitotic arrest in the genital ridge. This process is disrupted somehow during the formation of testicular tumors (Stevens 1967a). Curiously, EC cells not only continue to proliferate but they also have the ability to differentiate into a wide variety of cell types including representatives of the three primary germ layers (Stevens 1967b). We now know that EG cells, like ES and EC cells, are plutipotent stem cells and can give rise to cells derived from all the three primary germ layers. EG cells also share with ES cells the ability to give rise to germline chimeras when introduced into a host blastocyst. It was not long before such pluripotent stem cell lines were derived from human PGCs, work that we carried out in collaboration with Mike Shamblott in John Gearhart 's lab at John's Hopkins Medical School (Shamblott *et al.,* 1998). The development of pluripotent human stem cells could have a major impact on the treatment of human disease and Anne has been a champion of pluripotent stem cells, both in terms of stimulating the science and in terms of explaining the science and the ethics to the lay community - including politicians!

The finding that PGCs can give rise to pluripotent stem cells was fascinating because we and many others (including Anne) had previously found that when PGCs are transferred into a host blastocyst they do not give rise to somatic or germline chimeras. Some of this work we carried out with Anne and Mia Buehr at the MRC, MDU and some in collaboration with Martin Evans and Liz Robertson at Cambridge University. The fact that PGCs never gave rise to even somatic chimeras explains why our work, like that of all the others who tried the same experiment, remains unpublished. These data suggested (to those of us that knew about them) that PGCs themselves are restricted in developmental potency, perhaps even nullipotent. Therefore, in culture, as in the development of testicular tumors, a PGC gives rise to a pluripotent stem cell. Understanding how and why this transition occurs could tell us a lot about the molecular mechanisms regulating developmental potency in mammals. More eloquently put: " *It seems that some restrictions of developmental potential must have occurred in the ancestry of the germ cells. …..it is hard to see how a truly totipotent cell line could be maintained throughout embryogenesis, except that any cell line that contributes to a germ cell population is ultimately totipotent. Changes in the pattern of transcription occur during differentiation; these may be irreversible, but subsequent differentiation may bring about a series of further changes that eventually recreate the original pattern. In the carrot, every somatic cell is capable of giving rise to an entire new carrot plant, including germ cells. We do not think that is true for a mouse, but perhaps we are merely ignorant of the right environmental cues to bring about the requisite changes*." Although we are still largely "*ignorant of the right environmental cues (to manipulate developmental potency)"*, the ability to manipulate developmental potency in PGCs with defined growth factors has provided important clues as to the "*environmental cues*" and signals involved. The development of animal cloning by nuclear transplantation and the development of pluripotent stem cells from PGCs have at least provided the experimental scalpels with which to dissect the problem.

Oocyte maturation and meiotic chromosome segregation

Germ cells have a second attribute (other than developmental totipotency) that sets them apart from the somatic cells. That is the ability to undergo the unique meiotic division process. Arguably, meiosis is one of the most crucial events in the life of a nascent individual. The first meiotic division in the oocyte is an especially important step. If that division occurs correctly, then a normal egg is produced that can be fertilized and go on to complete normal development. If that division goes awry the usual result is the production of an aneuploid embryo that is unable to complete development. Occasionally, such aneuploid embryos will complete fetal development and give rise to an individual with chromosomal alterations. Why the incidence of fetal aneuploidy rises with advancing maternal age in human populations remains a mystery and a continuing human health risk.

In female mammals, germ cells enter meiosis in the embryo and then arrest at the prophase of meiosis I (see (Clarke 1998) for review). Oocytes can remain arrested in prophase for the entire reproductive lifespan of the animal. In mice groups of prophase-arrested oocytes are periodically recruited to resume meiosis. Oocytes undergo maturation into a fully formed egg arrested at metaphase of the second meiotic division (MII) and are ovulated. Resumption of meiosis involves a dramatic process of nuclear and cytoplasmic reorganization. Classically, resumption of meiosis is detected by nuclear envelope or germinal vesicle (GV) breakdown (GVBD**)** and chromosome condensation (reviewed in (Clarke 1998)). Concomitant with these events the microtubule cytoskeleton is transformed from a long interphase network running throughout the oocyte cytoplasm into short M-phase arrays or asters nucleated from microtubule organizing centers (MTOCs) (Clarke 1998). Co-ordinated regulation of these events is crucial for establishing the first meiotic spindle and ordered chromosome segregation.

