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Characteristic Features of Male Germline Development in Primates

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Abstract

The germline is constituted by all cells that have the potential to transmit their genetic information to the next generation. The germline can be considered as a defined sequence of genetic, cellular, and developmental processes recurring in each generation in order to ensure the continuity of a species-specific reproduction program. Although basic mechanisms of germline development in mammals are highly conserved, relatively slight yet relevant modifications of germline development evolved in different groups of mammals to adapt the entire process to the specific requirements of and conditions in each species. This review highlights selected aspects that illustrate germline adaptations and characteristics in primates mainly in comparison to the mouse, which is the best investigated mammalian model organism in reproductive biology.

The germline is constituted by all cells that have the potential to submit their genetic information to the next generation. The cycle of the germline in mammals is characterized by the recurring sequence of (1) fertilization, i.e., fusion of 2 haploid gametes, resulting in (2) a diploid phase of early embryonic development, which leads to (3) the specification and separation of germ cells (primordial germ cells; PGCs) from somatic cells, followed by (4) several rounds of mitotic divisions of the germ cells. Then (5) the germ cells enter meiosis, i.e., the genetic reduction division, resulting in (6) haploid cells forming gametes again. Fusion of the haploid male and the female gametes completes the cycle of the germline and begins the next generation (Fig. 1). This sequence of events occurs in all mammals. However, different species exhibit specific adaptations and characteristics of 1 or more phases of the cycle of the germline. Remarkably, the cycle of the germline is an extremely robust process that works "endlessly". On the other hand, certain dynamics of the germline is essential for evolution since only modifications of the germline cells can lead to (genetically or epigenetically) inherited traits possibly resulting in better-adapted offspring and, hence, supporting species diversion. In light of these conflicting goals, namely germline stability and genome dynamics, some selected characteristic features of male germ cell development in pri-

