

Foreword

The germ cell genome threads continuously from generation to generation via the sperm and egg. These gametes combine at fertilization to produce the totipotent zygote (one-cell embryo) that gives rise to each generation. Thus, the germ cell lineage is immortal and has extraordinary developmental and, perhaps, therapeutic potential. The germ cell field has generated several exciting discoveries and innovations during the past 15 years, which provide valuable insights into the mechanisms that regulate developmental potency and stem cell function. This monograph discusses the implications of these discoveries for understanding the fundamental biology of germline stem cells as well as their potential for human stem cell-based therapies.

The germ lineage exhibits an extraordinary breadth of developmental potentials during fetal, perinatal, and adult stages of life. Primordial germ cells (PGCs) first arise in the epiblast stage embryo and eventually migrate via the hindgut to colonize the gonads. Under normal, *in vivo* circumstances, these PGCs will give rise to oogenic or spermatogenic lineages. However, in some *in vivo* and *in vitro* situations, PGCs can adopt a pluripotent phenotype. Peter Donovan (Chap. 1) discusses the acquisition of pluripotency by PGCs and the implications for understanding the regulation of developmental potency, germ cell development, and testicular cancer. In males, PGCs that colonize the gonad become enclosed within pre-Sertoli cells of the seminiferous cords and give rise to pro-spermatogonia (aka: gonocytes). Pro-spermatogonia migrate to the seminiferous tubule basement membrane and establish the pool of spermatogonial stem cells (SSCs). SSCs are restricted to spermatogenic lineage development and balance self-renewing and differentiating divisions to maintain spermatogenesis. However, these adult tissue stem cells retain some developmental plasticity. Marco Seandel, Ilaria Falcatori, and Shahin Rafii (Chap. 2) describe conditions for converting spermatogonia from postnatal testes to pluripotent stem cells. They also discuss the potential for therapeutic application of testis-derived pluripotent stem cells compared to other pluripotent cell sources (e.g., embryonic stem cells and induced pluripotent stem cells). In converse, Sonya Schuh-Huerta and Renee Reijo Pera (Chap. 3) describe the conversion of pluripotent stem cells into the germ lineage. *In vivo* investigations of human germ cell development are inherently problematic. Therefore, pluripotent to germ cell conversion,

in vitro may provide a unique window for understanding the mechanisms of germ lineage commitment and development. In addition, pluripotent cells may one day be a source of *in vitro*-derived gametes (eggs or sperm) with potential application in the human fertility clinic.

The pool of spermatogonial stem cells (SSCs) in the postnatal testis gives rise to undifferentiated transit-amplifying progenitor spermatogonia followed by differentiating spermatogonia. Collectively these spermatogonial populations comprise the foundation of the spermatogenic lineage. Dirk de Rooij (Chap. 4) describes spermatogonial morphology and the kinetics of renewal and differentiation that maintain spermatogenesis throughout postpubertal life. Investigations of SSCs are complicated because there are no morphological and biochemical features that can distinguish these stem cells from other undifferentiated spermatogonia (except possibly whole mount analysis of clone size, described in Chap. 4). Jonathan Schmidt and Ralph Brinster (Chap. 5) describe SSC transplantation as a definitive bioassay for spermatogonial stem cells and SSC culture as a valuable *in vitro* tool for SSC expansion and mechanistic investigations. While transplantation is a valuable bioassay that has accelerated the pace of SSC research in the past two decades, it is technically challenging and has an inherent 2–3 month delay to analysis. Makoto Nagano and Jonathan Yeh (Chap. 6) have exploited the SSC culture system to establish a simpler and faster *in vitro* “cluster-forming activity (CFA) assay” to quantify SSC activity. While the CFA assay does not replace transplantation as a bioassay and does not recapitulate complete spermatogenesis, it can accelerate SSC research by providing a rapid and reliable readout during early experimental development. These experimental tools have enabled investigators to begin dissecting the mechanisms that regulate SSC renewal and differentiation. Olga Ocón-Grove and Jon Oatley (Chap. 7) explore the roles of extrinsic factors emanating from the local testicular microenvironment (niche) and the interplay between extrinsic and internal factors in the regulation of SSC fate decisions. Shosei Yoshida (Chap. 8) discusses the functional and anatomical features of the SSC niche and provides additional insights about molecular mechanisms regulating SSC behavior.

Treatments for some human diseases (e.g., cancer) can damage the spermatogonial stem cell pool and cause infertility. Gunapala Shetty, Gensheng Wang, and Marvin Meistrich (Chap. 9) describe the effects of gonadotoxic therapies in the testis, including the kinetics of germ cell demise and the potential for spermatogenic recovery. They also examine the effects of endocrine modulators on spermatogenic recovery and discuss potential clinical implications. Stefan Schlatt, Jose Rodriguez-Sosa and Ina Dobrinski (Chap. 10) describe ectopic xenografting of testis tissues from immature animals into immune-deficient mice as a means to recapitulate spermatogenesis and recover fertilization-competent sperm. This is an experimentally tractable approach to study spermatogenic lineage development in species that are less amenable to experimental manipulation, including humans. Grafting techniques may have application for preserving the fertility of prepubertal boys whose future fertility is at risk due to gonadotoxic treatments. In the final chapter, the editors of this volume, Brian Hermann and Kyle Orwig (Chap. 11) summarize progress applying SSC transplantation in a nonhuman primate model of male infertility and

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considerations for translation to the human fertility clinic. Methods for isolating, preserving, and transplanting SSC in nonhuman primates and humans are described and may provide important preclinical insights.

The editors are grateful to the prominent researchers who have made important contributions to the germ cell field and provided chapters for this effort. We also thank the experts who reviewed chapters prior to publication.