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INVITED RESEARCH HIGHLIGHT

Sperm Biology

Another piece of the meiosis puzzle

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Meiosis, the process by which the ovary and testis produce haploid gametes from initially diploid cells, is essential for sexual reproduction in mammals. This process, initially described in the late 19th and early 20th century using model organisms such as sea urchins, worms and fruit flies, continues to attract extensive interest from reproductive biologists due to its centrality in gametogenesis. The simple observational techniques used to initially describe meiosis have in more recent times given way to mechanistic molecular, cellular and biochemical approaches. A good example of this is the recent report by Wang *et al.*[1] which uses a new culture system to advance our understanding of mechanisms underlying retinoic acid (RA) effects on testicular meiosis and provides a novel experimental tool to facilitate future progress in this field.

It has been known for almost a century that Vitamin A is essential to support spermatogenesis,[2,3] and more recent work has suggested that RA, the active metabolite of Vitamin A, regulates many critical processes in the testis, including the onset of meiosis.[2,3] This has led to extensive work on the production and sites of action of RA in the developing testes to understand the gamut of its actions on spermatogenesis and specifically meiosis.

In vitro systems that recapitulate *in vivo* testicular processes have played important roles in the development of our understanding of testicular function. Initial *in vitro* techniques used involved organ culture of the testis or testicular fragments, as well as purification and culture of individual cell types. For example, the development of techniques for obtaining pure Sertoli cell cultures, predominately from neonatal and juvenile rodents,[4] greatly facilitated our understanding of factors regulating proliferation and function of these cells.

One obvious goal of *in vitro* systems involving the testis was the development of culture methodologies that would allow spermatogenesis or at least key facets of this process to be obtained *in vitro*. This goal proved elusive for many years because of problems inherent in developing culture systems that would recapitulate a process as complex as spermatogenesis, as well as difficulties in the identification, isolation, and propagation of germ cells such as the spermatogonial stem cells (SSCs) that were essential for these types of studies. However, by the early 21st century, reliable techniques for the manipulation of these SSCs had been developed.[5,6] This occurred first in rodents[5,6] but was soon followed by techniques for the identification and long-term culture of human SSCs.[7]

A number of culture systems have been described in recent years that have used testicular explants, Sertoli cell/germ cell co-cultures, primordial germ cells, embryonic stem cells, or induced pluripotent stem cells as the starting point to obtain differentiation of germ cells into the spermatogenic lineage to study the gamete development and meiosis.[8–11] However, culture systems involving explants or Sertoli cell/germ cell co-cultures by their nature involve multiple cell types and limit the ability to follow and analyze meiosis in the germ cells in these cultures. These culture systems frequently had a low efficiency of germ cell differentiation, and in some cases, haploid germ cells were not obtained. Importantly, there were no culture systems described so far in which SSCs alone could be used as the starting point for meiosis studies, which is important because these cells can be grown in large quantities and are also amenable to various types of genetic modification.

A recent report by Wang *et al.*[1] demonstrated that RA alone could induce meiosis and result in the production of leptotene/zygotene spermatocytes from cultured mouse SSCs. Notably, initial experiments involved growing SSCs on a feeder layer of cells, but later results showed that the SSCs grown in the absence of other cells and in serum-free conditions were capable of undergoing meiosis. This finding suggested the somewhat surprising conclusion that RA signaling alone was sufficient to induce meiosis. Co-culture with Sertoli cells increased the efficiency of meiotic induction in the mouse spermatogonia, possibly due to Sertoli cell production of RA, which has previously been shown to be involved in initiation of meiosis.[3]

The use of a monoculture of SSCs in the work of Wang *et al.*[1] allowed specific gene expression in the SSCs during meiosis to be evaluated, something that was difficult in previous work where more than one cell type was used in the culture. The authors used RNAseq to identify several thousand genes that were either up- or down-regulated by RA, and RA effects on the majority of these seemed to be direct. This group not only confirmed the role of some genes known to be involved in meiosis, such as the RA target *Stra8*, but also identified a number of other genes involved in various facets of meiosis that had not previously been shown to be involved in this process. The genes revealed to be direct or indirect targets of RA included genes involved in spermatogonial differentiation and meiosis initiation (e.g., *Stra8, c-Kit*), but also genes involved in other processes such as self-renewal of SSCs, showing that those gene networks are also part of the complex signaling events involved in RA induction of meiosis.

In summary, the development of a new serum-free culture system by Wang *et al.*[1] in which RA alone is able to drive meiosis in pure cultures of mouse SSCs provides a new and

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powerful tool to explore the signaling events involved in the initiation and progression of meiosis. The initial experiments with this system have already provided important insights into this process, including the finding that RA alone can drive this process. This work has also revealed the extensive nature of the changes in gene expression that accompany RA administration and has advanced our knowledge of the diverse group of gene networks whose expression is altered by this treatment. Despite the importance of meiosis in spermatogenesis, our mechanistic knowledge of how this process worked has been relatively limited. The work of Wang *et al.*[1] provides an important step forward in our efforts to understand exactly how meiosis is regulated, and the powerful new culture system for examining RA effects on meiosis will be an important tool for this group and others in future work to more clearly understand the mysteries of meiosis.

COMPETING INTERESTS

The authors declared no competing interests.

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