Surprisingly, very little is known about the molecular mechanisms that regulate the first meiotic division in mammals. Indeed, many other aspects of meiotic regulation in mammals remain to be fully characterized. Twenty years ago Anne McLaren posed these questions: "*how the signal for meiosis is transmitted and received; how meiotic arrest is achieved*...". Since *Germ Cells and Soma* was written, some of the genes involved in regulating meiosis in males and females have been identified. Some of these genes have been identified by cloning genetic loci associated with infertility in humans and mice. Others have been identified fortuitously through creation of knockout mice that resulted in animals that are infertile. Our own approach to identifying genes involved in regulating meiosis has been to look for homologies between meiotic regulation in lower eukaryotes such as yeast, flies and worms and that which occurs in mammals. The control of meiosis has been extensively studied in these organisms and many sterile mutants have been identified. The mechanisms that regulate resumption of meiosis have been at the center of the cell cycle field for several decades. GVBD is regulated by maturation or M-phase promoting factor or MPF, the complex of the cyclin-dependent kinase-1 (CDK1 or p34cdc2) and cyclin B that together form an active histone H1 kinase (Gautier *et al.,* 1988). In mitosis, the kinase activity of p34cdc2 is regulated by a variety of factors including: association with homologs of the yeast suc1/Cks1 proteins, association with different cyclin partners, reversible phosphorylation and by regulated destruction of its' cyclin partner. Like mitosis, meiosis is regulated in part by reversible phosphorylation. Although association of p34cdc2 with cyclin partners controls the localization of the protein, the kinase activity of p34cdc2 is also tightly regulated by phosphorylation. Phosphorylation of the p34cdc2 subunit regulates (in part) the H1 kinase activity of MPF that in turn regulates cell cycle transition from prophase to M-phase and M-phase to anaphase (Gould and Nurse 1989). The phosphorylation state of p34cdc2 is controlled by kinases (including the Wee1 and Mik1 kinases) (Gould and Nurse 1989) (Lundgren *et al.,* 1991) and the cdc25 dual-specificity phosphatase (Russell and Nurse 1986) (Millar *et al.,* 1991) (Strausfeld *et al.,* 1991) (Lee *et al.,* 1992).

Since Cdc25 proteins are mitotic inducers they represent important candidates for regulating MPF activation at GVBD in meiosis. In fact studies in *Drosophila* demonstrated a key role for one of the two fly cdc25 homologs in regulating meiosis.

Based on these observations, Dineli Wickramsinghe in our lab began to identify Cdc25 homologs in mammals and to study their function. In mammals, three cdc25 homologs, Cdc25A, Cdc25B and Cdc25C, have been identified to date and their roles are still being elucidated. Cdc25A likely regulates G1/S phase of the cell

cycle by its action on complexes of CDK4 and cyclin E (Sebastian *et al.,* 1993) (Hoffmann *et al.,* 1994). In contrast, Cdc25C is thought to regulate G2/M phase progression through its activity on complexes of p34cdc2/cyclin B (Strausfeld *et al.,* 1991) (Lee *et al.,* 1992). The role of the Cdc25B phosphatase remains unclear as it has been suggested to act both at G1/S and at G2/M (Sebastian *et al.,* 1993) (Galaktionov *et al.,* 1995a) (Galaktionov *et al.,* 1995b) (Nishijima *et al.,* 1997). The three genes are widely expressed in distinct but partially overlapping patterns during development, gametogenesis and in the adult animal. In the adult ovary Cdc25A is primarily expressed in oocytes, Cdc25C in somatic cells and Cdc25B in both cell types (Wickramasinghe *et al.,* 1995) (Wu and Wolgemuth 1995). These studies suggest that the Cdc25 genes have both overlapping and unique functions in mammals (Wickramasinghe *et al.,* 1995) (Wu and Wolgemuth 1995) (Kakizuka *et al.,* 1992). Previous studies have suggested that one physiological function of Cdc25C in vertebrates is to activate MPF at resumption of meiosis (Dunphy and Kumagai 1991) (Gautier *et al.,* 1991) (Kumagai and Dunphy 1991). To determine the role of the Cdc25 genes in mammalian gametogenesis we carried out targeted disruption of the genes in mice. Mice lacking Cdc25B are viable and males are fully fertile. However, we found that females were completely sterile even though the ovary contained normal oocytes enclosed in follicles. Ovulated oocytes from Cdc25B knockout mice remain arrested at prophase of meiosis. Isolated GV-stage oocytes from Cdc25B -/- animals never resume meiosis in culture. Our observations on mice lacking Cdc25B suggest that it is absolutely required for GVBD. Thus mice lacking Cdc25B provide a unique resource for the study of meiotic maturation in mammals.

