Male-driven de novo mutations in haploid germ cells

Marie-Chantal Grégoire, Julien Massonneau, Olivier Simard, Anne Gouraud, Marc-André Brazeau, Mélina Arguin, Frédéric Leduc, and Guylain Boissonneault*

Department of Biochemistry, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC, Canada J1E4K8

*Correspondence address. E-mail: guylain.boissonneault@usherbrooke.ca

Submitted on December 13, 2012; resubmitted on March 12, 2013; accepted on March 16, 2013

ABSTRACT: At the sequence level, genetic diversity is provided by de novo transmittable mutations that may act as a substrate for natural selection. The gametogenesis process itself is considered more likely to induce endogenous mutations and a clear male bias has been demonstrated from recent next-generation sequencing analyses. As new experimental evidence accumulates, the post-meiotic events of the male gametogenesis (spermiogenesis) appear as an ideal context to induce de novo genetic polymorphism transmittable to the next generation. It may prove to be a major component of the observed male mutation bias. As spermatids undergo chromatin remodeling, transient endogenous DNA double-stranded breaks are produced and trigger a DNA damage response. In these haploid cells, one would expect that the non-templated, DNA end-joining repair processes may generate a repertoire of sequence alterations in every sperm cell potentially transmittable to the next generation. This may therefore represent a novel physiological mechanism contributing to genetic diversity and evolution.

Key words: DNA damage / DNA replication/repair / spermiogenesis / polymorphism / gene mutations

The replication hypothesis for male-biased mutation

Although many genetic disorders are transmitted as pre-existing mutations, a significant fraction of deleterious germline mutations are created de novo. Being rare, some mutations, however, may be important for the generation of genetic diversity that contributes to adaptive evolution. Germline mutations may arise from several endogenous or exogenous mechanisms in both male and female. However, owing to the many more replication cycles of spermatogonia throughout the male reproductive life and the constant replenishment of gametes, it has become rather intuitive that male germline must have a higher propensity for spontaneous mutations as originally proposed by Haldane (1947). Replication was initially suspected because the male-to-female mutation rate ratio is found correlated with the male-to-female ratio of the number of cell divisions (Vogel and Motulsky, 2010; Hurst and Ellegren, 1998). Aging is therefore expected to increase this so-called ‘male bias’ as the total number of cell divisions in oogenesis remains constant while there is a linear increase in total spermatogonial cell division with age. As the difference in the number of chromosomal replications between male and female increases, the proportion of paternally derived base substitutions would be expected to increase as well. A very recent study using whole genome sequencing of 78 parent–offspring trios revealed that the de novo mutation rate reaches $1.2 \times 10^{-8}$ per nucleotide per generation or ~60 mutations per offspring on average. This study confirmed that 75% of these mutations arise from the father and that this proportion dramatically increases with paternal age at conception, rising by about two mutations per year (Kong et al., 2012). Alteration of the male gamete genetic integrity during aging may therefore contribute to the etiology of genetic diseases and it is worth noting that over 20 autosomal dominant disorders have been reported to be associated with advanced paternal age (Glaser and Jabs, 2004; Sayres and Makova, 2011). Base substitution is likely the most prominent type of male-biased mutation to arise from mitotic cell divisions because of the mis-incorporation of nucleotides by DNA polymerase (Johnson et al., 2000). Other types of mutations such as insertions and deletions (indels) were also found to be more biased from whole genome studies in rodents (Makova et al., 2004). Since indels can occur from strand slippage during replication, this lent further support to the potential contribution of the higher number of cell divisions in the male mutation bias.

Other mechanisms for male-biased mutations

The replication hypothesis, however, may represent an oversimplification since it is now well known that DNA lesions may arise from many different mechanisms and the contribution of some replication-independent factors may be significant. Oxidative damage is one...
Mechanisms of DNA mutation during spermiogenesis

The presence of DSBs in this haploid context would necessarily prevent homologous recombination to be used as a reliable, templated DNA repair mechanism that depends on sister chromatids as this is the case during the S phase in somatic cells. Non-homologous end-joining repair (NHEJ) processes must therefore be used in order to repair DSBs in spermatids but, based on studies in somatic cells, these mechanisms are associated with limited insertions or deletions at the repair site which alter the DNA sequence, although the structural integrity of the DNA is restored. Even from a homogeneous set of starting DNA ends as substrate, NHEJ creates important variations in the non-templated addition at the two DNA ends (Lieber, 2010). There are two different NHEJ pathways known to date that nevertheless display a similar potential to induce mutations. The canonical pathway, known as DNA-PKcs-dependent NHEJ uses DNA ligase IV, KU70, KU80 and XRCC4 to complete the DNA repair. NHEJ may proceed without some of the canonical factors using PARP1, DNA ligase III and XRCC1 as the alternative ‘back-up’ mechanism still known as B-NHEJ (Iliaakis, 2009). In addition to having to rely on error-prone DNA repair systems, the chromatin remodeling context is likely to create an impediment to the repair process and, not surprisingly, the overall DNA repair capacity was found to decrease as spermatids progress through their differentiation program (Olsen et al., 2005; Marchetti and Wyróbek, 2008). At the moment, the extent and distribution of DSBs is unknown but a random distribution would be expected to produce a different set of mutations in each spermatid leading to a wide repertoire of genetic polymorphism given the large population of spermatozoa produced over time. In addition to the chromosome reshuffling provided by meiosis, each offspring would also inherit from a given set of mutations induced by the chromatin remodeling in the spermatid of origin. The particular nature of spermatid chromatin does not allow any useful prediction with respect to whether randomly distributed versus clustered DNA
strand breaks (hotspots) can be found. It is known, however, that structural heterogeneity in spermatid chromatin establishes domains of general sensitivity to endogenous and exogenous nucleases that could be generated by the transient open chromatin structure at given loci (Barratt et al., 2010; Johnson et al., 2011). It stands to reason, therefore, that heterogeneity exists in spermatid chromatin structure and that some strand breaks possibly arise at specific loci.