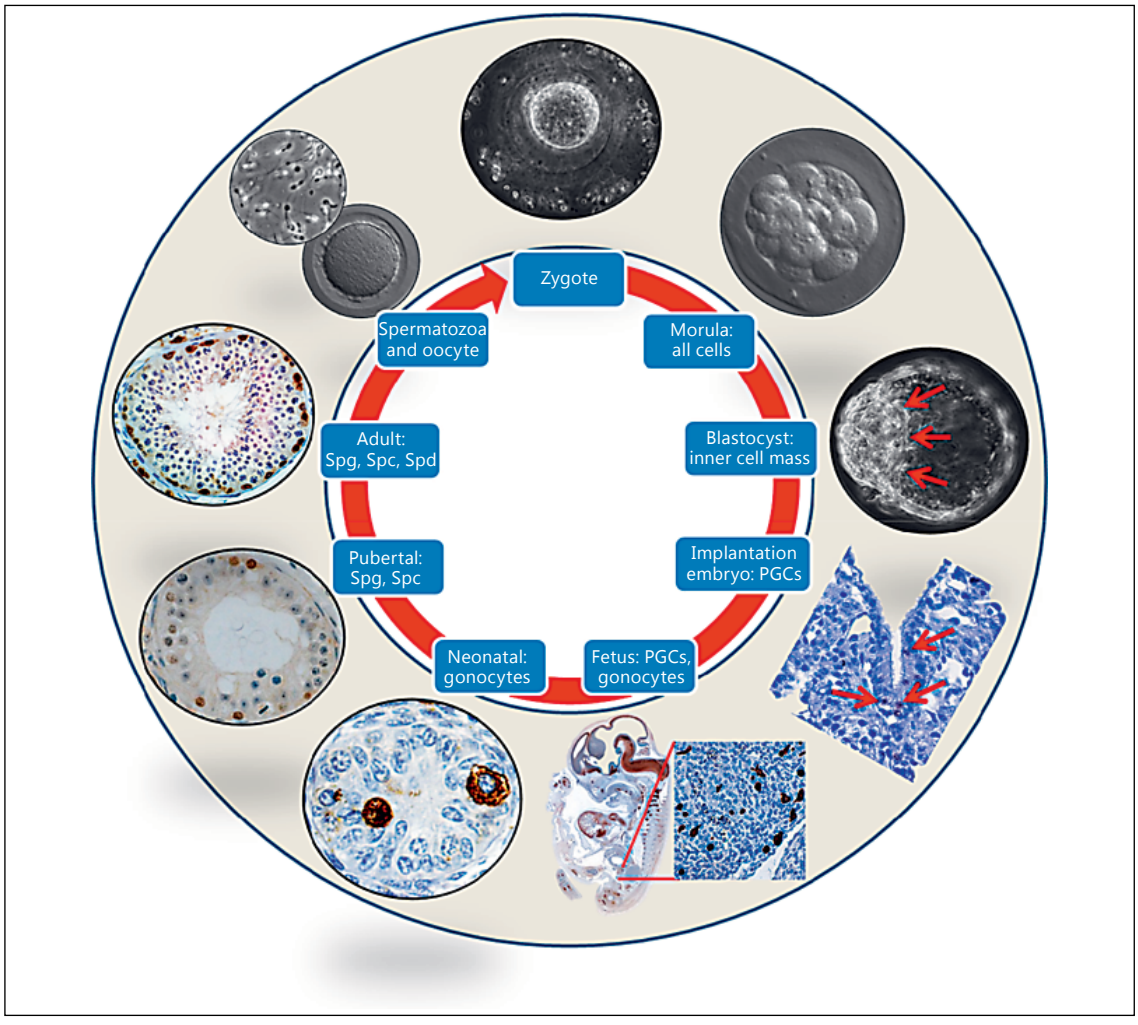

Fig. 1. Illustration of the cycle of the germline. All cells that have the potential to transmit their genetic information to the next generation belong to the germline. Germline cells are mentioned in the blue boxes. A new generation is initiated by the formation of a zygote, i.e., the fertilized oocyte. In the morula-stage embryo, the individual cells (blastomeres) are not yet specified. They are all potential progenitor cells of the prospective germ cells. The blastocyst consists of 2 clearly distinguishable cell populations: the inner cell mass cells (highlighted by red arrows) and the outer cell layer, i.e., the trophoblast. The germ cells develop from the inner cell mass cells. In the implantation embryo the first specified germ cells, called PGCs, occur. In the somite-stage embryos they are frequently located in the epithelium of the developing hind gut (red arrows). In the fetus, most PGCs have entered the forming gonad. The magnified area shows the distribution of germ cells in the gonadal primordium. In the neonatal testis the germ cells are mostly gonocytes, which are premeiotic germ cells located mostly in the center of the developing seminiferous tubule. At that stage, the tubule has no lumen. The gonocytes were stained using immunohistochemistry. In the pubertal testis, the seminiferous tubules form a lumen, and spermatogonia (Spg; stained brown) start to proliferate, giving rise to meiotic spermatocytes (Spc). In the adult testis, there are premeiotic Spg, meiotic Spc, and postmeiotic spermatids (Spd). Undifferentiated spermatogonia were stained by immunohistochemistry. Elongated spermatids are positioned on the apical (adluminal) surface of the germinal epithelium. Spermatozoa and oocytes are the gametes released from sexually mature male and female gonads, respectively. Fusion of the gametes initiates the next cycle of the germline. The different stages are illustrated using micro images (which are not shown to scale) of the Platform Degenerative Diseases of the DPZ.

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mates will be discussed in this chapter in comparison with the best-characterized mammalian species, the mouse (*Mus musculus*). The major focus will be on genetic, cellular, and developmental aspects. The term primate includes non-human primates (NHP) and humans, since both belong to the taxonomical (biological) order of primates.

The Totipotent and Pluripotent Stages of the Germline

After fertilization in mammals, the cells of the embryo are first totipotent and after only very few cell divisions their differentiation potential is restricted to pluripotency. A cell is totipotent if it can form a whole organism. In contrast, pluripotency is the ability of a cell to differentiate into all cell types of the body, but pluripotent cells lack the potential to form a whole organism. Both totipotent as well as pluripotent cells belong to the germline, and both states require a finely tuned temporal and spatial control [1, 2]. Interestingly, the molecular regulation of pluripotency and the signal transduction pathways associated with this developmental state are not fully conserved in different mammalian species as has been shown for pluripotent cells of human and mouse origin [3, 4]. Recently, marmoset monkey and mouse pluripotent cells directly isolated from embryos were also compared [5]. Some factors that have important roles in the mouse system [6, 7], like *KLF2* and *NR0B1* (also known as *DAX-1*), were also highly expressed in the mouse preimplantation embryo [5] but were basically undetectable in the marmoset monkey (*Callithrix jacchus*) preimplantation embryo [5]. Also, the pluripotency-associated gene *FBX015*, although not essential for mouse development and fertility [8], shows dramatic differences in the expression levels in mouse and monkey preimplantation embryos. These selected examples highlight molecular differences in the regulation of pluripotency and, thereby, also differences in the maintenance of the germline at the mouse and primate preimplantation embryo stage. When the expression of components of specific signal transduction pathways was analyzed, many of them were found to be present in both species. However, transcripts encoding components of the TGF-β/NODAL, FGF, and WNT signaling pathways, all important for different early developmental processes in mammals [9–11], were differentially expressed [5], suggesting that the utilization of specific signal transduction pathways is different between mouse and primate preimplantation embryos. Recent groundbreaking studies on the cynomolgus monkey (*Macaca fascicularis*) postimplantation embryo also revealed clear differences between primate and mouse epiblast [12], which is the embryonic precursor tissue of the PGCs, and PGC specification between the cynomolgus monkey and the mouse [13]. These comparative approaches employing mouse and primate allowed differentiation between basic pathways involved in the regulation of pluripotency in mammals and those mechanisms that apparently exhibit evolutionary plasticity [14]. In summary, general features of the early phase of embryonic development are conserved. However, there are also clear differences between the mouse and the primate regarding the regulation of pluripotency in preimplantation and implantation embryos. At present, the phenotypic and particularly the functional consequences of these molecular findings can only be speculated on, since functional and gene deletion studies are very demanding and challenging in NHP.