Important clues to mechanisms for controlling the activity of p34cdc2 and Cdc25 have recently come from groundbreaking studies on the DNA damage response. When DNA damage occurs, DNA damage-response pathways are activated and transduce signals through the Ataxia Telengectasia Mutated (ATM) Kinase that result in cell cycle arrest (reviewed in Nurse, 1997; Weinert 1997). This allows for the repair of damaged DNA and, therefore, for the maintenance of genome integrity. Cell cycle arrest following DNA damage is regulated by phosphorylation of inhibitory sites on p34cdc2 (Y15) causing cell cycle arrest at the G2/M phase checkpoint (see for example, (Furnari *et al.,* 1997)). Recent data suggests that an important mechanism for inducing cell cycle arrest following DNA damage is through regulated inhibitory phosphorylation of Cdc25 by the Chk1 kinase and the related Chk2 Kinase also known as Cds1 (Furnari *et al.,* 1997) (Peng *et al.,* 1997) (Sanchez *et al.,* 1997) (Chaturvedi *et al.,* 1999).

The mechanism for inhibition of p34cdc2 activity in the DNA damage response provides an important model for how MPF activity may be regulated in mammalian meiosis.

Phosphorylation of Cdc25 leading to its' inactivation may be through regulation of its interaction with the 14-3-3 proteins, a class of ubiquitously-expressed proteins that bind to many components of signaling pathways, including phosphatases such as Cdc25 (Conklin *et al.,* 1995) (Peng *et al.,* 1997). It has been proposed that 14-3-3 protein binds specifically to and sequesters Cdc25 phosphorylated on Ser216 (mouse Cdc25B; S321) thereby preventing it activating p34cdc2 (Draetta and Eckstein 1997) (Jessus and Ozon 1995). Because the upstream regulatory elements of the14-3-3 gene contain p53-binding sites, upregulation of p53 following DNA damage has been speculated to cause transcriptional activation of the 14-3-3 gene. 14-3-3 protein would then be available to sequester Cdc25, thereby preventing Cdc25 entering the nucleus to activate p34Cdc2. These studies suggest that Cdc25 proteins are key components in the pathway that links the DNA damage-response to cell cycle arrest (see (Nurse 1997) (Weinert 1997), for reviews).

Further support for the idea that DNA damage response elements function in meiosis comes from studies in a variety of animal species. First, mice lacking the ATM kinase, a key component of the DNA damage response, have defects in meiotic chromosome synapsis (Barlow *et al.,* 1996) (Elson *et al.,* 1996) (Xu *et al.,* 1996) (Plug *et al.,* 1997) (Barlow *et al.,* 1997b) (Barlow *et al.,* 1997a). Second, the Chk1 protein is expressed on meiotic chromosomes in male mice in a manner dependent on the ATM kinase (Flaggs *et al.,* 1997). Third, disruption of a Chk1 homolog, Chk2, in worms by RNA-mediated interference demonstrates that Chk2 is essential for meiosis (Higashitani *et al.,* 2000). Fourth, in prophase-arrested Xenopus oocytes, a dominant-negative Chk1 protein facilitates GVBD induced by progesterone (Nakajo *et al.,* 1999). Taken together these data suggest that components of the DNA damage response pathway are involved in regulating meiosis in a variety of animals species. Although studies in the Xenopus oocytes point to the role of the Chk1 protein in regulating prophase arrest and GVBD in vertebrates these studies have not been extended to mammalian oocytes. Such studies could point to evolutionary conservation of mechanisms or, alternatively, to divergent mechanisms of controlling meiotic maturation. In either case, the results of such studies in mammalian oocytes will yield important new information about meiotic maturation. The hypothesis that such a pathway regulates the activity of Cdc25B at GVBD of female meiosis in mice will be tested in our future experiments**.** These studies together with those from a variety of other labs go some way to answering the question that Anne posed twenty years ago " *how (is) meiotic arrest achieved*?"

