Regulatory divergence of X-linked genes and hybrid male sterility in mice

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Postzygotic reproductive isolation is the reduction of fertility or viability in hybrids between genetically diverged populations. One example of reproductive isolation, hybrid male sterility, may be caused by genetic incompatibility between diverged genetic factors in two distinct populations. Genetic factors involved in hybrid male sterility are disproportionately located on the X chromosome. Recent studies showing the evolutionary divergence in gene regulatory networks or epigenetic effects suggest that the genetic incompatibilities occur at much broader levels than had previously been thought (e.g., incompatibility of protein-protein interactions). The latest studies suggest that evolutionary divergence of transcriptional regulation causes genetic incompatibilities in hybrid animals, and that such incompatibilities preferentially involve X-linked genes. In this review, we focus on recent progress in understanding hybrid sterility in mice, including our studies, and we discuss the evolutionary significance of regulatory divergence for speciation.

Key words: chromosome substitution strain, hybrid male sterility, regulatory divergence, X chromosome

INTRODUCTION

Decreased reproductive fitness in interspecific or subspecific hybrid animals is referred to as hybrid sterility, and is widely observed in natural populations and laboratory crosses. Hybrid sterility partially or completely impedes genetic exchange between evolutionarily diverged populations, and the cessation of gene flow accelerates the genetic divergence between the populations. Thus, hybrid sterility is a major cause of reproductive isolation, and contributes to speciation (Coyne and Orr, 2004). Hybrid sterility may be caused by genetic incompatibilities between evolutionarily diverged interacting genes (or genomic factors), which are known as “Dobzhansky-Muller incompatibilities” (Bateson, 1909; Dobzhansky, 1936; Muller, 1942). Although it was originally assumed that Dobzhansky-Muller incompatibilities occur between polymorphic gene products (e.g., proteins), accumulating data suggest other possibilities, including incompatibilities between trans-acting regulatory factors (e.g., transcription factors) and cis-regulatory elements (e.g., promoters and enhancers) of their target genes (Haerty and Singh, 2006).

Interestingly, regulatory divergence occurs more frequently in male reproductive genes than in other genes, because cis- and trans-regulatory factors coevolve rapidly under the pressure of sexual selection (Haerty and Singh, 2006; Gordon and Ruvinsky, 2012; Llopart, 2012). Hybrid sterility largely follows “Haldane’s rule” whereby the heterozygous sex (XY or ZW) is more severely affected than the homozygous sex (XX or ZZ) (Haldane, 1922). For instance, hybrid sterility in mammals affects males more severely than it does females. Haldane’s rule infers the involvement of the sex chromosomes in hybrid sterility. Indeed, many quantitative trait loci (QTLs) for hybrid male sterility in mice were mapped on the X chromosome (Oka et al., 2004; Storchová et al., 2004; Good et al., 2008; White et al., 2011; Oka and Shiroishi, 2012), and limited gene flow on the X chromosome was observed between adjacent species or subspecies in natural hybrid zones (Tucker et al., 1992; Payseur et al., 2004; Teeter et al., 2008; Macholan et al., 2011; Janoušek et al., 2012). Such a disproportionate effect of X-linked loci on interspecific or inter subspecific hybrid fitness is called as “the large X effect” (Coyne and Orr, 1989). The large X effect is mainly attributed to the hemizygosity of the X chromosome in males, because this state accelerates the accumu-
ulation of male-beneficial recessive mutations on the X chromosome owing to the immediate exposure of the phenotype to natural selection (Rice, 1984). However, conflicting forces exist on the X chromosome. Owing to the different number of X chromosomes between sexes, the X chromosome should have acquired some compensatory functions, such as X-inactivation in female somatic cells and in male meiotic and postmeiotic germ cells, where the underlying molecular mechanisms are quite different between sexes (Lifschytz and Lindsley, 1972; Burgoyne et al., 2009; Sin and Namekawa, 2013). Consequently, male-biased X-linked genes tend to be expressed during the premeiotic phase (Wang et al., 2001; Khil et al., 2004), whereas those expressed during meiotic and postmeiotic phases are driven out onto the autosomes by retrotransposition (Khil et al., 2004, 2005; Sin et al., 2012). The X chromosome also evolved multicopy genes that can escape sex chromosome inactivation in male germ cells (Mueller et al., 2008, 2013; Sin et al., 2012). Thus, the X-linked male-biased genes have evolved by balancing conflicts.