Embryonic Specification of Primordial Germ Cells in Primates and the Mouse

In the mouse, about 45 PGCs can be detected around embryonic day (E) 7–7.25 by alkaline phosphatase staining [15, 16]. Lawson and Hage

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[15] also showed through lineage tracing experiments that PGCs are not finally specified in the mouse before E6.5. However, around E6.25–6.5, marked expression of *fragilis*, a gene important for germ cell specification, was found in nascent PGCs, followed by expression of *stella* around E7 [17]; *stella* is germ cell specific. However, recent data may suggest that the activation of a germ cell-characteristic gene expression program may start before E7, possibly already around E3.5–4 [18]. Hence, in the mouse there is a period of currently unknown length during which cells of the implanted embryo have the competence to differentiate into germ cells. The final specification, however, apparently occurs only around or even shortly after E7. These early implantation stages are difficult to obtain from humans (for ethical and practical reasons) as well as from NHP. Nevertheless, very recent in vitro as well as in situ studies delineated this phase of primate germ cell specification. Instructive in vitro systems employing human pluripotent stem cells suggested significant differences between mouse and human germ cell specification [19–22]. In the mouse, it has been shown that the interplay of BLIMP1 (also known as PRDM1), PRDM14, and AP2γ (TFAP2C) initiates a unique cellular program resulting in the specification of PGCs [23]. These factors synergistically suppress the ongoing somatic differentiation and activate the reexpression of pluripotency- and germ cell-specific genes [23]. *PRDM14* is essential for PGC specification in mice [24]. Contrastingly, the role of *PRDM14* in human germ cell specification seems to be only minor since this gene was only minimally expressed in in vitro assays of germ cell development compared to the mouse [21]. Remarkably, Sasaki et al. [13] showed that cynomolgus monkey PCGs did express *PRDM14* in the context of natural embryos. This discrepancy between monkey and human *PRDM14* expression in PGCs may either represent a species-specific difference (human vs. cynomolgus monkey) or may reflect the different approaches of PGC investigation, i.e., pluripotent stem cell based for human PGC development and natural embryo based for monkey PGC investigation. A clear difference between primate and mouse PGC specification is the role of *SOX17*. This transcription factor is a key regulator of human PGC specification [19] and acts upstream of *BLIMP1*. In the absence of SOX17, no germ cell-like cells were obtained from pluripotent stem cells, highlighting the role of SOX17 in human germ line specification [19]. Importantly, *SOX17* has also been shown to be highly expressed, together with *TFAP2C*, in cynomolgus monkey PGCs [13]. In striking contrast, *SOX17* has no apparent role during germ cell specification in mice [19]. In general, the pluripotent stem cell-based system for the (functional) investigation of primate PGC development appears to be a very useful alternative for potential in vivo studies in NHP for the following reasons. First, Sasaki et al. [22] compared the gene expression signatures of their in vitro-derived PGC-like cells with those of PGCs isolated from monkey embryos and found significant matching of both gene signatures, nicely validating the in vitro data. Second, SOX2 is a core pluripotency transcription factor expressed by mouse PGCs, and it is essential for mouse PGC development in vivo [25]. In striking contrast to the mouse, human PGCs neither express SOX2 during in vivo development, nor upon in vitro culture [26], while OCT4 and Nanog as additional core pluripotency factors [27] are expressed [28]. This pattern was recently confirmed by Sasaki et al. [13] for cynomolgus monkey PGCs and in our lab for marmoset monkey PGCs [29; unpubl. data], suggesting that a lack of SOX2 expression is a common characteristic of primate PGCs. Importantly, *SOX2* was expressed in human pluripotent stem cells, but was undetectable or strongly repressed in human PGC-like cells derived from pluripotent stem cells [19, 30], which nicely recapitulates the dynamics of SOX2 expression during human and cynomolgus monkey PGC

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development in vivo. In summary, molecular players essential for mouse PGC development like SOX2 appear to have only minor or even no role in primate PGC formation. In contrast, SOX17 is essential for human PGC formation, but not in the mouse. These examples highlight clear differences in the molecular regulation of PGC specification and development between primates and the mouse.

