1 | INTRODUCTION

In the fertilized egg, the DNA inherited from the egg and sperm is replicated, after which the egg undergoes cell proliferation. During the differentiation and proliferation of one generation of germ cells, approximately 70 base substitutions occur in chromosomal DNA. Among germ cells in which base substitutions occur during differentiation and proliferation due to mitosis, some haploid cells escape base substitutions after meiosis. Somatic cells die after continual cell division, whereas germ cells continue to divide by accurately replicating their DNA, as homologous recombination and meiosis are essential for cellular maintenance and passing genetic traits to offspring. We cloned the genes specifically expressed in haploid male germ cells to compare the characteristics of germ cells and somatic cells. The Genome Project revealed that genes encoding about 25,000 proteins are expressed in humans and the mouse. Recently, 2017 intronless genes were identified in the mouse genome. It was reported that 99% of human protein-coding genes align with homologues in the mouse and 80% are clear 1:1 orthologs. Genes specifically expressed in haploid germ cells have been cloned and their genomic structures analyzed. Genomic structural analyses of 246 cloned genes revealed 51 intronless genes. These results revealed that many intronless genes are expressed and function in haploid male germ cells (Table 1).
Spermatogenesis occurs in a filamentous tube called the seminiferous tubule within the testis, and germ cell differentiation can be divided into three main stages: proliferation and differentiation of spermatogonia, which are the male germ stem cells, meiosis of spermatocyte cells, and morphogenesis of haploid germ cells. The testis contains seminiferous tubules composed of germ cells surrounded by Leydig cells. In addition, lymphatic vessels, capillaries, and a small number of macrophages are present. 

The seminiferous tubule wall consists of the basement membrane, connective tissue and surrounding fibroblasts, and myoid cells attached to the outside of the wall. Almost all genes are expressed in the brain and testis. 

This is thought to be because gene expression is necessary to support the various differentiated cells of the brain and testis, the latter including differentiated cells from spermatogonia to spermatids and the cells supporting spermatogenesis.

### 3 | GENES EXPRESSED IN SPERMATOGENESIS

In mice, the differentiation of male germ cells (spermatogenesis), from spermatogonia to spermatozoa (spermatids) via meiosis, begins immediately after birth and takes approximately 35 days to complete. 

With the progress of gene analysis technologies such as differential display, subtracted testis-specific libraries, and microarray analysis, genes specifically expressed in the testis have been identified. 

Approximately 2300 testicular germ cell-specific genes are distributed across various chromosomes. 

By producing gene-disrupted mice, the functions of these genes were analyzed in vivo; the gene-disrupted mice produced fertile sperm, even if one function was lost in many of these specific genes. 

Histones are replaced by transition nuclear proteins (TNPs), which are ultimately replaced by protamine in spermiogenesis. Two TNPs, TNP1 and TNP2, are expressed in sperm nucleation. Loss of either the TNP1 or TNP2 gene produces fertile sperm, but loss of both genes results in a failure to form fertile sperm nuclei. TNP1 and TNP2 partially complement each other. 

These results indicate that the roles of individual testicular germ cell-specific genes may be complemented by the functions of other genes. Spermatogenesis is maintained by the redundancy and complexity of germ cell-specific genes. From this, it can be understood that the roles of individual genes in the body can be understood that the roles of individual genes in the body can be complemented by the functions of multiple genes that maintain germ cell differentiation.

### 4 | INTRONLESS GENES IN MALE GERM CELLS

Intronless genes are produced by retrotransposition. The Genome Project revealed that human and mouse chromosomes each contain approximately 2000 functional retrotransposed genes, many of which are expressed in haploid spermatids. Olfactory receptor (OR) genes are intronless genes expressed in somatic cells and sperm. 

It is believed that the original genes were duplicated to establish multiple different OR genes; however, intronless genes code a wide variety of functions in cells, including germ cells. 

Twenty-five genes expressed specifically in spermatogonia are located on the X chromosome, and retrotransposition of genes, mainly from the X chromosome to autosomal chromosomes, and vice versa, occurs frequently. 

For example, two intronless genes on autosomal chromosomes, phosphoglycerate kinase 2 and pyruvate dehydrogenase subunit E2, are thought to be derived from the retrotransposition of ancestral genes on the X chromosome via reverse transcription, which might be a mechanism to avoid X-chromosome inactivation during spermatogenesis. 

However, intronless genes such as phosphoglycerate mutase family member 4 (PGAM4) and NFKB activating protein-like (NKAP-L) are crucial for spermatogenesis and are located on the X chromosome, but the relationship between X-chromosome inactivation escape and the production of intronless genes remains unclear.

