

reductions by PP2 and U7 may have been generated via different mechanisms, given that the two drugs had varying effects on sperm incorporations and pronuclear differentiations. Moreover, confocal imaging revealed Ca²⁺ oscillations were blocked by U7 but not by PP2. Collectively, such data fail to support the view that SFK signaling is required for either GVBD or for initiating fertilization-induced Ca²⁺ oscillations in *Cerebratulus* and instead suggest that PP2-mediated inhibitions of polar body formation and cleavage involve undetermined drug effects on processes other than oscillation generation.

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Program/Abstract # 342

The mammalian Doublesex homolog DMRT1 controls the mitosis versus meiosis decision in males

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Germ cells are uniquely capable of undergoing either mitotic divisions, like other cells, or meiotic divisions that permit gametogenesis. In mammals meiosis is triggered by retinoic acid (RA), which activates genes including the meiotic inducer *Stra8*. Fetal males avoid meiosis by degrading RA in the fetal testis. When meiosis begins in males at puberty it requires RA and *Stra8*, but how these are controlled in spermatogonia has been unknown. We have found that the Doublesex-related transcription factor DMRT1 determines whether spermatogonia undergo mitosis or initiate meiosis. Spermatogonia lacking DMRT1 have abnormally active RA signaling and prematurely enter meiosis, independent of the normal spermatogenic cycle. Chromatin immunoprecipitation and other approaches show that control of meiotic initiation by DMRT1 involves direct transcriptional regulation of key RA metabolic enzymes and *Stra8*. Analysis of vitamin A depleted animals that lack RA reveals that DMRT1 also controls at least one retinoid-independent meiotic inducer. These results establish DMRT1 as an essential and direct regulator of the mitosis versus meiosis switch. The DM domain gene family to which DMRT1 belongs is deeply conserved in metazoan sexual regulation, and thus our findings also may have implications for meiotic control outside of mammals.

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Program/Abstract # 343

The RNA-binding protein Nanos2 is required to maintain spermatogonial stem cells

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In mice, spermatogenesis is initiated from a small number of stem cells belonging to undifferentiated spermatogonia. However, it remains unclear 1) which types of spermatogonia actually act as the stem cells and 2) how is the stem cell function regulated. Nanos, a zinc-finger RNA-binding protein, has been proposed as a conserved factor for germline stem cell function. In adult testes, Nanos2 is predominantly expressed in a subset of undifferentiated spermatogonia. However, the majority of *Nanos2*-null germ cells die by apoptosis before birth, hindering functional studies of Nanos2 during sperma-

togenesis. With the use of transgenic mouse strategies, I found that the RNA-binding protein Nanos2 is a key regulator for the maintenance of spermatogonial stem cells. Lineage-tracing analyses revealed that *Nanos2*-expressing spermatogonia self-renew and generate the entire spermatogenic cell lineage. Conditional disruption of postnatal *Nanos2* depleted spermatogonial stem cell reserves, whereas mouse testes in which *Nanos2* had been overexpressed accumulated spermatogonia with undifferentiated, stem cell-like properties. Thus, Nanos2 is expressed in self-renewing spermatogonial stem cells and maintains the stem cell state during murine spermatogenesis.

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Program/Abstract # 344

Stage-specific expression of the homeodomain protein Cux1 in Sertoli cells and spermatids during spermatogenesis

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The homeodomain protein Cux1 exists as multiple isoforms. The 200 kDa Cux1 protein is highly expressed in the developing kidney, where it functions to regulate cell proliferation. A 55 kDa Cux1 isoform is expressed exclusively in the testes. Transgenic mice ectopically expressing the 200 kDa Cux1 protein develop transient multiorgan hyperplasia, including the testes. We determined the pattern and timing of Cux1 protein expression in the developing testes. Cux1 expression was continuous in Sertoli cells of prepubertal testes, but became cyclic when spermatids appeared. In mature mice, Cux1 was highly expressed only in round spermatids at stages IV–V of spermatogenesis, in both spermatids and Sertoli cells at stages VI–X, and only in Sertoli cells at Stage XI. In Cux1 transgenic mice there were significantly fewer tubules expressing Cux1 in both Sertoli cells and spermatids and significantly more tubules expressing Cux1 in either spermatids or Sertoli cells. Moreover, Cux1 was not expressed in proliferating cells in testes from either wild type or transgenic mice. Thus, unlike the role of the somatic form of Cux1 in cell proliferation, the testis-specific form of Cux1 is not involved in cell division and appears to play a role in signaling between spermatids and Sertoli cells.

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Program/Abstract # 345

Inhibitory action of *Xenopus* dicalcin on sperm-egg interaction during fertilization

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To contribute to the study of sperm-egg interaction in the course of fertilization, we have isolated and characterized *Xenopus* dicalcin in *Xenopus* eggs. *Xenopus* dicalcin is localized markedly in the egg-coating envelope (called vitelline envelope; VE), and exhibits a Ca²⁺-dependent binding to two glycoproteins that constitute polymeric filaments of VE. Since these VE glycoproteins are considered to function as sperm-receptors, we examined the effect of dicalcin on sperm-VE binding, sperm-VE penetration, and fertilization *in vitro*. Preincubation of *Xenopus*