Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *DNA Habitats and Their RNA Inhabitants*

Transpositional shuffling and quality control in male germ cells to enhance evolution of complex organisms

Andreas Werner,[1] Monica J. Piatek,[1] and John S. Mattick[2]

[1]RNA Biology Group, Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle, United Kingdom. [2]RNA Biology and Plasticity Group, Garvan Institute of Medical Research, and St Vincent's Clinical School, University of New South Wales, Darlinghurst, Australia

Address for correspondence: John Mattick PhD, Garvan Institute of Medical Research 384 Victoria Street, Darlinghurst, NSW 2010, Australia. j.mattick@garvan.org.au. Andreas Werner PhD, RNA Biology Group, Institute for Cell and Molecular Biosciences, Newcastle University, Framlington Place, Newcastle, NE2 4HH, United Kingdom. andreas.werner@ncl.ac.uk

Complex organisms, particularly mammals, have long generation times and produce small numbers of progeny that undergo increasingly entangled developmental programs. This reduces the ability of such organisms to explore evolutionary space, and, consequently, strategies that mitigate this problem likely have a strategic advantage. Here, we suggest that animals exploit the controlled shuffling of transposons to enhance genomic variability in conjunction with a molecular screening mechanism to exclude deleterious events. Accordingly, the removal of repressive DNA-methylation marks during male germ cell development is an evolved function that exploits the mutagenic potential of transposable elements. A wave of transcription during the meiotic phase of spermatogenesis produces the most complex transcriptome of all mammalian cells, including genic and noncoding sense–antisense RNA pairs that enable a genome-wide quality-control mechanism. Cells that fail the genomic quality test are excluded from further development, eventually resulting in a positively selected mature sperm population. We suggest that these processes, enhanced variability and stringent molecular quality control, compensate for the apparent reduced potential of complex animals to adapt and evolve.

Keywords: spermatogenesis; transposon; endo-siRNA; RNA-processing bodies; evolution

Introduction

The evolution of organisms by natural selection relies on the generation of genetic and phenotypic variability and the selection of the subset of the population that is best able to survive and reproduce, with the reciprocal loss of those individuals whose characteristics render them less competitive. The basic algorithm that drives evolution is therefore "generate variation and test" (the *evolutionary algorithm*), which is ultimately a probability function.[1] Those lineages that can run the algorithm most efficiently and stringently (i.e., those that have short generation times and large progeny sizes, with strong competition for survival) will have an intrinsic strategic advantage in exploring and locating habitable space.

This reciprocally creates a challenge for multicellular organisms, which have comparably long generation times and few progeny. This problem is amplified as developmental complexity increases and the ability to run the normal evolutionary algorithm is slowed by orders of magnitude in time. The dilemma is compounded by the difficulty of introducing and testing adaptive changes in complex genetic programs. Ostensibly, in mammals and especially in primates, there has been a profitable trade-off between reproductive volume and reproductive success, the latter based on cognitive advancement, which requires significant investment in nurturing progeny. Nevertheless, in the absence of compensatory strategies, it seems that the implicit price of developmental complexity and cognitive capacity is slowed evolution.

Consequently, there must be a strong background (secondary or strategic) selective advantage in finding innovative solutions to ameliorate the problem, by optimizing the frequency of relevant variation and improving selection filters. Thus, extant complex organisms will be expected to have developed

doi: 10.1111/nyas.12608

 © 2014 The Authors. *Annals of the New York Academy of Sciences* published by Wiley Periodicals Inc. on behalf of The New York Academy of Sciences.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