In this review, we first summarize studies of hybrid sterility in mice, most of which address the large X effect. We then address why the X chromosome has a disproportionately large effect on hybrid sterility by showing that, compared with autosomes, the X chromosome has unusual biological characteristics which influence its gene content. We next introduce recent advances in the study of hybrid male sterility, brought about by transcriptome analysis. Finally, we speculate about future prospects in the field.

THE LARGE X EFFECT ON HYBRID STERILITY

Many of the pioneering studies on the genetic basis of hybrid male sterility have been performed on species or subspecies of the fruit fly, Drosophila, in which the role of the X chromosome is prominent (Coyne and Orr, 2004). For example, in a genome-wide introgression analysis of 142 Drosophila mauritiana chromosome segments (mean size: 1.5 Mb) in an otherwise D. sechellia genetic background, 27 of 45 (60%) X-linked introgressions were found to cause hybrid male sterility whereas only 17 of 97 (18%) autosomal introgressions did so (Masly and Presgraves, 2007).

The importance of the X chromosome in the genetic basis of reproductive isolation has also been documented in laboratory crosses of mouse strains. The two house mouse subspecies, Mus musculus domesticus and M. m. musculus, diverged from a common ancestor approximately 0.5 million years ago (Boursot et al., 1996; Suzuki et al., 2004; Chevret et al., 2005; Geraldes et al., 2008; Duvaux et al., 2011). Laboratory crosses between M. m. domesticus and M. m. musculus often yield fertile males but sterile females, following Haldane’s rule (Forejt, 1996). For example, F1 hybrid males generated from a cross of a female of the M. m. musculus-derived inbred strain PWD and a male of the laboratory strains C57BL/10 or C57BL/6j, which are predominantly derived from M. m. domesticus, show sterility in the hybrid genetic background (Forejt and Iványi, 1974; Forejt, 1996). F1 hybrid males from the reciprocal cross do not show sterility, suggesting that the M. m. musculus X chromosome is incompatible with dominant alleles of the M. m. domesticus autosomes (Flachs et al., 2012). Subsequent genetic studies showed that QTLs for male sterility, Hstx2 and Hst1, were located on the X chromosome (between 64.9 and 69.6 Mb) and chromosome 17, respectively (Gregorová et al., 1996; Trachtulec et al., 1997; Bhattacharyya et al., 2013, 2014). Hst1 was mapped in the t-complex that occupies the proximal third of chromosome 17. The t-haplotype is a variant form of the t-complex and accumulates mutations related to embryonic lethality and male sterility (reviewed in Sugimoto, 2014). However, hybrid sterility caused by Hst1 was found to be distinct from the t-sterility (Forejt and Ivanyi, 1974). Hst1 was later identified as the locus of the meiotic PR-domain gene (Prdm9) encoding histone 3 methyltransferase (Mihola et al., 2009). However, the combination of the M. m. musculus allele of Hstx2 and the M. m. domesticus allele of Hst1/Prdm9 could not recapitulate the hybrid male sterility in the genetic background of the C57BL/6 strain, suggesting that M. m. musculus alleles of other interacting genes are involved in the genetic mechanism of sterility in (PWD × C57BL/6) F1 males (Dzur-Gejdosova et al., 2012).

