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Review

Molecular events and signalling pathways of male germ cell differentiation in mouse

Moïra Rossitto, Pascal Philibert, Francis Poulat, Brigitte Boizet-Bonhoure*

Genetic and Development Department, Institute of Human Genetics, CNRS UPR1142, Montpellier, France

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ABSTRACT

Germ cells, the precursors of gametes, represent a unique cell lineage that is able to differentiate into spermatozoa or oocytes depending on the chromosomal sex of the organism. In the mammalian embryonic gonad, commitment to oogenesis involves pre-meiotic DNA replication and entry into the first meiotic division; whereas, commitment to spermatogenesis involves inhibition of meiotic initiation, suppression of pluripotency, mitotic arrest and expression of specific markers that will control the development of the male germ cells. The crucial decision made by the germ line to commit to either a male or a female fate has been partially explained by genetic and *ex vivo* studies in mice which have implicated a complex network of regulatory genes, numerous factors and pathways. Besides the reproductive failure that may follow a deregulation of this complex network, the germ cells may, in view of their proliferative and pluripotent nature, act as precursors of potential malignant transformation and as putative targets for exogenous environmental compounds. Our review summarizes and discusses recent developments that have improved our understanding on how germ cell precursors are committed to a male or a female cell fate in the mouse gonad.

Contents

* Corresponding author at: Institute of Human Genetics, Genetic and Development Department, CNRS UPR1142, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France.
E-mail addresses: Moira.Rossitto@igh.cnrs.fr (M. Rossitto), Pascal.Philibert@igh.cnrs.fr (P. Philibert), Francis.Poulat@igh.cnrs.fr (F. Poulat), Brigitte.Boizet@igh.cnrs.fr (B. Boizet-Bonhoure).

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1. Introduction

The sexual development of mammalian germ cells leads to the generation of future gametes, either oocytes in the female or spermatozoa in the male. This haploid cell-producing biological process is critical for reproductive function and consequently for the transfer of genetic information from generation to generation.

Germ cell precursors arise from the proximal epiblast at 6.25 days *post coitum* (dpc) and acquire the competence to become primordial germ cells (PGCs) while the somatic program of development is actively repressed [1]. Paracrine signalling molecules, such as bone morphogenetic proteins (Bmps) -2, -4, -8 that are members of the transforming growth factor β (Tgfβ) family, are required for PGC specification, localization and survival [2]. The specified PGCs then express PGC-specific markers such as alkaline phosphatase, stage-specific embryonic antigen (Ssea1), developmental pluripotency associated 3 (Dppa3 or Stella) [3] and RNA binding proteins Nanos3 [4] and Dnd1 [5], that are involved in germ cell survival. *Oct4* expression is also necessary for PGC specification and, together with Sox2 and Nanog, is associated with pluripotency that is required for PGCs to generate pluripotent progeny [6]. In addition, Prdm1 (also Blimp-1) and Prdm14 have been shown to be involved in the acquisition and the maintenance of pluripotency in PGCs at these early stages [6,7].

Once specified, PGCs start moving into the embryonic endoderm through the hindgut towards the future genital ridges, from 8.0 to 10.5 dpc. This active movement is directed by somatic cell-secreted chemoattractants, such as the growth factor Kit ligand [8] or the chemokine Cxcl12 [9] that bind to their respective receptor expressed by the PGCs, the *cKit* encoded tyrosine kinase receptor and Cxcl4. These factors are believed to act together with fibroblast growth factor (Fgf) and Bmp molecules to promote survival during migration and also be involved in stimulating PGC proliferation [10,11]. In both sexes, PGCs colonize the genital ridges at around 10.5 dpc and continue proliferating until 13.5 dpc. During these stages, DNA methylation of the germ cell genome is erased until, once in the gonad, the PGCs undergo a complete demethylation at imprinted and non-imprinted genes leading to the pluripotency of many developmentally regulated genes (for review [12]) (Fig. 1).