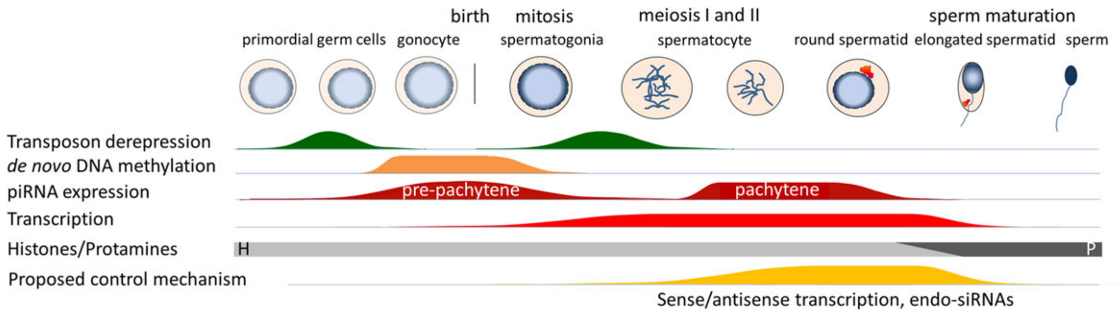

Figure 1. Schematic representation of spermatogenesis. The timing of developmental processes relevant to transposon derepression and resilencing are indicated in the lower panels. Accordingly, TEs undergo a first round of derepression during fetal germ cell differentiation that triggers piRNA expression and *de novo* DNA remethylation. This course of events is thought to be specific for animals, because piRNAs are only found in the animal kingdom. In mammals, a second round of TE derepression initiates at the mitotic phase as a consequence of either active or passive genome-wide demethylation. The relaxation of repressive chromatin marks enables transposition but also triggers a wave of transcription that promotes sense/antisense RNA expression and the synthesis of pachytene piRNAs. We propose that the majority of the transcripts are stored in the chromatoid body (indicated in round and elongated spermatids). siRNA (and piRNA)–Argonaute complexes (RISCs) search for their complementary target RNAs in the chromatoid body (CB) if the corresponding transcript is present; if the target RNA is not found in the CB, the RISCs enter the nucleus to interfere with the maturation process of the spermatid.

progressively more sophisticated strategies to improve their ability to search evolutionary space, and therefore enhance their long-term competitiveness. By extension, successful (that is, extant) lineages will have selected and retained these capacities.

Thus, there must be a selective advantage not just for new adaptive traits per se, but also, and more generally and potentially more powerfully, for mechanisms that optimize the search for evolutionary innovation and adaptation. Here, we make the case that an important part of this strategy has been to increase emphasis on the generation and selection of genetic variants in male germ cells. Undifferentiated spermatogonia are produced in enormous numbers, especially in mammals, where the ratio of spermatogonia compared to ova and zygotes is in the order of 10^9 or higher. This large number of cells allows for extensive experimentation giving even highly unlikely events—that is, beneficial mutations—a greater chance to occur. Conversely, the vast experimental output with predominantly negative or silent changes must undergo exquisitely tight screening to ensure that sperm with deleterious changes are eliminated. To put it another way, all the "money" is on a very limited number of "bets," since a single cell fertilizes the egg and gives rise to a single offspring, of which there are not many produced in a reproductive lifetime.

The scenario that mutational variation and initial selection and quality control have been transferred in substantial part from the zygote to the sperm fits with the known temporal events in spermatogenesis, including DNA demethylation, the activation of transposition, its subsequent suppression by Piwi-interacting RNA (piRNA)–mediated pathways, the genome-wide wave of transcription that is followed by chromatin compaction, and large-scale apoptosis of immature sperm cells.[2–4] We propose that these processes represent stages of a developmental program to enable the mobilization of transposable elements (TEs), which, through quasi-random insertion, promote variation in the genome. The transcriptional burst in the meiotic phase of spermatogenesis that produces the most complex transcriptome of all tissue, including the brain,[5] represents the next important event in the proposed developmental program. Accordingly, the germ line–specific program[6] enables pervasive transcription as the prerequisite for genomic quality screening to reduce the deleterious side effects of transposon insertions and recombination errors (Figs. 1 and 2). Intriguingly, the proposed stringent quality-control mechanism also helps to explain how complex organisms, humans in particular, can thrive in a highly mutagenic environment.[7]

Increasing variability: optimizing part 1 of the evolutionary algorithm

There are two general mechanisms for the creation of new raw material for evolution: gene duplication