Hybrid male sterility between M. m. domesticus and M. m. musculus was also observed in a M. m. domesticus genetic background (Storchová et al., 2004; Good et al., 2008; Bhattacharyya et al., 2014). Males of a chromosome substitution strain (CSS), B6.PWD-ChrX, which carries a PWD-derived X chromosome in a C57BL/6 genetic background, show sterility and the responsible locus, Hstx1, was mapped to the same region as Hstx2 (Storchová et al., 2004; Bhattacharyya et al., 2014). Interestingly, the PWD allele of Hstx2 in the hybrid genetic background and the PWD allele of Hstx1 in the B6 genetic background exhibit different phenotypes during spermatogenesis. The former state causes aberrant meiotic pairing of heterosubspecific homologous chromosomes and failure of sex chromosome inactivation, resulting in meiotic arrest (Dzur-Gejdosova et al., 2012). On the other hand, the latter state causes defects during the postmeiotic stage and spermigenesis (Storchová et al., 2004). Another study, in which X-linked chromosomal segments from the PWD strain were introgressed into a genetic background of the wild M. m. domesticus-derived LEW/EiJ strain, detected multiple X-linked QTLs for reduced testis weight, decreased sperm count and abnormal sperm head morphology. Highly significant QTLs were accumulated in the proximal half of the X chromosome between markers DXMit26 and DXMit119 (Good et
Hybrid male sterility was also observed in laboratory crosses between *M. m. domesticus* and *M. m. molossinus* (Takagi et al., 1994; Oka et al., 2004). *M. m. molossinus* is descended from the normal hybrid of the two subspecies, primarily *M. m. musculus* and, to lesser degree, *M. m. castaneus* (Yonekawa et al., 1980, 1988; Moriwaki, 1994; Sakai et al., 2005). F1 hybrid males from crosses between C57BL/6 and MSM, which is an inbred strain derived from wild *M. m. molossinus*, are fertile, but subsequent intercrosses cause male sterility (Oka et al., 2004). Similar to B6.PWD-ChrX, male sterility was also observed in the CSS B6-ChrX<sup>MSM</sup>, which carries an MSM-derived X chromosome in a C57BL/6 genetic background (Oka et al., 2004). Another CSS, B6-ChrXT<sup>MSM</sup>, which carries the distal half of the X chromosome (from 86 Mb) in a C57BL/6J genetic background, also showed reduced testis weight but was subfertile. These observations suggest that hybrid male sterility between C57BL/6 and MSM is attributable to the improper epistatic interaction between the MSM-derived X chromosome and B6-derived autosomal recessive alleles. Genetic studies showed that the genes responsible for abnormal sperm head morphology and reduced testis weight, which are major phenotypes of B6-ChrX<sup>MSM</sup> males, were located on distinct X-linked loci at 65 Mb and 147 Mb, respectively (Oka et al., 2004). Subsequent studies demonstrated that an MSM-derived pair of autosomes, chromosomes 1 and 11, restored male fertility in a B6-ChrX<sup>MSM</sup> strain, while MSM-derived chromosome 1 restored testis weight in a B6-ChrXT<sup>MSM</sup> strain (Oka et al., 2007, 2014).

Hybrid male sterility caused by improper epistatic interaction between the X chromosome and autosomal recessive genes was also reported between *M. m. domesticus* and *M. m. castaneus* (White et al., 2012). F1 males from the two reciprocal crosses between the *M. m. domesticus* -derived WSB strain and *M. m. castaneus* showed high levels of fertility, but a large proportion of F2 males displayed severe defects in male reproductive phenotypes. QTLs for testis weight were detected in the pseudautosomal region and on chromosomes 2 and 4 (White et al., 2012). The pseudautosomal region has been previously linked to meiotic arrest and F1 sterility in crosses between *M. spreitus* and C57BL/6J (Guénét et al., 1990; Matsuda et al., 1991, 1992; Hale et al., 1993).

Reduced fitness of wild *M. m. domesticus* and *M. m. musculus* hybrid mice has been observed. *M. m. domesticus* ranges across Western Europe and the Middle East, whereas *M. m. musculus* ranges throughout Eastern Europe and Northern Asia (Guénét and Bonhomme, 2003). In nature, *M. m. domesticus* and *M. m. musculus* form a hybrid zone in the center of Europe that has resulted from secondary contact between the two subspecies, representing the early stage of reproductive isolation in which limited gene flow occurs between populations.