One of the critical events following GVBD is the restructuring of the microtubule cytoskeleton to form the MI spindle, which in turn has a critical role in orchestrating the events of chromosome segregation. Compelling evidence suggests that many of the events of meiosis, like mitosis, are regulated by reversible phosphorylation. Ganesan Gopalan in our lab began a screen for kinases that might regulate meiosis and identified a new family of mammalian kinases some of which are highly expressed during meiosis (Gopalan *et al.,* 1997) (Gopalan *et al.,* 1998) (Gopalan *et al.,* 1999). These kinases are related to the yeast *increase in ploidy* (*Ipl1)* and fly *aurora* (*aur*) genes that are involved in regulation of chromosome segregation. Temperature-sensitive (ts) *ipl1* mutant yeast cells missegregate chromosomes severely and die at elevated temperatures. *Ipl1* encodes a serine/threonine kinase and abolition of *Ipl1* gene function results in severe non-disjunction (Francisco *et al.,* 1994). Loss of function of the kinase encoded by the fly *aurora* gene results in failure of centrosome separation leading to the formation of monopolar spindles (Glover *et al.,* 1995). We have termed these proteins the Ipl1- and aurora-related kinases (IAKs), and they play important roles in regulating spindle architecture, chromosome segregation and cytokinesis. The first mammalian member of this family, IAK1, is a new component of the mitotic centrosomes and spindle of mammalian cells (Gopalan *et al.,* 1997) (Kimura *et al.,* 1997).

Expression of IAK1 in *ipl1* mutant, but not wildtype, yeast cells causes defects in chromosome segregation and spindle architecture suggesting that it can interfere with components of the yeast chromosome segregation pathway (Gopalan *et al.,* 1997). Consistent with these observations, overexpression of IAK1 in fibroblasts is transforming and results in multiple asters and the generation of aneuploid cells (Zhou *et al.,* 1998) (Bischoff *et al.,* 1998). These data demonstrate that IAK1 is an important regulator of centrosome function, spindle formation and chromosome segregation in mitosis. The important role that this kinase family also plays in meiosis was first described by Jill Schumacher in our lab studying IAK homologs in C. elegans (Schumacher *et al.,* 1998a) (Schumacher *et al.,* 1998b). We disrupted the function of the two C. elegans IAK1 homologs, AIR-1 and AIR-2, by RNA-mediated interference. AIR-1 is localized to mitotic centrosomes of developing C.elegans embryos similar to the localization of IAK1 in mammalian cells. Disruption of AIR-1 function in oocytes led to the development of severely aneuploid embryonic cells and embryonic lethality (Schumacher *et al.,* 1998a). These data demonstrate that disruption of AIR-1 function causes chromosome segregation defects in meiosis that lead to embryonic lethality. The second *C. elegans* IAK1 homolog, AIR-2 is localized, not to centrosomes and the mitotic spindle, but rather to meiotic chromosomes and midbody microtubules (Schumacher *et al.,* 1998b). Disruption of AIR-2 function results in defects in polar body extrusion, continued polar body replication and embryonic lethality at the one cell stage (Schumacher *et al.,* 1998b). These data suggest an important role for these kinases in meiosis. Compelling evidence from a variety of organisms supports that idea. First, temperature-sensitive (ts) *ipl1* mutants in yeast are defective in sporulation, suggesting that the Ipl1 protein is required for meiosis or spore formation in yeast (Clarence Chan, personal communication). Second, in *Xenopus*, an Ipl1 and aurora-related protein, pEg2, is present in oocytes and egg extracts (Roghi *et al.,* 1998) (Andresson and Ruderman 1998). In mitotic cells, pEg2, like its mammalian relative IAK1, is localized to the centrosomal region of the cell and the centrosome-proximal region of the mitotic spindle. Immunoelectron microscopy shows that pEg2 is localized around the pericentriolar material at prophase and on the spindle microtubules in anaphase (Giet and Prigent 1998). Injection of the wildtype kinase accelerates appearance of new MOS protein, activation of Mitogen-Activated Protein Kinase (MAPK) and meiotic maturation (Andresson and Ruderman 1998). Overexpression of pEg2 dramatically reduces the concentration of progesterone required to trigger oocyte maturation (Andresson and Ruderman 1998). These data suggest that pEg2 may function minimally in the MOS/MAPK signaling pathway required for resumption of meiosis in Xenopus (Andresson and Ruderman 1998) (Roghi *et al.,* 1998). Taken together these data suggest that the IAK kinases likely play an important role in regulating meiotic chromosome segregation. The identification of new components of the meiotic spindle such as IAK1 should allow for a better understanding of how the spindle is organized and regulated to orderly segregate chromosomes. Understanding IAK1 function in mammalian meiosis and its relationship to known meiotic regulators such as MPF and MAPK should both improve our understanding of how these kinases function to orderly segregate chromosomes and ultimately to understand how this process goes wrong.

