Y Chromosome Evolution and Functional Specialization

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The Y chromosome is one of the sex chromosomes found in males of animals of different taxa, including insects and mammals. Among all chromosomes, the Y chromosome is characterized by a unique chromatin landscape undergoing dynamic evolutionary change. Being entirely heterochromatic, the Y chromosome as a rule preserves few functional genes, but is enriched in tandem repeats and transposons. Due to difficulties in the assembly of the highly repetitive Y chromosome sequence, deep analyses of Y chromosome evolution, structure, and functions are limited to a few species, one of them being Drosophila melanogaster. Here researchers survey comparative evolutionary history of the fly and human Y chromosomes, and functions of Y-linked piRNA clusters ensuring sex-specific piRNA silencing.

Drosophila | Y chromosome | piRNA pathway | rDNA | intron gigantism | azoospermia | transposable elements

1. Introduction

Compared to the autosomes and the X chromosome, the Y represents a unique chromatin landscape undergoing dynamic evolutionary change. The Y chromosomes of different animals almost entirely consist of heterochromatin and, as a rule, contain few functional genes, but are enriched in simple repeats and transposons. Deep analysis of Y chromosomes is restricted to a few species, one of them being *Drosophila melanogaster*. The Y chromosome of *D. melanogaster* comprising about 40 Mb of heterochromatic DNA contains about 14 protein-coding genes (**Figure 1**) mainly acquired from the autosomes, whereas 80% of its sequence is represented by tandem repeats [1][2][3][4]. Many functional Y-linked genes are found to be duplicated and most of these duplicated copies are pseudogenes not supported by natural selection. Satellites, or simple tandem repeats, presumably constitute about 65% of the entire Y chromosome, and Long Terminal Repeats (LTR) and Long Interspersed Nuclear Element (LINE) transposable elements comprise 18% and 7% of the total Y sequence, respectively. The Y chromosome has a 1.4–1.8-fold enrichment in retrotransposon content compared to 10% of LTRs and 5% of LINEs in the rest of the genome [4]. Whereas Y chromosomes exhibit high structural divergence between even closely related species [5], shared developmental trajectories provide convergent evolution of Y chromosomes across different organisms.

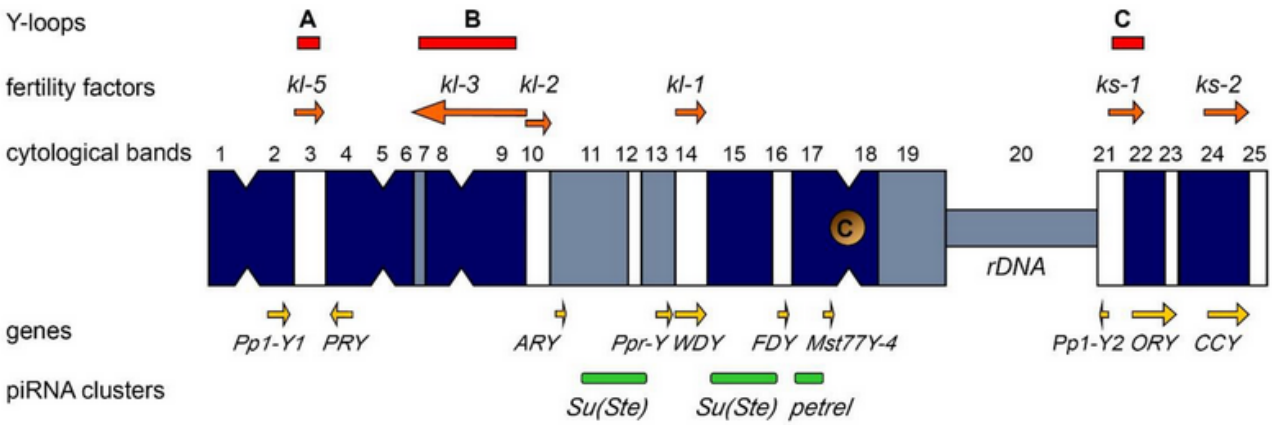

Figure 1. The scheme of the Y chromosome of *D. melanogaster*. Cytological bands are shown in accordance with Hoechst 3325B banding, white bands represent no fluorescence. From top to bottom: cytological location of fertility factor genes (orange arrows) and corresponding Y-loops (red bars); Y chromosome scheme with cytological bands indicated; cytological location of the centromere (C), *rDNA* loci, and several genes (yellow arrows); cytological location of largest piRNA clusters (green bars). The scheme is modified from [6] according to data from [2][4][7].