Natural populations from multiple transects of the hybrid zone, in countries such as Denmark, Germany, the Czech Republic and Bulgaria, show a limited gene flow of X-linked genetic markers between the western population of *M. m. domesticus* and the eastern population of *M. m. musculus* (Tucker et al., 1992; Payseur et al., 2004; Teeter et al., 2008; Macholan et al., 2011; Janoušek et al., 2012). This implies that the X chromosome is prominently involved in the establishment of reproductive isolation between the mouse subspecies in nature.

The genomes of classical laboratory strains, including C57BL/6, are predominantly (> 90%) derived from *M. m. domesticus*, and the remainder is mostly of *M. m. molossinus* origin (Yang et al., 2007, 2011; Takada et al., 2013). Although genome blocks originating from *M. m. molossinus* have been introgressed into the autosomes of the classical laboratory strains, the X chromosome rarely contains such introgressed blocks (Yang et al., 2007, 2011; Takada et al., 2013). This may be a consequence of selection against X-linked *M. m. molossinus* alleles in an *M. m. domesticus* genetic background, which might decrease male reproductive ability.

**ACCUMULATION OF MALE REPRODUCTIVE GENES ON THE X CHROMOSOME**

The X chromosome emerged from ancestral autosomes in the common ancestor of placental and marsupial mammals (therians), and dramatically differentiated from autosomes (Potrzebowski et al., 2008). Sexual antagonism that is beneficial for one sex, but deleterious for the other, has accelerated the accumulation of male-biased genes on the X chromosome owing to its hemizygosity in males (Rice, 1984; Charlesworth et al., 1987). On the other hand, inactivation of the X chromosome in male germ cells has influenced the gene content of the X chromosome. The mammalian sex chromosomes are evolutionarily diverged except for one small region of common sequence, the pseudautosomal region, where the heterogeneous sex chromosomes form the synopsis during the first meiotic prophase. Errors in chromosome synapsis are detected by an error-sensing mechanism, known as a meiotic checkpoint, and meiotic cells with asynapsis are eliminated by apoptosis (Burgoyne et al., 2009). Owing to this protection, the sex chromosome evolved a specific heterochromatic domain, called the sex body or XY body, which is transcriptionally inactive during male meiosis; this is termed “meiotic sex chromosome inactivation” (MSCI) (Handel, 2004; Burgoyne et al., 2009). The inactivated state of the sex chromosomes is maintained into the spermatid stage, and is referred to as “postmeiotic sex chromatin” (PMSC) (Namekawa et al., 2006; Sin et al., 2012). Indeed, the majority of X-linked testis-expressed genes (212 of 351) were repressed in pachytene spermatocytes (Sin et al., 2012). Thus, two opposing
One strategy against sex chromosome inactivation is retrotransposition of X-linked genes to autosomes, where they can be expressed in the meiotic and postmeiotic phases (Emerson et al., 2004; Potrzebowski et al., 2008, 2010). When the expression profiles of X-retrotransposed housekeeping genes, 20 in human and 27 in mouse, were investigated, X-retrotransposed genes were found to be highly expressed in meiotic spermatocytes and postmeiotic round spermatids, while their X-linked parental genes showed a low level of expression in these cells, compensating for the lowered transcription of X-linked parental genes during MSCI and PMSC (Sin et al., 2012). Another strategy is amplification of the copy numbers of X-linked genes. Recent studies show that X-linked multicopy genes tend to escape MSCI and/or PMSC, ensuring a sufficient level of expression of reproductive genes, although such multicopy genes are a minority of X-linked genes (Mueller et al., 2008, 2013; Sin et al., 2012).