Chromatin remodeling in spermatids involves massive withdrawal and degradation of histones that should leave transient free DNA supercoils (Boissonneault, 2002) (Fig. 1). Such a high degree of free superhelical density is likely to generate non-B-DNA structures which can be responsible for breakpoint hotspots and chromosomal rearrangements (Wang et al., 2008). For instance, Z-DNA, characterized by a left-handed instead of a typical right-handed double helical structure, is generated within regions of high negative supercoiling and may serve as a recognition signal for DSB formation (Kha et al., 2010). High density of free supercoils independent of replication can produce cruciform extrusion that may also act to signal breakpoints involved in translocations (Inagaki et al., 2009). Interestingly, the break points of a well-known recurrent non-Robertsonian translocation, t(11;22)(q23;q11), are concentrated within regions harboring palindromic AT-rich repeats. All of eight studied cases of such de novo translocations were found to be of paternal origin and linked to spermatogenesis (Ohye et al., 2010). Analyses of sperm samples from 10 donors indicated that there was no age-dependent increase in the frequency of this de novo translocation and no increase in translocation frequency was observed in follow-up studies (Kato et al., 2007). Since aging is associated with a greater number of cell divisions, this suggests that these translocations are independent of replication. Thus, the possibility of gross-chromosomal rearrangements in spermatids deserve further consideration as it may represent one additional mechanism by which the chromatin remodeling process contributes to diversity.

**Future studies**

Compared with somatic cells, a general attenuation in spontaneous mutation frequency was previously observed in spermatogenic cells of young mice based on the functional analysis of the lacI reporter transgene used as a retrievable mutational target (Kohler et al., 1991). Although this strongly supports the concept that germ cells are in a ‘protected’ state relative to somatic cells, some key steps of spermatogenesis must nevertheless remain responsible for the transmission of a significant number of de novo transgenerational mutations and the clear male bias reported so far. The lacI reporter transgene system, such as that of the Big Blue® mouse used in these studies, rely on a multicity concatemer of the lacI gene on a single chromosome (Hill et al., 1999). The mutation potential of the chromatin remodeling in spermatids will have to be established on a much larger genome-wide scale or in the vicinity of the DSBs, providing a more systematic approach. In this context, the use of such a reporter transgene system will be limited because of the weaker probability that these hotspots be found within the inserted transgene.

The capture of DNA at strand breaks followed by next-generation sequencing should allow genome-wide mapping of DNA strand breaks in elongating spermatids (Leduc et al., 2011b). The distribution of strand breaks between coding and non-coding regions will be of special interest for evolutionary perspective especially if one can establish the intrinsic mutagenic activity of the chromatin remodeling at these hotspots. Because of the end-joining repair process likely involved, search for indels should probably be emphasized since

---

**Figure 1** The three major differentiation states of the haploid spermatids (mouse model). Round spermatids harboring somatic-like chromatin (left). The chromatin remodeling in elongating spermatids involves withdrawals of most of the nucleosomes (curved arrow) and DNA strand breaks (thick arrows, middle). In the late steps and following chromatin remodeling, the non-templated end-joining repair of DSBs is expected to create short sequence alteration (red).
only substitutions were the focus of the whole genome transgenerational studies published so far.

Conclusions

Overall, there is now substantial evidence that the chromatin remodeling steps in spermatids may not be genetically inert but could represent an evolutionary conserved, replication-independent, process that may act more specifically to introduce de novo mutations, indels or even chromosomal rearrangements such as translocations. Its contribution to the genetic landscape, diseases and evolution will now be possible with improvement in the sensitivity of next-generation sequencing that allows the monitoring of mutations occurring at low frequency.

Authors’ roles

M.-C.G. and G.B. wrote the manuscript. J.M., O.S., A.G., M.-A.B., M.A. and F.L. provided editorial comments.

Funding

The experimental work related to this article was made possible by grants from the Canadian Institute of Health Research (grant # MOP-93781) and from the Natural Sciences and Engineering Council of Canada (grant # 155182). M.-C.G is the recipient of a scholarship from FRQ-S (Québec).

Conflict of interest

None declared.

References


Marchetti F, Wyrobek AJ. DNA repair decline during mouse spermiogenesis results in the accumulation of heritable DNA damage. DNA Repair (Amst) 2008;7:572–581.


Sayres MAW, Makova KD. Genome analyses substantiate male mutation bias in many species. Bioessays 2011;33:938–945.