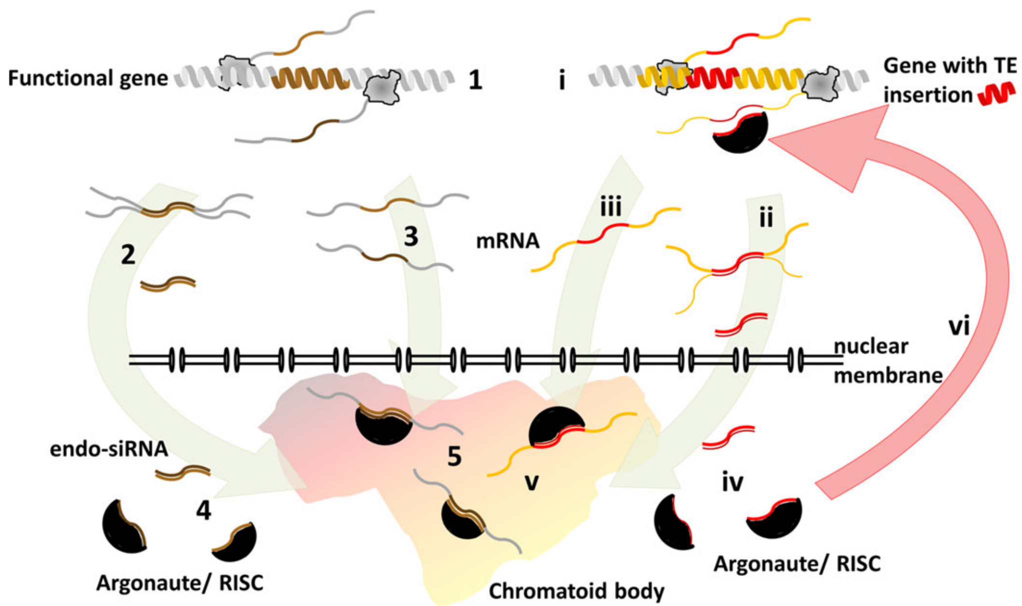

Figure 2. Schematic representation of the proposed endo-siRNA–based control mechanism. The left side (1–5) shows the mechanism applied to a nonmutagenized gene; the right side represents a gene that has been damaged by a transposon insertion. During the first step, the genes are transcribed in both directions (1 and i) generating fully processed complementary RNA. The sense/antisense mRNAs can either hybridize and become processed into endo-siRNAs (2 and ii) or exported and stored in the chromatoid body (3 and iii). The gene with the transposon insertion, however, produces little, unstable, or incorrectly spliced or folded sense mRNA (represented as a thin line) that fails to reach the chromatoid body (iii). The endo-siRNAs reach the cytoplasm, where both strands are incorporated into a complex with an Argonaute protein (RISC) (4 and iv). RISCs search for and bind to their complementary targets, which are sequestered in significant numbers in the chromatoid bodies (5 and v). If a RISC complex fails to hybridize to a target in the chromatoid body, it will remain mobile and eventually find its target in primary RNAs at the transcribed locus (vi). We propose that the nuclear RISC eventually interferes with the further maturation of the sperm, thus eliminating cells with deleterious TE insertions.

and transposition. While there is innovation in protein space, the proteome of mammals is remarkably stable, and many homologous and orthologous proteins only differ by a few codons between species. It is generally (although not universally) acknowledged that most phenotypic innovation and adaptive radiation in animals occurs by alterations to the regulatory superstructure rather than the repertoire of protein components.[8–10] Transposition can efficiently mobilize modular regulatory cassettes into both the genome and transcriptome and is therefore well suited to enable innovation. Consistent with this idea, high levels of transposon-derived RNA are expressed in germ cells, and the enzymes required for integration can be visualized in postmeiotic spermiocytes.[11] Novel male germ line insertions are detectable that cause significant variation of the transposon landscape in closely related animals and even within strains of the same species.[12] The detection of random transposition events in individual germ cells, however, is challenging, since these cells lack clonal expansion and retrotransposition events may in fact be underestimated. Retrotransposition is also reported to occur in early embryos at a high level, leading to mosaicism in clonally expanded stem cells.[13] It is likely that the system is itself selectively tuned to avoid transposition into protein-coding sequences, which is logical in evolutionary terms and supported by the nonrandom distribution of TEs in the mammalian genome.[14] The pattern of insertion suggests that promoter regions of protein-coding genes are preferentially targeted, while the 3′ ends and introns are affected to a lesser extent.[12] Intriguingly, protein-coding sequences, mutagenesis of which often has catastrophic consequences,[15] remain untouched.

Our hypothesis predicts a developmental program that promotes genomic innovation through

TE transposition to secure long-term competitiveness. A window of genomic innovation is opened during the early stages of spermatogenesis, which occur during embryogenesis in humans and mice. Genome-wide active demethylation occurs and enables mobilization of various classes of transposable elements.[16] The process is terminated by piRNA-driven genome-wide DNA *de novo* remethylation.[17] A second window of relaxed transposon suppression seems to occur during the meiotic phase of spermatogenesis in adult testis of mammals.[18] The emerging underlying mechanisms involve DNA hypomethylation, germ line–specific promoter usage, and a subset of pachytene piRNAs.[18–20]