Interestingly, independent studies show that the evolution of the mammalian X chromosome is bimodal (Zhang et al., 2010; Mueller et al., 2013; Soumillon et al., 2013). Recently emerged genes that are primate- or rodent-specific are preferentially located on the X chromosome, which harbors ~14% of all recently emerged genes (Zhang et al., 2010). Most of the recently emerged X-linked genes have male-biased functions and are multicopy genes that are not affected by PMSC (Zhang et al., 2010). Compared with their non-X-linked counterparts, the recently emerged X-linked genes show a significantly high rate of base and amino acid substitutions (Zhang et al., 2010). Moreover, expression levels of the recently emerged X-linked genes in testis diverge more than those of non-X-linked counterparts in comparisons both between rat and mouse and between human and chimpanzee (Zhang et al., 2010; Sin et al., 2012). Accelerated protein evolution and transcriptional evolution of the recently emerged X-linked genes imply positive selection. A high proportion of older genes that evolved immediately after the divergence of placental mammals (human and mouse) and marsupials (opossum) are also X-linked (~13%) (Zhang et al., 2010). However, in contrast to recently emerged X-linked genes, older X-linked genes mainly consist of single-copy genes and are preferentially expressed in the premeiotic stage, which suggests that they are subjected to sex chromosome inactivation (Khil et al., 2004; Zhang et al., 2010; Mueller et al., 2013). Population genetics theory predicts that the amount of genetic diversity is dependent on the rate of mutation and the effective population size (Ellegren, 2009). Most mutational changes in DNA are thought to occur through replication errors during cell division. Since spermatogenesis in mammals involves more cell divisions than oogenesis, the mutation rate in the male germ line is higher than that in the female germ line (Miyata et al., 1987; Vicoso and Charlesworth, 2006). While autosomes spend an equal time in males and females, the X chromosomes spend only one-third of their time in males and two-thirds of their time in females. Thus, the net mutation rate of the X chromosome should be lower than that of the autosomes (Vicoso and Charlesworth, 2006). However, the combined effect of several different evolutionary forces influences the genetic diversity in the X chromosome (Ellegren, 2009). For example, the X chromosome experiences stronger positive selection than the autosomes, because beneficial recessive mutations will be directly selected in the heterogametic sex when they are X-linked. As a consequence, this increases the rate of adaptive evolution, a situation often referred to as the “faster-X effect” (Charlesworth et al., 1987). The faster-X effect is also observed for microRNAs (miRNAs). In mammalian species, including human and mouse, the density of miRNAs on the X chromosome is two-fold greater than that on autosomes (Guo et al., 2009). X-linked miRNAs are preferentially expressed in testes and show a significantly high substitution rate compared with autosomal miRNAs (Zhang et al., 2007; Guo et al., 2009). Male reproductive genes often undergo rapid evolution, which is presumably driven by sexual selection that acts on traits related directly to mating or reproductive success (Swanson and Vaquero, 2002; Torgerson and Singh, 2003, 2006; Wang and Zhang, 2004; Dean et al., 2009). Thus, the two forces, the faster-X effect and sexual selection, reinforce the rapid evolution of male reproductive genes on the X chromosome (Torgerson and Singh, 2003, 2006).
regulatory element that alters a binding motif for a transcrip-