2. Preparing germ cells for differentiation

2.1. Germ cell differentiation is driven by the somatic environment

After their arrival into the undifferentiated bipotential gonads, PGCs are exposed to somatic signals that will promote either a male or a female development. In the undifferentiated gonad of an XY mouse, somatic sex determination is induced at 10.5 dpc by the expression of the Y chromosome-linked *Sry* gene. At its 11.5 dpc expression peak, Sry leads to the upregulation of *Sox9* expression and the subsequent differentiation of the somatic cell precursors into Sertoli cells (for review [13]) (Fig. 1). Otherwise, in the absence of Sry, expression of *R-Spondin1* (*Rspo1*)/*Wnt4* through β-catenin signalling and *FoxL2* in the XX somatic precursors, drive the gonads towards a female phenotype. The commitment to the male pathway involves the down-regulation of genes associated to female development, and vice versa [14–16]. These differentiating cells then contribute to the differentiation of the other somatic cell lineages before influencing the germ cell lineage by producing signals that promote male or female commitment thus leading to spermatogenesis or oogenesis, respectively [17]. Both XY and XX germ cells are able to respond to either male or female gonadal events up until 12.5 dpc after which their fate is fixed [17]; some studies have shown that isolated XY germ cells remain competent to enter into meiosis until 14.5 dpc [18] and even 15.5 dpc [19]. Fate determination in the germ cell lineage becomes apparent between 12.5 and 15.5 dpc and involves a repression of pluripotency and a commitment to differentiate as one of two specialized gamete precursors: spermatogonia or oogonia [20,21].

2.2. Dazl: a competence factor that prepares germ cells for differentiation

After specification and migration, the transition from PGCs to "gametogenesis-competent cells" referred to as the licensing for gametogenesis, has been shown to require the RNA binding protein Dazl, a competence factor that contributes towards the initiation of sex-specific differentiation of the male and female germ cells [22]. *Dazl* expression is initiated during PGC migration and continues in developing germ cells. In the absence of Dazl in the C57BL/6 genetic background, XY and XX germ cells fail to activate genes required for male-specific and female-specific differentiation, respectively [23]. Germ cells in foetal $Dazl^{-/-}$ testes do not engage in spermatogenesis, and fail to express *Nanos2*, to arrest in G_0/G_1 and to remethylate their genomes. This process was found to be not cell-autonomous but rather induced by the somatic genital ridge; indeed, Gata4-induced genital ridge formation was necessary and sufficient to induce expression of *Dazl* and *Mvh* (*Vasa*) and consequently, licensing of germ cells [24]. Dazl was shown to act as a translational repressor, suppressing pluripotency, somatic differentiation and apoptosis in proliferating PGCs [25]. Amongst its targets, are genes involved in pluripotency in PGCs, such as *Sox2*, *Sall4*, and *Suz12* which suppresses somatic differentiation in the germ cells; indeed, 16.5 dpc $Dazl^{-/-}$ gonads were shown to aberrantly express these proteins. Furthermore, Dazl was shown to suppress translation of *Caspase 2, 7* and *9* mRNAs, suggesting a direct regulation of apoptosis in PGCs [25]. Other potential mRNA targets for Dazl include *Mvh/Vasa* [26].

Altogether, the crucial roles of Dazl in controlling translation of several key mRNAs, suggest its involvement in the downregulation of pluripotent genes at the time of germ cell differentiation. As Dazl was shown to control the translation of critical components of spindle function in mouse oocyte meiosis MI and MII [27], Dazl might also be involved in cell cycle regulation during PGC proliferation and early gonadogenesis.

2.3. Coordinated timing of germ cell differentiation by Prc1

In the absence of *Rnf2*, a component with *Ring1* of the Polycomb repressive complex 1 (Prc1), expression of *Stra8*, *Rec8*, *Scp3*, *Hormad2* and genes involved in retinoic acid (RA) metabolism, are upregulated in female 11.5 dpc PGCs, inducing prematurely arrested proliferation and entry into meiotic prophase. In cultured 11.5 dpc genital ridges, Prc1 suppressed retinoic acid-induced *Stra8* via the direct binding of its component Rnf2 onto the *Stra8* promoter at 11.5 and 12.5 dpc [28]. Furthermore, Ring1 and Rnf2 increased Oct4-positive PGC number in 11.5 dpc gonads of both

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