As long as TEs are predominantly a threat to genome stability, enhanced transposition is an undesirable by-product of DNA hypomethylation at the onset of meiosis (and also during other phases of germ line and somatic development) and amounts to genomic "Russian roulette" with little biological rationale.[3] If, however, the innovative potential of TE activation is emphasized, DNA demethylation promotes the search for a novel competitive edge. Intriguingly, the methylation status of intracisternal A particles (IAPs, a highly active family of retroviral elements in rodents) depends on the nutritional status of the animal, and hypomethylation can be reversed with a diet rich in methyl donors (e.g., choline, methionine).[21] TEs could therefore act as sensors and respond to sustained shortage of food with increased transposition and an intensified search for genomic innovation.[22]

Mammals, which arguably display the highest level of organismal complexity, allow transposition twice during spermatogenesis and have evolved an efficient filter to screen for deleterious mutations (as we will argue in the next section). The mechanism involves pachytene piRNAs and endogenous siRNAs (endo-siRNAs) derived from sense/antisense transcription of genic loci and other pervasive transcription and enables a positive selection of cells that continue the development into mature sperm cells.[23,24] During sperm development, maturation, fertilization, and early embryogenesis, the number of potential offspring gradually decreases and is progressively phenotypically refined, as outlined below. Indeed, the strategy actively exploiting evolutionary space only works with an extremely robust screening mechanism in place to ensure only the fittest sperm make it through to the egg.

Improving selection filters: optimizing part 2 of the evolutionary algorithm

It has been known for many years, although never satisfactorily explained, that transcription in the testis is more extensive than in any other tissue, even the brain, which is far more complex than any other somatic tissue.[5,25] The finding that many of the mRNAs produced in testis are not translated—in-depth analysis of the transcriptome and proteome show little overlap—indicates that the act of transcription or the RNA itself (and not the encoded protein) are of biological importance.[26,27]

Another clue to the relevance and importance of this transcription is provided by analysis of the pattern of antisense transcription, which is qualitatively different from other tissues and favors the formation of endo-siRNAs and piRNAs from natural antisense transcripts (NATs).[19,24,28,29] The fact that these NATs share complementarity with their cognate mRNAs[30–33] make the related piRNAs and endo-siRNAs perfect guides to target effector complexes to protein-coding transcripts. We propose that sense–antisense hybrid formation and germ cell–specific RNA interference represent key events in the genomic quality-control mechanism that is essential to mitigating the consequences of enhanced transposition. Accordingly, genomic damage (inflicted by transposon insertion or other mutagenic events) triggers a cascade to eliminate the cell if transcripts from the affected gene fail to reach the cytoplasm (Fig. 2). Recent findings on piRNAs[34] and endo-siRNAs,[23] as well as evidence from the fields of RNA interference and transcriptomics (reviewed in Refs. 30, 31, and 35), provide the conceptual framework for our model. In other words, a protein-coding mRNA is deemed "fit for purpose" if it is exported in adequate amounts to the cytoplasm—a test that poorly transcribed, misfolded, mis-spliced, or unstable mRNAs fail.

The following paragraph explains the proposed control mechanism in a scenario where a protein-coding mRNA shares complementarity with a processed antisense transcript; nonetheless, the mechanism could be relevant to other transcriptional units that produce complementary RNA. Interestingly, both endo-siRNAs and pachytene piRNAs originate from protein-coding genes that are significantly enriched in antisense transcripts, suggesting that the two branches of RNA interference

may be intertwined via common testis-specific effector proteins.[19,36] The molecular basis and genomic background of the mechanism in Figure 2 is explained in more detail in other papers.[33,37] In the first step of the proposed sequence of events (Fig. 2), both sense transcript and antisense transcript are generated, spliced, and fully processed. In fact, the mRNA-like structure of many antisense transcripts has been confirmed experimentally.[38,39] Thereafter, the complementary transcripts have two options. In the first, they are exported to the cytoplasm and sequestered in the chromatoid body (CB), a prominent and dynamic RNA-processing center in (haploid) spermiocytes that harbors a plethora of short and long RNAs including mRNAs as well as components of the RNA interference (RNAi) machinery and enzymes of the nonsense-mediated decay pathway.[40,41] Alternatively, the hybrids are processed into endo-siRNAs and/or piRNAs by nuclear dicer or an undefined endonuclease.[42] The sense/antisense-derived small RNAs are exported to the cytoplasm, where they form a complex with Argonaute proteins.[41,43,44] These so-called pre-RISCs (RNA-induced silencing complexes) can bind the small RNAs in both orientations, meaning that two versions of the complex are formed, one complementary to the sense transcript and the other complementary to the antisense transcript.[45–47] Both pre-RISCs will search for their complementary target molecule and find it either within the chromatoid body or—if absent there—at the transcription site in the nucleus.[34,48–50] Nuclear RISC has been implicated in transcriptional gene silencing in various mammalian systems,[48–52] and we propose that the inflicted chromatin mark will abort further development of the spermiocyte. A comparable strategy of endo-siRNAi–based screening of genetic material has been described for the licensing of novel genes in *Caenorhabditis elegans*,[53] as well as for programmed DNA rearrangement and elimination in lower eukaryotes.[54,55]