Sex chromosomes are thought to play an important role in the production of new genes. As in autosomal chromosomes, mutations that occur due to gene retrotransposition in female germ cells during ontogenesis can be repaired by homologous recombination between the two X chromosomes, and this process can eliminate harmful mutations. In contrast, homologous recombination does not occur within most regions of the Y chromosome due to large differences between the X and Y chromosomes, except within small homologous regions. Genetic changes that occur in germ cells during ontogenesis might be transmitted to future generations without being repaired by meiosis. Therefore, sex chromosomes with retrotransposed intronless genes may have been passed on to the next generation via male germ cells, eventually spreading to various chromosomes. The presence of intronless germ cell-specific genes may be explained as follows. Although DNA mutations, including retrotransposons, occur in various cells during ontogenesis, they must occur in specific germ cells during the early stage of embryonic development to be transmitted to future generations. In particular, retrotransposition occurs only in genes transcribed into mRNA. If a gene expressed in germ cells is retrotransposed to another genomic location in addition to being expressed from its original location, it is more likely that the expressed gene will

| Cells expressing genes                  | Number of genes | Number of intronless genes | Rate of intronless genes | References |
|----------------------------------------|-----------------|----------------------------|--------------------------|------------|
| Germ cell specific                     | 246             | 51                         | 20%                      | 5          |
| Somatic cell specific or non-specific  | ≥25 000         | 1966                       | 8%                       | 4          |

**TABLE 1** Rate of intronless genes in the genes encoding mouse proteins
be functional in germ cells than in somatic cells, as the original gene has a defined function in germ cells (Figure 1). Furthermore, spermatids lose most of their cytoplasm, and the histones of most chromosomes are replaced by protamines in the nucleus to reduce splicing. Therefore, intronless genes may be crucial for gene expression in and regulation of cellular differentiation.

### 5 | FUNCTION OF INTRONLESS GENES

Intronless genes include genes related to various cell properties such as cytoskeleton,21 signal transduction,22 chromatin formation,23 and metabolic enzymes24 in humans.25 In fact, intronless genes encoding spermatid-specific isoforms, which differ from somatic enzymes, are expressed in spermatids during almost all stages of the glycolytic pathway, from glucose to acetyl-CoA production (Figure 2).26 In addition, SCOT-t, an intronless gene encoding an energy-metabolizing enzyme, has been identified.27 Some genetic polymorphisms associated with male infertility have been identified, and an association between genetic polymorphisms in androgen receptors or the human leukocyte antigen system and infertility has been reported.28,29 In a single-nucleotide polymorphism (SNP) analysis of haploid germ cell-specific intronless genes in a Japanese cohort,25 SNPs in SCOT-t30 and PGAM418 were found to occur at significantly higher rates in male infertility patients. Four single-nucleotide polymorphisms were reported in SCOT-t: one in the 3’ noncoding region and three in the coding region causing predicted amino acid substitutions (Table 2).30 Homozygotes for the minor allele of the c.854T/G SNP at aa 285 (L285R) were found significantly more often in infertile patients. The minor allele of the c.75G/C SNP at aa 25 (W25C) in PGAM4 on the X chromosome was also found significantly more often in infertile patients and showed reduced enzymatic activity.18 These results indicate that analysis of haploid sperm cell-specific intronless genes may be useful in understanding infertility.

HASPIN is another characteristic intronless gene of interest.31 HASPIN is predominantly expressed in male germ cells and regulates

![Figure 1](https://example.com/figure1.png)

**Figure 1** Schematic representation of the heredity of intronless genes in male germ cells. Most multicellular organisms are composed of various differentiated tissues (thick arrow). The cells of the body are divided into somatic (thick blue arrow) and germ (thick red arrow) cells. Retrotransposed genes in somatic cells are not inherited. Alternatively, retrotransposed genes in germ cells are passed on to the next generation unless the gene is incompatible with germ cells. A retrotransposed gene is inherited only when it gains suitable expression and function in the next generation of somatic cells. Circles indicate retrotransposed genes that adapted to cells; black circles indicate inactivated genes; cross marks indicate maladapted retrotransposed genes. A transposed gene in a germ cell will not be inherited by the next generation if any abnormality occurs in cell differentiation in the next generation (upper right). A gene is considered a pseudogene if it is inactive in all cells. [Colour figure can be viewed at wileyonlinelibrary.com]