Defective cell cycle control in the oocyte can have a drastic effect on oogenesis and ovarian physiology. The ordered segregation of chromosomes during female meiosis is critical for the development of the fetus and for the health of the offspring (Hassold *et al.,* 1996). Defects in meiotic chromosome segregation, most usually caused by defective MI spindle formation, can cause infertility, early fetal loss, birth defects and the transmission of chromosomal abnormalities conferring disease susceptibility to the next generation (Bishop *et al.,* 1996) (Hassold *et al.,* 1996). Chromosomal abnormalities are thought to be the leading cause of mental retardation (Hassold *et al.,* 1996). The incidence of aneuploidy in embryos and fetuses increases dramatically with advancing maternal age and may also be associated with environmental factors (Hassold *et al.,* 1996). In addition, defects in meiotic cell cycle control can cause parthenogenetic activation of oocytes leading to the development of ovarian teratoma (Eppig *et al.,* 1996) (Hirao and Eppig 1997). Further studies on the molecular mechanisms regulating meiosis in mammals will provide important information for the diagnosis and treatment of a variety of human conditions, including infertility, persistent fetal-loss, ovarian teratocarcinogenesis and birth defects including cancer susceptibility and mental retardation.

Conclusion

The germ cells have a special place in the life cycle of animals because they must be able to retain the ability to re-create the organism, the property known as developmental totipotency, but at the same time be able to differentiate into the highly specialized gametes. Indeed the male and female gametes in mice couldn't be much more different from one another. Historically, the analysis of germ cell development and homeostasis is one of the oldest disciplines in biology. It seems obvious why. Germ cells are set aside from the soma during development and then undergo a dramatic period of migration in order to reach the place in which they finally reside for the rest of their life. Tied to the events of somatic sexual differentiation, the differentiation of an indistinct looking primordial germ cell into a highly specialized egg or sperm is a complex biological problem. And then there is meiosis, a unique form of cell division that has served animal species so well and yet its' successful completion is so critical for the survival of the embryo to come. The germline is indeed a unique lineage with many unusual features. Not least of these is the fact that germ cells pass information from generation to generation. So too with the scientists studying them. Many of us studying these cells have been fortunate over the years to have received encouragement and help from Anne McLaren even though at times we were in essence her competitors. It is not uncommon to see her at a meeting engaged in a spirited discussion with young students and postdocs (or more senior scientists!) with a twinkle in her eye making a forceful point. But also she will be encouraging and suggesting experiments, all with an eye on publication and the extension of our understanding of these incredible cells. An insight into her philosophy of the conduct of science is seen in *Germ Cells and Soma* where, at the end of the book, she proposed three conjectures about germ cell development "*with the aim of stimulating research designed to prove me wrong*" (McLaren 1981). She hasn't often been wrong in our experience but it's not a bad philosophy to follow!

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