2. Comparative Evolutionary History of the Fly and Human Y Chromosomes

2.1. Y Chromosome Differentiation and Functions in Flies

The Y chromosome is a sex chromosome found in males of different groups of animals, including mammals and Diptera. Whereas in mammals the development of an organism according to the male type is determined by the presence of a functional Y chromosome, in *Drosophila*, sex is determined by the ratio of the number of X chromosomes to the number of the autosomes: normally, the presence of two X chromosomes triggers development according to the female type, and one—according to the male type [8][9]. Thus, individuals with the XXY genotype are female in flies and male in mammals, while X0 are female in mammals and male in flies. In *Drosophila* with the X0 karyotype, there are no severe structural or functional body disorders, except for male sterility [6]. Fly Y chromosome is not involved in sex determination. In Diptera, the Y chromosomes arose from the autosomes repeatedly (**Figure 2**a), which provides good material for studying parallel processes of convergent evolutionary development [10][11][12][13]. Relatively young, newly formed Y chromosomes of flies, being formed from tens of thousands of years to a few million years ago, maintain the structure and genes of the ancestral autosome, while most of the genes on the old fly Y chromosomes have been acquired subsequently due to a transfer from the autosomes or the X chromosome. Old Y chromosomes such as in *D. melanogaster* that presumably have been persisted for long time (several decades of millions of years) are often highly heterochromatic, contain a large amount of repetitive DNA, and their genes undergo degeneration [10][12].