According to this hypothesis, the pass/fail criterion for a bidirectionally transcribed gene is whether the transcripts make it to the CB. Any transposon insertion that significantly reduces transcription or interferes with processing or nuclear export will deplete the affected transcript from the CB. As a consequence, the specific pre-RISC will enter the nucleus and find its target on the nascent sense transcript, and the sperm harboring the mutation will be eliminated (Fig. 2). Intriguingly, mouse testis displays very efficient nonsense-mediated decay (NMD) and expresses the highest level of NMD core proteins among 13 tissues including the brain.[56] This proposed transcriptional proofreading checks the retention of a cogent transcriptional landscape, and eliminates, albeit crudely, a large proportion of all nascent spermatocytes, contributing to the massive attrition at this point.[57] Cell viability is probably the most effective and active filter: if the resulting mutation results in apoptosis, the mutation is terminal.

Interestingly, substantial attrition of oocytes occurs during female embryonic germ line development; the process is linked to transposon mobilization and occurs at meiotic prophase I. The expression of a transposon-encoded protein particle (L1RNP) is monitored, and high levels provoke attrition of oocytes.[58] As a result, female germ cells are selected for low transposon activity; in contrast, the mechanism we propose for male germ cells applies to later stages, permitting transposon integration and the generation of genome variability.

The positively selected sperm population has to survive further tests, the first of which may be environmental stability such as temperature variance, physical stress, or pathogen defense.[57,59] Added to this is physical fitness: properly developed sperm must have characteristics necessary for mobilization to run the gantlet—which may also broadly explain the long journey that nascent spermatocytes have to undergo through seminiferous tubules—and to effectively compete to reach and fertilize the egg before others. Finally, there is selection during embryogenesis, where many fertilized eggs do not advance past early stages of gestation, resulting in high early miscarriage rates in humans. These later selection filters, however, screen for cellular performance, and molecular faults will go unnoticed if the phenotypic consequences of the defects are efficiently compensated or only manifest later during development. The proofreading mechanism we suggest safeguards early steps of protein synthesis: it eliminates mutations that prevent the mRNA of the affected gene from reaching the cytoplasm and being translated. This is an intriguingly simple strategy; a protein-coding gene that cannot produce a protein is hardly beneficial to the emerging organism, and its elimination will enhance the chances for better candidates.

To summarize, we propose that mammals have optimized the evolutionary algorithm by opening an additional window of opportunity for TE transposition to promote variability in the huge numbers of developing sperm. The transcriptomes of the randomly mutagenized cells are afterwards tested by an RNAi-based mechanism for deleterious defects in RNA synthesis and maturation. In combination with the physical challenges involved in reaching the egg, this molecular quality control ensures that the developmental cogency of the offspring is at least maintained. Intriguingly, the proposed stringent quality-control mechanism also provides an elegant solution to the debated problem of how complex organisms, humans in particular, can thrive in a highly mutagenic environment. Current estimates of mutation rates predict that, in the absence of purifying selection of germ cells, deleterious changes will accumulate within a few generations to a level incompatible with a fit and thriving population, referred to as the *mutation paradox* in humans.[7] Ironically, the creationist movement uses the positively selected sperm population as prime evidence for the existence of a divine force in evolution.

Conclusion

The potential for transferring some of the experimental strategies in evolution to sperm in animals, especially mammals, is appealing both logically and evidentially. It makes the obvious and largely confirmed prediction that individual organisms, such as humans and mice, will display individual transposon profiles, including novel insertions.[9,60]

Enhanced transposition to promote variability may also occur at other stages of the life cycle, and for other reasons. There is evidence of transposon mobilization in embryogenesis[61] and in the brain,[62,63] the latter potentially to create somatic diversity. In addition, different classes of transposons may have evolved different roles in the evolutionary dynamics of germ lines and the plasticity of somatic cells in animals.[22] Preliminary evidence suggests that this may have been accompanied by the coevolution of genes, like the RNA-editing enzymes APOBEC (apolipoprotein B mRNA–editing enzyme, catalytic polypeptide-like) and the Rett syndrome–related MeCP2 (methyl CpG binding protein 2), to domesticate and regulate the process.[64,65] In any case, we suggest that the addition of transpositional experimentation and selection in sperm has been an important, and thus far unappreciated, aspect of the evolution of evolvability, especially in mammals.