The Role of the Amnion and the Extraembryonic Ectoderm

The extraembryonic ectoderm (ExE) plays an important role during mouse PGC specification [23]. PGCs respond to BMP4, which is secreted from the ExE during embryonic days E6.5–7.5 [31]. Mouse embryos lacking BMP4 fail to develop PGCs; they do not contain any germ cells [32]. These data suggest that the ExE is essential for proper PGC specification and development in the mouse. However, since the anatomy and morphology of the mouse and the primate embryo are fundamentally different, with a flat germ disc in primates and an egg cylinder in the mouse [4, 33], there is no direct structural counterpart of the mouse ExE in the primate embryo [34]. Recently, it was shown that the nascent (pregastrulation) amnion is the origin of PGCs in the cynomolgus monkey [13]. Giving rise to PGCs appears to be a unique function of the amnion in primates [13]. Furthermore, this study also provided insights into (potential) inductive processes regulating PGC specification and determination in primates. The amnion itself as the PGC-generating tissue produces BMP4 and WNT3A, to which the PGCs may respond [13]. In summary, the significant anatomical differences between mouse and primate embryos at the time of PGC specification [4] and the amnion as the origin of primate PGCs [13] demonstrate significant species-specific adaptations in primates compared to the mouse.

Gonocytes and Spermatogonial Stem Cells

PGC specification and their initial development occur outside the gonads and even outside the embryo proper. In order to constitute a functional gonad, the PGCs have to be translocated from their extraembryonic position into the developing gonad, which is free of germ cells during the first steps of its differentiation. Only little is known about the primate (-specific aspects of) PGC migration and translocation [26, 29, 35–38]. Upon arrival in the male gonad, postmigratory PGCs are surrounded by cells forming the somatic parts of the developing testicular cords, which eventually become the seminiferous tubules in the adult testis. Upon inclusion in the testicular cords, the PGCs are called gonocytes. The gonocyte stage developmentally links the PGC stage with the spermatogonial stage and involves phases of relative quiescence, proliferation, migration from a central position within the testicular cord to the basement membrane of the developing germinal epithelium, and finally differentiation to a spermatogonial stem cell [39, 40]. However, in mice, a subpopulation of gonocytes directly initiates spermatogenesis in order to fuel the first wave of sperm production without passing through an undifferentiated spermatogonial stage [41]. Again, only little is known about the molecular regulation of the development of gonocytes in primates [40, 42–45]. However, one aspect is evident: in primates there is an extended phase of relative quiescence of the gonocytes between the beginning of the gonocyte stage and the initiation of puberty, i.e., spermatogonia start to proliferate in order to initiate spermatogenesis. Although it has been shown that there is some germ cell proliferation and differentiation during the prepubertal period in the monkey [46] and in the human [47, 48] testis, this prepubertal period of relative testicular quiescence has no direct counterpart in rodents. In fact, a comparative analysis of the germ cell population in the neo- and postnatal human, marmoset, and rat testis

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showed that GC differentiation and loss of pluripotency marker expression in marmosets is remarkably similar to that in the human but fundamentally different from the situation in rats [40]. Based on their findings, McKinnell et al. [40] concluded that researchers and clinicians need to be very cautious when extrapolating the results from rodent studies into humans. In mice, no gonocytes were found on postnatal day 11 [46], and all germ cells had developed to spermatogonia or spermatocytes. The first haploid round spermatids were seen in postnatal day 21 mouse testes, and spermatogenesis was already completed by postnatal day 35 in the mouse [49]. This is in sharp contrast to the marmoset, which first produces sperm cells around 12 months after birth [50], and to the human testis, where puberty is usually initiated around the age of 11–13 years. Although some signals involved in the initiation and regulation of puberty have also been identified in NHP [51], there are still major gaps of knowledge in this field [52]. In general, an extended period of relative testicular quiescence before the initiation of the first meiotic wave occurs in primates, but not in rodents. These are relevant adaptations to the different reproductive strategies of primates as K-selected species with few offspring, long periods of gestation, long and intensive parental care, and a long period until the reproductive age is reached. In contrast, as r-selected species, rodents have high numbers of offspring, short periods of gestation, little parental care, and reach adulthood and sexual maturity early [53].