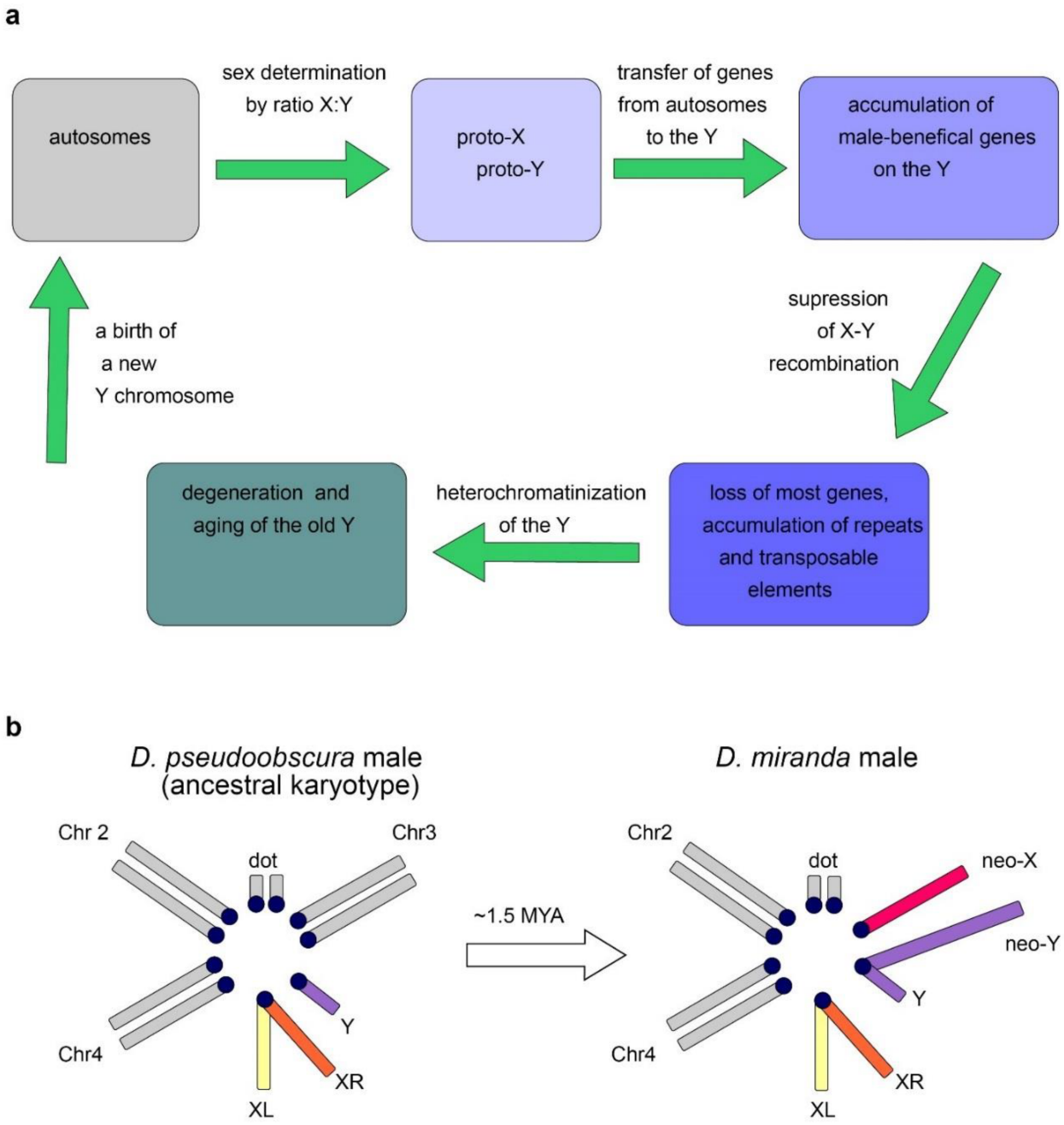

Figure 2. (**a**) Putative Y chromosome life cycle in genus *Drosophila*. It starts with the origin of proto-Y from the autosome, then goes throughout accumulation of genes necessary for spermatogenesis and male fitness, cessation of the X-Y recombination, degeneration of the bulk of acquired genes, heterochromatinization, aging, and arising from autosomes repeatedly. (**b**) The scheme of the karyotype of *D. miranda* males and its close relative species *D. pseudoobscura*, from which *D. miranda* diverged about 2 MYA. In *D. miranda*, the fusion of chromosome 3 with the ancestral Y chromosome created the neo-Y chromosome about 1.5 MYA. XL and XR indicate the left and right arm of the ancestral X chromosome. The scheme is modified from [14].

Drosophila miranda contains a pair of young neo-sex chromosomes that were born ~1.5 million years ago (MYA) after splitting from the closely related species *Drosophila pseudoobscura*. Its neo-sex chromosomes have been created by the fusion of the former autosome 3 with the ancestral, degenerated Y chromosome of this clade

(**Figure 2**b) [14]. Initial stages of Y evolution are characterized by massive amplification of distinct classes of genes. The neo-Y chromosome of *D. miranda* initially contained about 3000 protein-coding genes, but during its evolution, it has acquired over 3200 genes, primarily by tandem amplification of protein-coding genes that were ancestrally present on the chromosome. Testis-specific and dosage-sensitive genes appear to have amplified and have been fixed on the neo-Y to facilitate male fitness. The neo-X and neo-Y chromosomes in *D. miranda* still maintain a high homology across their length, with up to ~98% sequence identity in homologous regions [14][15]. It has been suggested that newly emerged sex chromosomes are a battleground for meiotic drive and X-Y inter-chromosomal conflicts [14][16]. In some *Drosophila* species, such as *D. pseudoobscura*, the old Y chromosome has become part of the autosome, while the neo-Y has been presumably formed de novo several MYA (**Figure 2**a) [12][17]. At the same time, the gene content of the Y chromosomes in *D. pseudoobscura* and *D. melanogaster* is completely different, except that both species maintain various Y-linked genes necessary for male fertility. The same pattern can be seen upon comparison of other Diptera species: despite the different evolutionary history, the functions of most Y-chromosomal genes are related to the male reproductive system [5][12][18].

2.2. Origin of the Y Chromosome in Mammals and Sex Determination

In many animals, sex is determined by a pair of heteromorphic X and Y chromosomes. According to modern concepts, sex chromosomes originate from an ancestral pair of autosomes, one of which acquires a sex-specific gene, which starts the process of differentiation of the sex chromosomes. In mammals, this event occurred only once in the common ancestor of marsupials and placentals prior to their splitting, about 160–180 MYA [19][20][21][22]. The proto-Y chromosome of all mammals (from kangaroo to human) arose from a single autosome in which one of the alleles of the *SOX3* gene, as a result of a mutation, became the sex-determining gene *SRY* [23][24]. However, the product of the sex determination gene only provides a switch, triggering a certain pathway of development. Unique evolutionary forces facilitated the selection and accumulation of male-beneficial mutations around the *SRY* locus, and the linkage between them was supported by selective pressure to avoid crossing over between the proto-Y and proto-X [25]. As a rule, if the dominant allele causes the development of a male, then the chromosome in which it is located becomes the Y chromosome (and its homolog is called the X). In birds, males are the homogametic sex (ZZ) and females are the heterogametic (ZW) [21][26].