Acknowledgments

We thank Noora Kotaja and Sushma Grellscheid for discussions and critical reading of the manuscript and acknowledge funding from the Australian National Health & Medical Research Council (Australia Fellowship 631668) to JSM and from The Dunhill Medical Trust to AW (Grant DMT SA10/0210). Monica J. Piatek is a recipient of a Ruth Jacobson Studentship.

Conflict of interest

The authors declare no conflicts of interest.

References

1. Dennett, D. 1995. *Darwin's Dangerous Idea: Evolution and the Meanings of Life*. New York: Simon & Schuster.
2. Yadav, R.P. & N. Kotaja. 2013. Small RNAs in spermatogenesis. *Mol. Cell. Endocrinol.* **382:** 498–508
3. Bao, J. & W. Yan. 2012. Male germline control of transposable elements. *Biol. Reprod.* **86:** 162, 1–14.
4. Laiho, A., N. Kotaja, A. Gyenesei & A. Sironen. 2013. Transcriptome profiling of the murine testis during the first wave of spermatogenesis. *PLoS One* **8:** e61558.
5. Soumillon, M., A. Necsulea, M. Weier, *et al.* 2013. Cellular source and mechanisms of high transcriptome complexity in the Mammalian testis. *Cell Reports* **3:** 2179–2190.
6. Hammoud, S.S., D.H. Low, C. Yi, *et al.* 2014. Chromatin and transcription transitions of mammalian adult germline stem cells and spermatogenesis. *Cell Stem Cell* **15:** 239–253
7. Lesecque, Y., P.D. Keightley & A. Eyre-Walker. 2012. A resolution of the mutation load paradox in humans. *Genetics* **191:** 1321–1330.
8. Carroll, S.B. 2008. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* **134:** 25–36.
9. Cowley, M. & R.J. Oakey. 2013. Transposable elements re-wire and fine-tune the transcriptome. *PLoS Genetics* **9:** e1003234.
10. Mattick, J.S. 2001. Non-coding RNAs: the architects of eukaryotic complexity. *EMBO Rep.* **2:** 986–991.
11. Ergun, S., C. Buschmann, J. Heukeshoven, *et al.* 2004. Cell type-specific expression of LINE-1 open reading frames 1 and 2 in fetal and adult human tissues. *J. Biol. Chem.* **279:** 27753–27763.
12. Nellaker, C., T.M. Keane, B. Yalcin, *et al.* 2012. The genomic landscape shaped by selection on transposable elements across 18 mouse strains. *Genome Biol.* **13:** R45.
13. Kano, H., I. Godoy, C. Courtney, *et al.* 2009. L1 retrotransposition occurs mainly in embryogenesis and creates somatic mosaicism. *Genes Develop.* **23:** 1303–1312.
14. Mouse Genome Sequencing Consortium, R.H. Waterston, K. Lindblad-Toh, *et al.* 2002. Initial sequencing and comparative analysis of the mouse genome. *Nature* **420:** 520–562.