According to the classical model, ongoing spermatogenesis in rodents is fueled by spermatogonial stem cells, which are called A single (A_s) spermatogonia [54]. These are individual cells that are believed to be the starting point of a new clone of differentiating germ cells. They are characterized by the expression of several marker proteins such as GFRα1 (the GDNF receptor), NGN3, PLZF [for a review, see 55], SALL4 [56], and LIN28 [57]. In primates, the different putative stem cell types were classified and named after their morphological appearance: A_{dark} are considered to represent reserve stem cells, and A_{pale} spermatogonia are considered to represent active stem cells in terms of sperm production [for a review, see 55]. However, 10 years ago Ehmcke and Schlatt [58] proposed a model of spermatogonial self-renewal and differentiation that was based on the assumption that the primate spermatogonial population is more plastic than previously thought. Moreover, the relatively simple mouse model was also recently profoundly revised [59]. In fact, these novel data suggest that an extended heterogeneous population of spermatogonia forms a flexible and to a certain extent reversible pool of cells with stem cell characteristics on the one hand, and on the other hand differentiating characteristics. These important findings may lead to a revised model of spermatogonial self-renewal, expansion, and differentiation in mammals. However, even if the unexpected finding of plasticity of the spermatogonia population in the mouse testis applies to all mammals, there are significant differences between the mouse and the primate spermatogonial stem cell pool. Regarding marker expression, this is very obvious for LIN28 [44] (Fig. 2). While many mouse spermatogonia strongly express this important developmental and pluripotency-associated factor [60], it could be detected only in an extremely minor subpopulation of primate spermatogonia [44]. Also, NGN3, an important lineage-specifying transcription factor in the pancreas [61], is already expressed in mouse spermatogonia from the A_s stage onwards, but is absent from rhesus monkey A_{dark} and A_{pale} stem spermatogonia [55]. Irrespective of the validity of the different models of spermatogonial self-renewal and differentiation, it appears that mouse spermatogonia are in a more primitive, gonocyte-like state compared to primate spermatogonia. Single cell transcriptome analysis in combination with functional assays will further clarify the mode of the spermatogonial system in different

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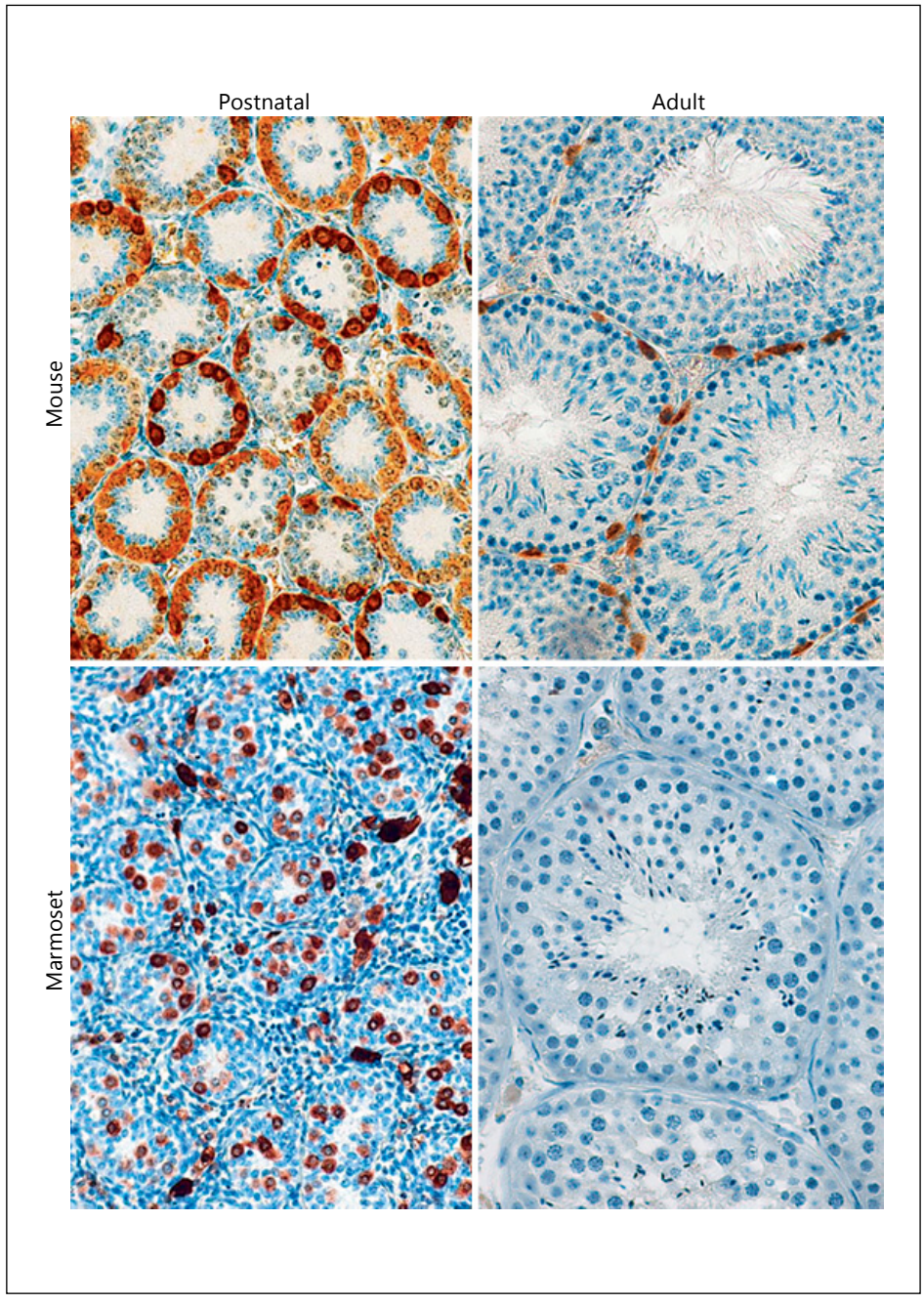