2.3. Evolutionary Factors and Forces Determining the Structure and Functional Specialization of the Y Chromosome

The loss of recombination leads to the inefficiency of natural selection and causes the ensuing accumulation of Y-linked loss-of-function mutations, chromosome-wide gene decay, and amplification of repetitive DNAs [27][28][29][30]. In parallel to the loss of genes, Y chromosomes have accumulated large amounts of DNA repeats, and the *D. melanogaster* old Y chromosome mainly consists of heterochromatin (**Figure 2**a) [4][21]. Despite the human Y chromosome having undergone a rapid decay early in evolution, its massive degeneration then dramatically stopped. Genes that remained intact currently show remarkable stability, and no human Y-linked genes have been lost during the last 44 million years [22][31]. The maintenance of human Y-linked genes is mainly associated with two functional categories: genes essential for male reproductive functions and dosage-sensitive ubiquitous

housekeepers [32]. Studies of males with Y deletions have allowed researchers to identify three ‘azoospermia factor’ (*AZF*) regions, *AZFa*, *AZFb*, and *AZFc*, and partially map within them the genes essential for spermatogenesis [33]. The *AZFa* deletions affecting the *DBY* gene cause the most severe azoospermia phenotype, exhibiting a complete loss of testis germline cells accompanied by the maintenance of somatic Sertoli cells (the so-called Sertoli Cell-Only Syndrome; SCOS) [34][35][36].

As in fruit flies, mammalian Y chromosomes also exhibit gene amplification, with the amplicon structures predominantly containing genes with testis-specific functions [37][38][39][40]. Due to the presence of repeating structures, local intra-chromosomal gene conversion is possible, as well as intra- and inter-chromatid exchange. These mechanisms partially compensate for the lack of recombination with the X chromosome by eliminating harmful mutations. At the same time, inter-chromatid recombination can in some cases lead to the formation of isodicentric chromosomes formed by homologous crossing over between opposing arms of palindromes on sister chromatids [41].

The loss of the ability to recombine plays a key role in establishing the structure of the Y chromosome [30][42]. Mutations that prevent recombination between proto-X and proto-Y, such as inversions, deletions, or accumulation of repeats, are supported by selection. Reducing the ability of recombination with the homologous X chromosome dramatically accelerated the evolution of the Y chromosome preventing the elimination of emerging mutations via crossing over, while the X chromosome has retained the ability to cross over in the homogametic sex. This led to the degeneration of most of the original Y-chromosomal genes, and multiple deletions caused a significant size decrease with a relative increase in the proportion of non-coding heterochromatic regions. The rapid evolutionary degeneration of the Y chromosomes, typical in a wide range of species, leads to the hypothesis that in the future the human Y chromosome may disappear altogether. This hypothesis is based not only on extrapolation, but is also indirectly supported by precedents in the evolution of some species including multiple fishes, reptiles, grasshoppers, cockroaches, and dragonflies [43][44][45]. However, other researchers claim that human Y degeneration stopped millions of years ago and currently nothing threatens Y chromosome survival [46].

2.4. Dosage Compensation System Contributes to Y-Linked Gene Maintenance

As a rule, a single gene copy appears to be enough to provide development and life-cycle maintenance of diploid animals; however, a small cohort of genes exhibits a high sensitivity in case of decreased gene dosage. This phenomenon is known as haploinsufficiency, and it is associated with many developmental disorders in human [47][48][49]. In male flies, the genes of the only X chromosome are overactivated in somatic tissues, eliminating the problem of haploinsufficiency and potentially lethal imbalance between the X and autosome transcriptional level in the two sexes. In contrast, in female mammals, inactivation of one of the two X chromosomes occurs. However, according to various estimates and in distinct types of human cells, 20–30% of genes of inactive X chromosome escape the inactivation [50][51]. In mammals, haploinsufficient Y-chromosomal genes have X-chromosomal homologues that avoid inactivation during dosage compensation in females, which indicates the need for their expression on both sex chromosomes to ensure normal functions in the body. Thus, in males, these dosage-sensitive genes cannot disappear from the Y chromosome without negative consequences, and they can survive