tion factor can influence the transcriptional activity of a single gene or, in some cases, a few neighboring
genes, whereas a mutation in a trans-regulatory factor that alters its structure influences the transcription of many target genes. Molecular studies have demonstrated that genetic variation of enhancers and promoters impacts on the binding affinity of transcription factors, which in turn fail to recruit other trans-regulatory factors or RNA polymerase II (PolII) and down-regulate gene expression (Kasowski et al., 2010; Pickrell et al., 2010; Kwasnieski et al., 2012; Reddy et al., 2012; Heinz et al., 2013). For example, a recent study investigated the binding of two major macrophage transcription factors, PU.1 and C/EBPα, in macrophages of two mouse strains using chromatin immunoprecipitation followed by massively parallel sequencing (ChIP-seq) (Heinz et al., 2013). The study showed that less than 1% of sites exhibited highly significant strain-specific binding of PU.1 or C/EBPα. These sites were correlated with differential expression of the nearest gene and were associated with strain-specific histone modifications such as H3K4me2 and H3K27ac, which respectively mark primed and active enhancer states. Another study investigated the binding of PolII and a transcription factor, nuclear factor xB (NFκB), in several human lymphoblast cell lines using ChIP-seq. Twenty-five percent of PolII binding sites and 7.5% of NFκB binding sites were found to be different between individuals, and these differences were often correlated with differences in gene expression (Kasowski et al., 2010). To understand the regulatory divergence between subspecies/species, many studies in mouse and Drosophila have attempted to deconstruct cis- and trans-regulatory components by comparing the allele-specific expression in F1 hybrids to expression in their parental subspecies/species (Wittkopp et al., 2004; McManus et al., 2010; Takahasi et al., 2011; Goncalves et al., 2012; Meiklejohn et al., 2014; Oka et al., 2014). The expression of genes is regulated by direct interaction of trans-acting regulatory factors with cis-regulatory elements. Polymorphic cis- and/or trans-regulatory components can interact properly in the nuclear environment of parental strains, which often results in differential expression between the parental strains (Fig. 1A). In the nuclear environment of F1 hybrids, several patterns of expression level should be observed. Differential expression of two alleles in F1 hybrids indicates functional differentiation in the cis-regulatory elements, implying polymorphism in sequences of the cis-regulatory elements (Fig. 1B). If trans-regulation diverges between parental subspecies/species, two alleles in F1 hybrids will be equally expressed at a level in the range of their parental expression under the assumption that trans-regulatory factors from both strains should affect both alleles equally in the same nuclear environment (Fig. 1B). If genetic divergence occurs both in cis and trans, expression of the two alleles will be different from the above patterns. Based on these criteria, a study showed that 43% and 2% of differentially liver-expressed non-imprinted genes (30% of genes) between C57BL/6 and CAST/Ei (M. m. castaneus) were attributed to cis-variants and trans-variants, respectively, whereas 55% could be attributed to regulatory divergence in both cis and trans (Goncalves et al., 2012). Most of the changes in expression induced by cis- and trans-variants were in opposite directions, implying that compensatory regulation of cis-variants to stabilize gene expression levels commonly occurred, following regulatory changes in trans-acting factors (Goncalves et al., 2012).