Fig. 2. Differential expression of the stem cell factor LIN28 in adult spermatogonia of marmoset monkey and mouse testes. In the immature postnatal testes, the gonocytes/spermatogonia are LIN28-positive in both species. In contrast, in the adult testes, only mouse spermatogonia are positive for LIN28, while the marmoset monkey spermatogonia (and those of all other NHP species tested so far) are generally negative for LIN28. Only very few primate spermatogonia express LIN28 [44].

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mammals, including primates, within the next few years.

One highly significant and undoubted difference between premeiotic germ cell expansion in the mouse and primate testis, however, is the number of mitotic cell divisions before the spermatogonia eventually enter meiosis. While there are 10 or 11 mitotic divisions of spermatogonia in the mouse before they enter meiosis, in primates there are significantly fewer premeiotic cell divisions: 5 in Rhesus monkeys and only 2 in humans (for a review, see Ehmcke and Schlatt [58]). This means that the size of the clones emerging from 1 stem cell is much bigger in the mouse than in the human. The rhesus monkey clone size is between that of the mouse and the human. This difference in clone size in turn also implies that the density of stem cells in the primate testis is much higher than in the mouse testis. It was hypothesized that the germ cell clonal size (which is inversely correlated with the stem cell density) determines whether a primate species predominantly exhibits a single- or a multistage organization of the cross-section of the germinal epithelium [62]. The human testis shows a multistage system in around 80% of all seminiferous tubule cross-sections [62], which corresponds to the small germ cell clone size in the human testis [63]. Similarly, the marmoset monkey also predominantly shows a multistage organization, with up to 5 stages per cross-section [62, 64]. This high number of stages per cross-section suggests a very small clone size and very few premeiotic mitotic divisions of spermatogonia in this species. In contrast, the macaque species showed a multistage organization of the germinal epithelium cross-section in only 20–33% of all cross-sections [62], correlating well with the larger number of premeiotic spermatogonial cell divisions [63]. Hence, in primates, the species-specific histological organization of the seminiferous epithelium most likely evolved depending on the number of premeiotic spermatogonial cell divisions. The fact that both the human and the marmoset exhibit a multistage system makes the marmoset monkey a very good model for human spermatogenesis [64]. Finally, when NHP are used as experimental animals in reproductive biology, it must be considered that some primate species, including the rhesus monkey, have seasonal testicular activity with a transient block of spermatogonial proliferation [65], while seasonality has never been described for the human and the marmoset testis.

Spermatogonial Stem Cell Culture

For more than 20 years, it has been possible to culture mouse spermatogonia [66]. Nagano et al. [66] demonstrated the spermatogonial stem cell identity of the cultured cells by transplantation into a recipient testis, where the cells repopulated the seminiferous tubule and initiated complete spermatogenesis. Since then, mouse spermatogonia have been cultured in many laboratories around the world. They were genetically manipulated in vitro and transplanted into appropriate recipients in order to generate genetically modified mice [67], and were converted to a pluripotent state resembling embryonic stem cells [68]. Furthermore, rodent in vitro spermatogenesis was intensely investigated and finally successful [69–73] even when initiated from cultured spermatogonial stem cell lines [74]. Altogether, the culture of mouse spermatogonial stem cells is well established. In the primate, it is still a matter of debate whether long-term culture and the expansion of spermatogonial stem cells, i.e., the culture of functional stem cells, is possible. While some studies could detect human [75, 76] and marmoset monkey [77, 78] spermatogonia only for very limited periods in culture, other reports described the long-term culture of human spermatogonia [79–82] and even their differentiation into haploid germ cells [83]. However, since some markers used in human long-term spermatogonial culture studies are not unequivocal germ cell markers, it remains a matter of conten-