under selective pressure [31][32][50][52]. Strict dosage requirements for sex-linked genes are demonstrated in the case of Turner syndrome (exhibiting X0 karyotype or mosaicism) and Klinefelter syndrome (XXY), since such genes have been haploinsufficient or overexpressed, respectively, in these karyotypes [51]. Turner syndrome is a genetic condition caused by complete or partial loss of the second sex chromosome in human [53][54]. Studies of manifestations of this syndrome indicate that the functions of the Y chromosome consist not only of ensuring the normal functioning of the male reproductive system. Due to the absence of the *SRY* gene, which is the key to triggering male-type development, patients with this syndrome are exclusively female, with multiple body disorders and cognitive impairment [54]. Individuals with Klinefelter syndrome are infertile as a result of excess gene dosage of X escape genes, and abnormal meiotic pairing of the sex chromosomes. An atypical number of X or Y chromosomes (XXY, XXX, or X) contributes to spatial chromosome conformation changes and leads to disruption of DNA methylation patterns of autosomal genes, causing distinct disease phenotypes: mental illness, cancer, and disrupted fertility [51].

2.5. Convergent Nature of the Evolution of Y Chromosomes

Despite their independent evolutionary origins in different species, Y chromosomes in species with heterogametic males have a number of similar features: they are usually smaller than X chromosomes, contain significantly fewer genes, most of which are related to the male reproductive system, and also have a relatively large number of repeats and significant areas occupied by heterochromatin. It has been proposed that such convergent evolution is due to the similar nature of the selection pressure. Another common feature—the acquisition of repetitive sequences and the loss of most of the original genes—is associated with accelerated Y evolution due to the loss of recombination with the X chromosome [27]. The difference between the evolution of the Y chromosome in mammals and Diptera is mainly that in Diptera the acquisition of new genes often significantly prevails over the loss of the original ones; although, both processes take place in both groups. Presumably due to slower changes in mammals, the evolutionary processes have not yet reached the point where the Y chromosome has lost all homology with the X chromosome.

3. Current Undestanding of *Drosophila* Y Chromosome Contribution in piRNA Biogenesis and Functioning of piRNA-Clusters

3.1. Brief Description of the piRNA System

The piRNA pathway provides both innate and adaptive immune system defense against the activity of transposable elements (TEs) leading to the protection of genome integrity in germinal tissues. It also participates in the maintenance of germline stem cells, regulation of protein-coding gene expression, the establishment of embryonic patterning (in Diptera), and transgenerational epigenetic inheritance [55][56][57]. Small non-coding piRNAs 23-35 nt in length associated with proteins of the PIWI subfamily are present in animals from fungi to humans [58][59][60]. piRNAs are generated from piRNA clusters, which are long precursors that are transcribed from heterochromatic

regions containing fragments of transposons. piRNA precursors are processed to generate small piRNAs in perinuclear nuage granules (**Figure 3**) [61][62][63][64].