However, the resolution to detect effects of trans-regulatory variants in F1 hybrids is sometimes limited, because trans-regulatory factors from both parental strains exist in the same nuclear environment, where variant trans-regulatory factors from one parental strain may affect the cis-regulatory elements from the other parental strain quantitatively (e.g., dose insufficiency) and/or qualitatively (e.g., low binding affinity). This limited resolution is improved in transcriptome analysis with CSSs. CSSs are strains that have a single pair of chromosomes from a different strain in the genetic background of a host strain. In comparing the expression between CSSs and the host strain, the regulatory effects of trans-variants can be ignored, because all trans-regulatory factors except those encoded by the substituted chromosome are derived from the host strain. This simplifies the decomposition of cis- and trans-regulatory components: all differential expression between CSSs and the host strain is attributed to cis-variants or trans-variants (Fig. 1C).

A recent microarray study reported that the X-chromosomal CSSs B6-ChrXMSM and B6-ChrXTMSM, which have MSM strain-derived whole or partial X chromosomes, respectively, in a host C57BL/6 genetic background, displayed differential expression in 20% of X-linked genes in prepubertal testes, as compared to the C57BL/6 strain (15% down-regulated and 5% up-regulated) (Oka et al., 2014). The up-regulation of X-linked genes was mainly attributable to MSM-derived cis-variants. In contrast, the down-regulation of X-linked genes was attributable to both MSM-derived cis-variants and cis-trans incompatibilities at similar frequencies. The cis-trans incompatibility occurred disproportionately on the X chromosome when compared with the autosomes (Oka and Shiroishi, unpublished). Interestingly, X-linked genes misregulated by cis-trans incompatibilities were preferentially expressed in spermatogonial stem cells, suggesting that male germline-biased X-linked genes are likely to cause cis-trans incompatibilities (Oka et al., 2014). cis-trans co-evolution frequently occurs in male-biased genes, and
Fig. 1. Illustration of the concept of cis- and trans-variants for gene expression regulation. (A) Cis-trans regulation in parental strains. Colors of the background indicate genetic backgrounds; blue and orange are strain A and B, respectively. Transcription of genes (rectangles) is regulated by cis-elements and trans-acting regulatory factors in each parental strain. (B) Regulation by cis-variants (left panel) and trans-variants (right panel) in F1 hybrids. For cis-variants, the expression levels of each parental allele depend on polymorphism of the corresponding cis-elements in the F1 hybrid (left graph). For trans-variants, if trans-acting regulatory factors from both parental strains act equally on monomorphic cis-elements, the same expression levels of both alleles are observed (right graph). (C) Regulation by cis-variants (left panel) and cis-trans incompatibilities (right panel) in a CSS. Strain A is a host strain and strain B is a donor strain. As for cis-variants, alleles from strain B in the CSS are expressed depending on polymorphism of strain B-derived cis-elements (left graph). In the situation of cis-trans incompatibilities, trans-acting regulatory elements from strain A are incompatible with cis-elements from strain B, resulting in down-regulation of the donor genes in the CSS (right graph).
may contribute to the breakdown of gene expression regulation in hybrid males (Landry et al., 2005; Haerty and Singh, 2006; McManus et al., 2010; Gordon and Ruvinsky, 2012). Thus, regulatory divergence caused by cis-trans incompatibilities could generally underlie the male hybrid sterility phenotypes (Landry et al., 2005; Haerty and Singh, 2006; Oka et al., 2014). In B6-ChrXMSM and B6-ChrXMSM testes, the differential expression of X-linked genes resulted in perturbation of global transcriptional regulation on autosomes by cascading deleterious effects (Oka et al., 2014). Of note, a previously reported QTL regulation on autosomes by cascading deleterious effects genes resulted in perturbation of global transcriptional regulation in hybrid males (Landry et al., 2005; Haerty and Singh, 2006; Oka et al., 2014). In B6-ChrXMSM and B6-ChrXMSM testes, the differential expression of X-linked genes resulted in perturbation of global transcriptional regulation on autosomes by cascading deleterious effects genes resulted in perturbation of global transcriptional regulation in hybrid males (Landry et al., 2005; Haerty and Singh, 2006; Oka et al., 2014). Of note, a previously reported QTL regulation on autosomes by cascading deleterious effects genes resulted in perturbation of global transcriptional regulation in hybrid males (Landry et al., 2005; Haerty and Singh, 2006; Oka et al., 2014).

FUTURE PERSPECTIVES

Genetic studies have detected many QTLs for hybrid male sterility between mouse subspecies/species. However, to date, no responsible X-linked genes have been identified in mice. Although X-linked QTLs for hybrid sterility showed high statistical significance, the severity of male reproductive phenotypes diminishes as the chromosomal regions containing the QTLs become narrow in the construction of congenic strains (Oka and Shiroishi, 2012). One possible explanation for this is the involvement of multiple X-linked genes responsible for hybrid male sterility. Another explanation is extensive misregulation on the X chromosome due to incompatibilities between cis- and trans-regulatory components. The larger the introgressed X-chromosomal segments are, or the larger the number of introduced genes from another subspecies is, the greater the impact on the transcriptional regulation of congenic males. Interestingly, X-linked genes seem to be more frequently misregulated than autosomal genes in spermatogonial stem cells, possibly as a consequence of rapid regulatory divergence between subspecies. Genes expressed in spermatogonial stem cells are not likely to directly contribute to reproductive ability. Therefore, it is unclear why cis-trans regulation for X-linked early germline genes shows such rapid evolution.

Recent genomic studies have focused on genetic variation of cis-regulatory elements and associated binding of trans-regulatory factors, histone modifications, and gene expression by means of genetic variation between mouse strains or human individuals using high-throughput sequencing. Future investigations will reveal the extent to which regulatory divergence contributes to divergence in gene expression between subspecies/species and to speciation.

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