15. Mattick, J.S. 2009. The genetic signatures of noncoding RNAs. *PLoS Genet.* **5:** e1000459.
16. Kimmins, S., N. Kotaja, I. Davidson & P. Sassone-Corsi. 2004. Testis-specific transcription mechanisms promoting male germ-cell differentiation. *Reproduction* **128:** 5–12.
17. Kuramochi-Miyagawa, S., T. Watanabe, K. Gotoh, *et al.* 2008. DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes Develop.* **22:** 908–917.
18. Soper, S.F., G.W. van der Heijden, T.C. Hardiman, *et al.* 2008. Mouse maelstrom, a component of nuage, is essential for spermatogenesis and transposon repression in meiosis. *Dev. Cell.* **15:** 285–297.
19. Gan, H., X. Lin, Z. Zhang, *et al.* 2011. piRNA profiling during specific stages of mouse spermatogenesis. *RNA* **17:** 1191–1203.
20. Gan, H., L. Wen, S. Liao, *et al.* 2013. Dynamics of 5-hydroxymethylcytosine during mouse spermatogenesis. *Nat. Commun.* **4:** 1995.
21. Cropley, J.E., C.M. Suter, K.B. Beckman & D.I.K. Martin. 2006. Germ-line epigenetic modification of the murine A(vy) allele by nutritional supplementation. *Proc. Nat. Acad. Sci. USA.* **103:** 17308–17312.
22. Sharif, J., Y. Shinkai & H. Koseki. 2013. Is there a role for endogenous retroviruses to mediate long-term adaptive phenotypic response upon environmental inputs? *Philos. Trans. R. Soc. Ser. B* **368:** 20110340.
23. Song, R., G.W. Hennig, Q. Wu, *et al.* 2011. Male germ cells express abundant endogenous siRNAs. *Proc. Nat. Acad. Sci. USA.* **108:** 13159–13164.
24. Carlile, M., D. Swan, K. Jackson, *et al.* 2009. Strand selective generation of endo-siRNAs from the Na/phosphate transporter gene Slc34a1 in murine tissues. *Nucl. Acids Res.* **37:** 2274–2282.
25. Ravasi, T., H. Suzuki, K.C. Pang, *et al.* 2006. Experimental validation of the regulated expression of large numbers of non-coding RNAs from the mouse genome. *Genome Res.* **16:** 11–19.
26. Kleene, K.C., J. Bagarova, S.K. Hawthorne & L.M. Catado. 2010. Quantitative analysis of mRNA translation in mammalian spermatogenic cells with sucrose and Nycodenz gradients. *Reproduct. Biol. Endocrinol.* **8:** 155.
27. Wang, G., Y. Guo, T. Zhou, *et al.* 2013. In-depth proteomic analysis of the human sperm reveals complex protein compositions. *J. Proteom.* **79:** 114–122.
28. Grinchuk, O.V., P. Jenjaroenpun, Y.L. Orlov, *et al.* Integrative analysis of the human cis-antisense gene pairs, miRNAs and their transcription regulation patterns. *Nucl. Acids Res.* **38:** 534–547.
29. Werner, A. & D. Swan. 2010. What are natural antisense transcripts good for? *Biochem. Soc. Trans.* **38:** 1144–1149.
30. Beiter, T., E. Reich, R.W. Williams & P. Simon. 2008. Antisense transcription: a critical look in both directions. *Cell Mol. Life Sci.* **66:** 94–112.
31. Faghihi, M.A. & C. Wahlestedt. 2009. Regulatory roles of natural antisense transcripts. *Nat. Rev. Mol. Cell Biol.* **10:** 637–643.
32. Werner, A. 2005. Natural antisense transcripts. *RNA Biol.* **2:** 53–62.
33. Werner, A., M. Carlile & D. Swan. 2009. What do natural antisense transcripts regulate? *RNA Biol.* **6:** 43–48.
34. Gou, L.T., P. Dai, J.H. Yang, *et al.* 2014. Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. *Cell Res.* **24:** 680–700.
35. Czech, B. & G.J. Hannon. 2011. Small RNA sorting: matchmaking for Argonautes. *Nat. Rev.* **12:** 19–31.
36. Werner, A., S. Cockell, J. Falconer, *et al.* 2014. Contribution of natural antisense transcription to an endogenous siRNA signature in human cells. *BMC Genom.* **15:** 19.
37. Werner, A. & J.A. Sayer. 2009. Naturally occurring antisense RNA: function and mechanisms of action. *Curr. Opin. Nephrol. Hypertens.* **18:** 343–349.
38. Katayama, S., Y. Tomaru, T. Kasukawa, *et al.* 2005. Antisense transcription in the mammalian transcriptome. *Science* **309:** 1564–1566.
39. Kiyosawa, H., I. Yamanaka, N. Osato, *et al.* 2003. Antisense transcripts with FANTOM2 clone set and their implications for gene regulation. *Genome Res.* **13:** 1324–1334.
40. Meikar, O., V.V. Vagin, F. Chalmel, *et al.* 2014. An atlas of chromatoid body components. *RNA* **20:** 483–495.
41. Meikar, O., M. Da Ros, H. Korhonen & N. Kotaja. 2011. Chromatoid body and small RNAs in male germ cells. *Reproduction* **142:** 195–209.
42. Doyle, M., L. Badertscher, L. Jaskiewicz, *et al.* 2013. The double-stranded RNA binding domain of human Dicer functions as a nuclear localization signal. *RNA* **19:** 1238–1252
43. Gonzalez-Gonzalez, E., P.P. Lopez-Casas & J. del Mazo. 2008. The expression patterns of genes involved in the RNAi pathways are tissue-dependent and differ in the germ and somatic cells of mouse testis. *Biochim. Biophys. Acta* **1779:** 306–311.
44. Modzelewski, A.J., R.J. Holmes, S. Hilz, *et al.* 2012. AGO4 regulates entry into meiosis and influences silencing of sex chromosomes in the male mouse germline. *Dev. Cell* **23:** 251–264.
45. Betancur, J.G. & Y. Tomari. 2012. Dicer is dispensable for asymmetric RISC loading in mammals. *RNA* **18:** 24–30.
46. Noland, C.L. & J.A. Doudna. 2013. Multiple sensors ensure guide strand selection in human RNAi pathways. *RNA* **19:** 639–648.
47. Alnumeir, S. & A. Werner. 2014. Generation of endo-siRNAs in *Xenopus laevis* oocytes. *Methods Mol Biol.* **1173:** 27–32.
48. Berezhna, S.Y., L. Supekova, F. Supek, *et al.* 2006. siRNA in human cells selectively localizes to target RNA sites. *Proc. Nat. Acad. Sci. USA.* **103:** 7682–7687.
49. Ohrt, T., J. Muetze, P. Svoboda & P. Schwille. 2012. Intracellular localization and routing of miRNA and RNAi pathway components. *Curr. Top Med. Chem.* **12:** 79–88.
50. Weinmann, L., J. Hock, T. Ivacevic, *et al.* 2009. Importin 8 is a gene silencing factor that targets Argonaute proteins to distinct mRNAs. *Cell* **136:** 496–507.
51. Gullerova, M. & N.J. Proudfoot. 2012. Convergent transcription induces transcriptional gene silencing in fission yeast and mammalian cells. *Nat. Struct. Mol. Biol.* **19:** 1193–1201.