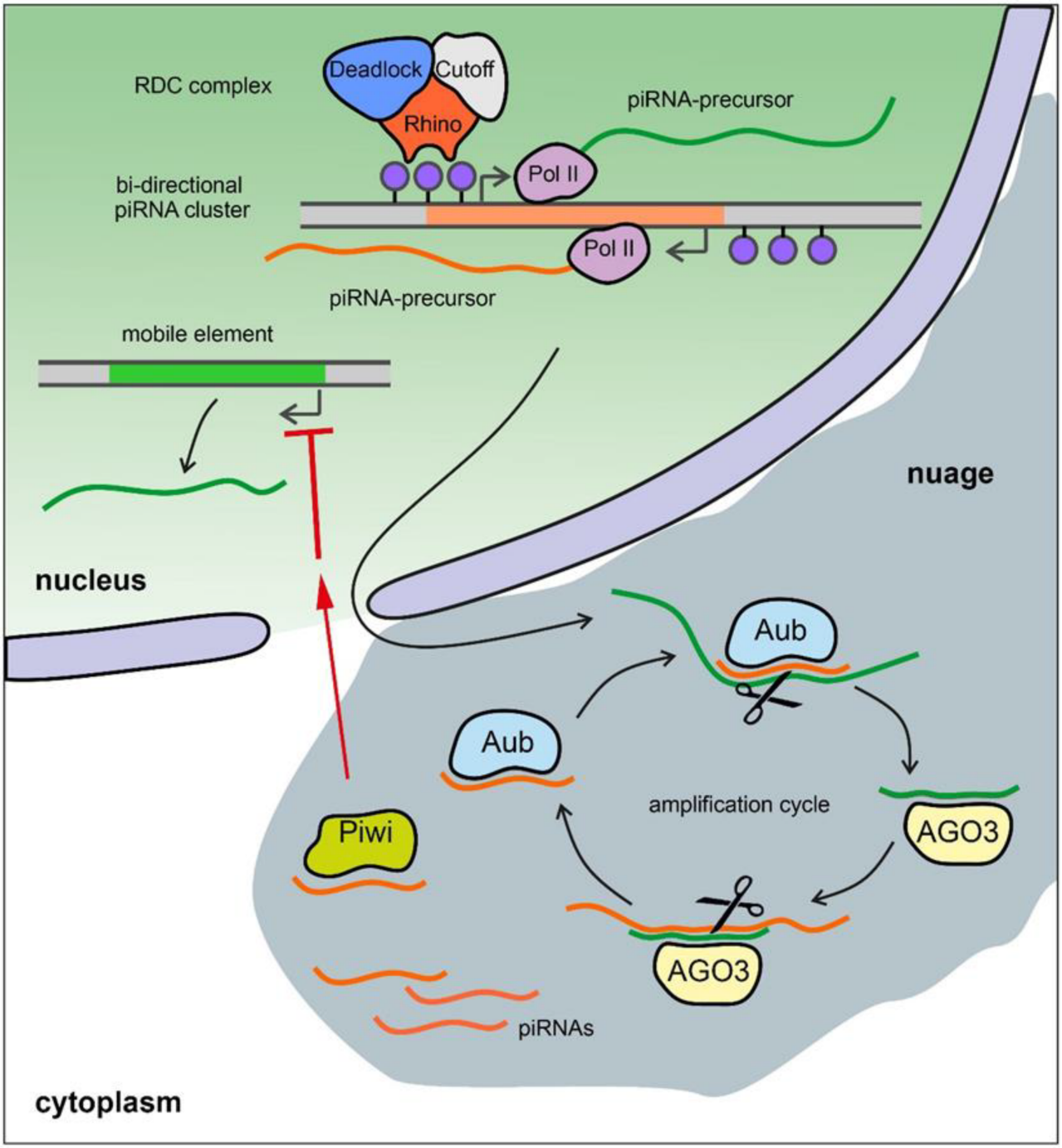

Figure 3. piRNA biogenesis in *Drosophila* germ cells. Bi-directional piRNA clusters are recognized by the RDC complex with the aid of histone modification H3K9me3 (blue dots) and are transcribed by Pol II machinery with the production of long unspliced transcripts of piRNA precursors. They are exported from the nucleus in the perinuclear nuage granules and are presumably cleaved by endonuclease Zucchini forming the 5′-end of the future piRNA (not shown). The cleaved transcripts are loaded into PIWI clade protein Aubergine (Aub) and then trimmed from the 3′-end by an unknown trimmer nuclease (not shown). Aub loaded with guide antisense piRNA recognizes and cleaves

the complementary sense transcript producing the 5′-end of a new sense piRNA. The new piRNA is loaded into PIWI clade protein AGO3 and, in turn, performs cleavage of the complementary antisense transcript. This step generates a new antisense piRNA that is identical or very similar to the initiating piRNA (ping-pong amplification cycle). Piwi proteins loaded by antisense piRNAs translocate into the nucleus where they suppress transcription of TEs with complementary sequences by a co-transcriptional repression mechanism.