**Table 2** Prevalence of single-nucleotide polymorphisms (SNPs) in SCOT-t in infertile or proven fertile populations

| SNPs type and position | Genotype | Fertile controls | Infertile cases | Fold increase in infertile cases | Statistical significance |
|------------------------|----------|-----------------|----------------|-------------------------------|--------------------------|
| c.113T/C (L38P)        | T/T      | 246 (94)        | 246 (96)       |                               |                          |
|                        | T/C      | 14 (5.4)        | 7 (2.7)        |                               |                          |
|                        | C/C      | 1 (0.4)         | 2 (0.8)        | X2                            | P < .54                  |
| c.854T/G (L285R)       | T/T      | 208 (80)        | 204 (80)       |                               |                          |
|                        | T/G      | 50 (19)         | 39 (15)        |                               |                          |
|                        | G/G      | 3 (1.1)         | 12 (4.7)       | X4                            | P < .018                 |
| c.1055C/G (T352M)      | C/C      | 251 (96)        | 238 (93)       |                               |                          |
|                        | C/T      | 8 (3.1)         | 11 (4.3)       |                               |                          |
|                        | T/T      | 2 (0.8)         | 6 (2.4)        | X3                            | P < .17                  |
| c.1651T/C              | T/T      | 209 (80)        | 205 (80)       |                               |                          |
|                        | T/C      | 49 (19)         | 38 (15)        |                               |                          |
|                        | C/C      | 3 (1.2)         | 12 (4.8)       | X4                            | P < .018                 |

aParentheses indicate amino acid substitution.
results may further elucidate the evolution of the genome and its essential features.

ACKNOWLEDGEMENTS
We thank Dr Yoshitake Nishimune (professor emeritus of the Research Institute for Microbial Diseases, Osaka University), Dr Akihiko Okuyama (former professor of the Department of Urology, Osaka University Graduate School of Medicine), and all our collaborators involved in this germ cell research.

CONFLICT OF INTEREST
The authors confirm that there are no conflicts of interest with the contents of this review.

ORCID
Hiromitsu Tanaka https://orcid.org/0000-0002-0170-5323

REFERENCES
1. Furusawa M. The disparity mutagenesis model predicts rescue of living things from catastrophic errors. Front Genet. 2014;5:421.
2. Tanaka H, Baba T. Gene expression in spermiogenesis. Cell Mol Life Sci. 2005;62:344-354.
3. Tanaka H, Yoshimura Y, Nishina Y, et al. Isolation and characterization of cDNA clones specifically expressed in testicular germ cells. FEBS Lett. 1994;355:4-10.
4. Sakharkar KR, Sakharkar MK, Culiat CT, et al. Functional and evolutionary analyses on expressed intronless genes in the mouse genome. FEBS Lett. 2006;508:1472-1478.
5. Kato Y, Nozaki M. Distinct DNA methylation dynamics of spermatozoa. Cytogenet Genome Res. 2003;103:58-62.
6. Fujii T, Tamura K, Masai K, et al. Use of stepwise subtraction to comprehensively isolate mouse genes whose transcription is upregulated during spermiogenesis. EMBO Rep. 2002;3:367-372.
7. Miyata H, Castaneda JM, Fujihara Y, et al. Genome engineering uncovers S4 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. Proc Natl Acad Sci U S A. 2016;113:7704-7710.
8. Zhao M, Shirley CR, Hayashi S, et al. Transition nuclear proteins are required for normal chromatin condensation and functional sperm development. Genesis. 2004;38:200-213.
9. Barnes IHA, Ibarra-Soria X, Fitzgerald S, et al. Expert curation of the human and mouse olfactory receptor gene repertoires identifies conserved coding regions split across two exons. BMC Genom. 2020;21:196.
10. Flegel C, Vogel F, Hofreuter A, et al. Characterization of the olfactory receptors expressed in human spermatozoa. Front Mol Biosci. 2016;2:73.
11. Wang PJ, McCarron JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. Nat. Genet. 2001;27:422-426.