52. Morris, K.V., S. Santoso, A.M. Turner, *et al.* 2008. Bidirectional transcription directs both transcriptional gene activation and suppression in human cells. *PLoS Genet.* **4:** e1000258.
53. de Albuquerque, B.F. & R.F. Ketting. 2013. Is this mine? small RNAs help to decide. *Dev. Cell* **27:** 599–601.
54. Coyne, R.S., M. Lhuillier-Akakpo & S. Duharcourt. 2012. RNA-guided DNA rearrangements in ciliates: is the best genome defence a good offence? *Biol. Cell/Under Auspices Eur. Cell Biol. Organ.* **104:** 309–325.
55. Kataoka, K. & K. Mochizuki. 2011. Programmed DNA elimination in Tetrahymena: a small RNA-mediated genome surveillance mechanism. *Adv. Exp. Med. Biol.* **722:** 156–173.
56. Zetoune, A.B., S. Fontaniere, D. Magnin, *et al.* 2008. Comparison of nonsense-mediated mRNA decay efficiency in various murine tissues. *BMC Genet.* **9:** 83.
57. Shaha, C., R. Tripathi & D.P. Mishra. 2010. Male germ cell apoptosis: regulation and biology. *Philos. Trans. R. Soc. Lond. Ser. B, Biol. Sci.* **365:** 1501–1515.
58. Malki, S., G.W. van der Heijden, K.A. O'Donnell, *et al.* 2014. A role for retrotransposon LINE-1 in fetal oocyte attrition in mice. *Dev. Cell* **29:** 521–533.
59. Baum, J.S., J.P. St George & K. McCall. 2005. Programmed cell death in the germline. *Sem. Cell Develop. Biol.* **16:** 245–259.
60. Burns, K.H. & J.D. Boeke. 2012. Human transposon tectonics. *Cell* **149:** 740–752.
61. Hackett, J.A. & M.A. Surani. 2013. DNA methylation dynamics during the mammalian life cycle. *Philos. Trans. R. Soc. Lond. Ser. B, Biol. Sci.* **368:** 20110328.
62. Baillie, J.K., M.W. Barnett, K.R. Upton, *et al.* 2011. Somatic retrotransposition alters the genetic landscape of the human brain. *Nature* **479:** 534–537.
63. Coufal, N.G., J.L. Garcia-Perez, G.E. Peng, *et al.* 2009. L1 retrotransposition in human neural progenitor cells. *Nature* **460:** 1127–1131.
64. Mattick, J.S. 2010. RNA as the substrate for epigenome-environment interactions: RNA guidance of epigenetic processes and the expansion of RNA editing in animals underpins development, phenotypic plasticity, learning, and cognition. *Bioessays* **32:** 548–552.
65. Muotri, A.R., M.C. Marchetto, N.G. Coufal, *et al.* 2010. L1 retrotransposition in neurons is modulated by MeCP2. *Nature* **468:** 443–446.