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tion at present whether the cultured cells are really functional germ cells. The only functional test currently possible would be transplantation of long-term cultured NHP germ cells into a recipient testis of the same species and subsequent generation of offspring from the long-term cultured and transplanted germ cells. This is currently not possible in humans for ethical reasons due to the very limited knowledge about the safety of the procedure for the potential offspring. Nevertheless, in the long term, this procedure may be a promising strategy to preserve the prospective fertility of male prepubertal cancer survivors [84], and a very promising spermatogonial stem cell transplantation study has already been conducted in NHP with cryopreserved, but not long-term cultured spermatogonia [85]. In general, the long-term primate spermatogonial cell culture has to be improved. The robust spermatogonial stem cell culture in mice may reflect the more primitive developmental state of the mouse spermatogonial stem cells compared to the primate counterparts. In this context, it is tempting to speculate that the recently established concept of a naïve and a primed state of pluripotent stem cells [86] can also be translated to mouse (naïve) and primate (primed) spermatogonial stem cells.

Considering Potential Limitations of Mouse Models of Human Male Infertility

There exist excellent reviews on the genetic causes of male infertility [87, 88]. Hundreds of mouse mutants exhibiting spermatogenic defects leading to infertility have been generated, illuminating the roles of many conserved mammalian fertility genes. However, although many of the mouse genes investigated in the context of infertility were also analyzed in infertile patients, only few mouse fertility genes were also found to be mutated in patients, like TEX11 [89]. Even if all mouse genes were to be mutated soon [90], it remains questionable whether these mice represent appropriate models of the majority of human infertile patients. This may be due to the following facts: (1) male infertility is a multifactorial disease, and the interplay of several factors in primates may be different from that in mice, (2) there might be significant epigenetic differences between mouse and primate germ cells, and (3) there are primate-specific genes that have no counterpart in the mouse genome. Importantly, the primate-specific transcripts are highly enriched in the brain and in the testis [91] (see also below). Therefore, it is likely that some aspects of male primate germ cell development as well as some cases of human infertility cannot be modeled in mice due to the absence of the relevant genes. In general, the aspect of the evolution of novel genes, particularly in the testis, has probably been underestimated until recently [92], except for the sex chromosomes [93–95].

The Genome Perspective: Primate-Specific Genes and Mobile Elements

In terms of the evolutionary perspective, genomes are highly dynamic, and several mechanisms for the birth of new genes have been identified [96]. While new protein-coding genes usually evolve from preexisting protein-coding genes, recently even "motherless" new protein-coding genes, which probably developed de novo from ancestral noncoding DNAs, were described in the human and ape genome [97]. Hence, during evolution, genome dynamics resulted in highly diverse genomes. Importantly, it was recently estimated that the human genome encodes more than 300 human-specific genes and around 1,000 primate-specific genes [91]. Since the number of human genes is less than 20,000, more than 5% of all human genes are primate specific. These new genes are predominantly implicated in brain and testis development and function. Within the testis, most of the "male

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genes" appear to be expressed by germ cells [98], but not by the somatic compartment of the testis. Consequently, testicular transcriptomes are also different in different species [98], and, probably even more strikingly, gene expression levels evolve faster in primates than in rodents [99]. Furthermore, primates exhibit a faster accumulation of alternatively spliced transcripts than other mammalian orders [100]. On the molecular level, the global removal of repressive epigenetic marks and a permissive chromatin state during meiosis may boost gene expression in male germ cells. This molecular environment in germ cells most likely represents the delivery room for novel genes, and based on this, the "out-of-the-testis hypothesis" of gene/genome evolution was established [98]. To summarize: (1) testicular germ cells are most likely the hotbed of genomic evolution; (2) there are hundreds of primate-specific genes with enriched activity in the testis; (3) the testicular transcriptome is significantly different from those of other organs, and (4) interestingly, there is a primate-specific acceleration of transcriptome evolution.