6 | FUTURE PERSPECTIVES

Analysis of the function of germ cell-specific intronless genes will shed light on the causes of male infertility. Additionally, the primary structures of the genomes of various organisms will be clarified and compared, together with the origins of intronless genes. These
16. Emerson JJ, Kaessmann H, Betran E, Long M. Extensive gene traffic on the mammalian X chromosome. *Science*. 2004;303:537-540.
17. McCarrey JR, Thomas K. Human testis-specific PGK gene lacks introns and possesses characteristics of a processed gene. *Nature*. 1987;326:501-505.
18. Okuda H, Tsujimura A, Irie S, et al. A single nucleotide polymorphism within the novel sex-linked testis-specific retrotransposed PGAM4 gene influences human male fertility. *PLoS One*. 2012;7:e35195.
19. Okuda H, Kiuchi H, Takao T, et al. A novel transcriptional factor Nkapl is a germ cell-specific suppressor of Notch signaling and is indispensable for spermatogenesis. *PLoS One*. 2015;10:e0124293.
20. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature*. 2003;423:825-837.
21. Soda T, Miyagawa Y, Fukuhara S, Tanaka H. Physiological role of actin regulation in male fertility: insight into actin capping proteins in spermatogenic cells. *Reprod Med Biol*. 2020;19:120-127.
22. Tanaka H, Iguchi N, Nakamura Y, et al. Cloning and characterization of human haspin gene encoding haploid germ cell-specific nuclear protein kinase. *Mol Hum Reprod*. 2001;7:211-218.
23. Tanaka H, Matsuoka Y, Onishi M, et al. Expression profiles and single-nucleotide polymorphism analysis of human HANP1/HIT2 encoding a histone H1-like protein. *Int J Androl*. 2006;29:235-239.
24. Tanaka H, Kohroki J, Iguchi N, et al. Cloning and characterization of a human orthologue of testis-specific succinyl CoA:3-oxo acid CoA transferase (Scot-t) cDNA. *Mol Hum Reprod*. 2002;8:16-23.
25. Nishimune Y, Tanaka H. Infertility caused by polymorphisms or mutations in spermatogenesis-specific genes. *J Androl*. 2006;27:326-334.
26. Welch JE, Schatte EC, O’Brien DA, et al. Expression of a glyceraldehyde 3-phosphate dehydrogenase gene specific to mouse spermatogenic cells. *Biol Reprod*. 1992;46:869-878.
27. Koga M, Tanaka H, Yonogida K, et al. Isolation and characterization of a haploid germ cell-specific novel complementary deoxyribonucleic acid; testis-specific homologue of succinyl CoA:3-Oxo acid CoA transferase. *Biol Reprod*. 2000;63:1601-1609.
28. Pan B, Li R, Chen Y, et al. Genetic association between androgen receptor gene CAG repeat length polymorphism and male infertility: a meta-analysis. *Medicine (Baltimore)*. 2016;95(10):e2878.
29. Tsujimura A, Ota M, Katsuyama Y, et al. Susceptibility gene for non-obstructive azoospermia located near HLA-DR and -DQ loci in the HLA class II region. *Hum Genet*. 2002;110:192-197.
30. Tanaka H, Miyagawa Y, Tsujimura A, Nishimune Y. Male infertility and single-nucleotide polymorphisms in the testis-specific succinyl CoA:3-oxoacid CoA transferase (SCOT-T/OXCT2) gene in a Japanese cohort. *Int J Reprod Fertil Sex Health*. 2018;001(51):1-6.
31. Tanaka H, Yoshimura Y, Nozaki M, et al. Identification and characterization of a haploid germ cell-specific nuclear protein kinase (Haspin) in spermatid nuclei and its effects on somatic cells. *J Biol Chem*. 1999;274:17049-17057.
32. Cao Z, Xu T, Tong X, et al. HASPIN kinase mediates histone deacetylase to regulate oocyte meiotic maturation in pigs. *Reproduction*. 2019;157:501-510.
33. Wang F, Dai J, Daum JR, et al. Histone H3 Thr-3 phosphorylation by Haspin positions Aurora B at centromeres in mitosis. *Science*. 2010;330:231-235.
34. Shimada M, Goshima T, Matsu H, et al. Essential role of autoactivation circuitry on Aurora B-mediated H2AX-pS121 in mitosis. *Nat Commun*. 2016;7:12059.
35. Huertas D, Soler M, Moreto J, et al. Antitumor activity of a small-molecule inhibitor of the histone kinase Haspin. *Oncogene*. 2012;31:1408-1418.
36. Tanaka H, Wada M, Park J. HASPIN kinase inhibitor CHR-6494 suppresses intestinal polyp development, cachexia, and hypogonadism in Apcmin/+ mice. *Eur J Cancer Prev*. 2020;29:481-485.
37. Yoshimura Y, Tanaka H, Nozaki M, et al. Nested genomic structure of haploid germ cell specific haspin gene. *Gene*. 2001;267:49-54.

How to cite this article: Tanaka H, Tsujimura A. Pervasiveness of intronless genes expressed in haploid germ cell differentiation. *Reprod Med Biol*. 2021;20:255–259. https://doi.org/10.1002/rmb2.12385