Retrotransposons are mobile self-replicating genetic elements that can spread over the genome via an RNA intermediate [101] and can be considered to resemble retroviruses. However, viruses spread horizontally between organisms. In contrast, retrotransposons spread vertically through the germ line. Alu elements are a subclass of retrotransposons of about 300 base pairs in length. Since there is no specific defense or removal mechanism for Alu sequences, there is a steady accumulation of Alu sequences during evolution [101]. Importantly, Alu sequences are unique to primates, and they comprise around 11% of the human genome [102], while all transposable elements together constitute around 50% of the human genome [103]. Alu germline activity is the highest of all human retrotransposons and significantly contributes to genome instability and, hence, genetic population diversity [101]. Furthermore, another family of retrotransposons, the hominid-specific SINE-VNTR-Alus (called SVA), are the youngest of these elements, constituting 0.13% of the genome and originating approximately 25 million years ago [104]. The SVA are present in the human genome in about 3,000 copies. It has been shown that the elements of this family also contribute to the development of new genes in primates [104]. These examples highlight that significant parts of the primate genome are specific to primates and do not have any counterpart in the genomes of other mammalian taxa. Furthermore, these primate-specific portions of the genome are clearly not "junk DNA," but have functional relevance [105] – also in terms of primate-specific evolution [106].

The Haploid Phase of the Germline

Postmeiotic spermatids condense their chromatin in preparation for their release from the germinal epithelium and their subsequent passage through the female reproductive tract. Because of the block of transcription in spermatids [107] and the release of the residual body (a droplet of unnecessary cytoplasm also containing RNA), it was previously thought that sperm cells are free of RNA transcripts. Therefore, the transcriptome of the spermatids and sperm cells was not appreciated for a long time. This changed in recent years, and a very interesting study detected the highly surprising number of 16,900 different gene transcripts in spermatozoa [108]. This is more than in whole liver and in Sertoli cells, for instance [108]. Many spermatozoa transcripts may represent non- or incompletely destructed and retained transcripts from round and elongating spermatids. However, even if only a small fraction of the 16,900 transcripts encode functional RNAs, it becomes more and more evident that these transcripts (respectively, their corresponding genes) have evolutionary [109] and fertility-related [110] relevance. That species-spe-

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cific differences also occur in the spermatozoa transcriptome is nicely reflected by the fact that protamine expression is different in man and rat. While 2 protamines (protamine 1 and protamine 2) are expressed in human spermatozoa at a specific ratio (0.98 P1/P2 in normozoospermic men) [111], in rat spermatozoa protamine 2 transcripts are barely present, and the corresponding protein was not detected in rat spermatozoa [112]. Deviation from the 0.98 P1/P2 ratio in humans is a useful parameter to estimate a reduction of the fertilizing potential of human spermatozoa [111]. The importance of the tightly controlled P1/P2 ratio in human sperm samples, on the one hand, and the evolutionary plasticity of the protamine system in mammals, on the other hand, suggest that fertility-relevant haploid expressed genes are regulated in a human-specific way. Unfortunately, only very limited data are available on protamines in NHP [113], and, to the best of my knowledge, P1/P2 ratios are yet to be determined in NHP.

Conclusion and Outlook

Primates and rodents differ in many relevant biological aspects, including neurobiology, life span, gestation period, and the number of progeny. This review highlights selected primate-specific features of germ line development, which are not as evident as other biological differences between primates and rodents, but which are probably not less significant with regard to reproduction, ontogenesis, and evolution. These include the differential regulation of developmental potency in the preimplantation embryo, the apparently different origins of PGCs in the primate and mouse postimplantation embryo, and the differential expression of some important (transcription) factors in primate and mouse premeiotic germ cells, namely SOX2, SOX17, and LIN28. Furthermore, the size and expansion of germ cell clones in the testis is regulated in a species-specific way – also within the order of primates – resulting in typical patterns of tubular cross-sections (preferentially single-stage organization in human and marmoset monkey vs. preferentially multistage organization in the macaques). Mouse spermatogonial stem cell culture is well established. In contrast, it remains controversial whether human and NHP long-term spermatogonial stem cell culture and expansion has thus far been successful. Regardless, the fact that the mouse spermatogonial stem cell culture protocols were not applicable to human and NHP spermatogonial stem cells may reflect biological differences between rodent and primate spermatogonial stem cells. Moreover, it must always be considered that the primate genome significantly differs from the rodent genome; there are up to 1,000 primate-specific genes, and there are also primate-specific mobile genetic elements. Taking into account that the primate-specific genes are particularly active in germ cells, this genomic difference between primates and rodents cannot be overestimated in germ cell biology. Furthermore, it appears reasonable to assume that upcoming research will uncover many additional primate-specific factors and characteristics of germline development, particularly in light of high-throughput transcriptome and epigenome analyses of single cells and the emergence of genetically modified monkeys. Finally, with regard to the investigation of infertility in humans, it is on the one hand important to realize potential limitations of non-primate animal models for primate (including human) germ cell biology and fertility. On the other hand, human geneticists, andrologists, and reproductive biologists may be well advised to consider and investigate specific aspects of the primate germline as causes of human infertility in more detail. It therefore appears to be essential to further investigate the primate – including the human – germline and germ cell biology in order to obtain better insights into human reproductive function and failure.

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