3.2. The Y Chromosome as a Major piRNA-Producing Genomic Region in the Fly Testes

The piRNA system in *D. melanogaster* exhibits a strong sexual dimorphism. TE-mapping piRNAs are known as the most abundant class of piRNAs in the ovaries, whereas only about 40% of piRNAs map to TEs in the testes, and the largest cohort of piRNAs map to protein-coding genes [7][65]. In the testes of *Drosophila*, almost half of all piRNAs originate from the piRNA clusters located on the Y chromosome (**Figure 1**) [7]. The largest number of piRNAs is generated from the Y-linked *Suppressor of Stellate* (*Su(Ste)*) repeats directed to silencing of the homologous tandem *Stellate* genes residing on the X chromosome [65][66][67]. The number of *Su(Ste)* repeats comprises more than 500 tandemly ordered copies residing in two cytolocations on the Y (**Figure 1**) [2][4][7]. The insertion of the defective transposon *hoppel* into the promoter is responsible for the initiation of antisense transcription of *Su(Ste)* repeats and their acquisition of piRNA cluster functions [66]. *Stellate* derepression in the case of deletion of most of *Su(Ste)* repeats or disruption of the piRNA system leads to the accumulation of needle-like protein aggregates in spermatocytes, disturbances of meiosis, and, as a result, a decrease in male fertility [66][68]. The *Stellate/Su(Ste)* system is species- and sex-specific for *D. melanogaster*. It was is shown that *Stellate* genes participate in male hybrid sterility of F1 progeny of crosses between *D. melanogaster* females and males of closely related *D. mauritiana*. The hybrid males possess maternal X-linked *Stellate* genes, but their paternal Y chromosome does not contain *Su(Ste)* repeats and the corresponding piRNAs are not generated. Derepression of *Stellates* in the testes of hybrid males leads to a meiotic catastrophe and complete sterility [65][68]. The Y chromosome of *D. melanogaster* also contains the *petrel* locus (**Figure 1**), which is a source of multiple piRNAs highly complementary to *pirate/CG12717 gene,* providing strong testis-specific silencing of this gene [7]. However, the functional significance of the repression of *pirate,*encoding a SUMO-isopeptidase*,* in the testes remains unclear to date. It appears that both in the cases of the *Stellate/Su(Ste)* and *pirate/petrel* pairs, their current evolutionary relationships are initially based on parallel acquisition or co-amplification of homologous genes on the sex chromosomes. On the whole, the mechanism of determination of genomic regions as piRNA clusters is poorly resolved.

3.3. The Y Chromosome in Other Species as a Source of piRNAs

The suppression of genes harmful for spermatogenesis appears to be one of the main functions of piRNAs originating from the Y chromosome of *D. melanogaster*. In mouse testes, novel polyadenylated non-coding RNAs called *Pirmy* and *Pirmy*-like transcribed from the long arm of the Y chromosome have recently been discovered [69]. Morphology- and sperm motility-related abnormalities have been found in two strains of Y-deleted mice with disrupted expression of *Pirmy* and *Pirmy*-like RNAs. The *Pirmy* and *Pirmy-like* RNAs serve as sources of piRNAs

that are complementary to 5′- and 3′-UTRs of several autosomal genes, that presumably contribute to fertility and sex ratio maintenance in the progeny. The proteins expressed from these autosomal genes are up-regulated in the sperm of Y-deleted mice and appear to be responsible for the disruption of sperm morphology and motility [69].

In *Bombyx mori*, females are the heterogametic sex (ZW), and the W chromosome is heterochromatinized and consists almost entirely of transposon sequences. piRNA from the *Fem* locus on the W chromosome functions as a suppressor of the *Masc* gene, which regulates sex-specific splicing of the *doublesex (dsx)* gene, which is necessary for sex determination in many insects [70]. Thus, small piRNAs from the Y or W chromosome can potentially be involved in sex determination, the resolution of intragenomic conflicts, reproductive isolation, and the regulation of gene expression for ensuring spermatogenesis [7][16][65][69][70][71].

Y-linked piRNA clusters and their functions in humans remain poorly understood [72][73]. High-throughput sequencing of piRNAs from three human adult testis samples and subsequent data analysis have revealed 28 putative piRNA-cluster candidate regions on the Y [74]. However, among them, only one uni-directional cluster contains a significant number of mapped piRNAs (45.4 rpkm). This locus includes remnants of SINE, LINE, and LTR TEs, and has a highly homologous region of the same size on the X chromosome. Due to the high level of heterochromatinization and a large number of repetitive elements, the human Y chromosome is not perfectly assembled, and data about piRNA clusters are not complete.

4. Conclusions and Perspectives

In most heterosexual eukaryotes Y chromosomes are maintained and perform various essential functions. These include sex determination, ensuring male fertility; correct segregation of meiotic chromosomes; epigenetic regulation of harmful elements; contribution to interspecies hybrid sterility; and other responsibilities. Studies of model organisms, *Drosophila* and mice, have fundamental significance for uncovering the shared properties of Y chromosomes of multiple species. The convergent nature of evolution of the Y chromosome allows researchers to consider that the data obtained in model organisms can be useful to a certain extent for the prediction of the human Y chromosome behavior in the future, as well as in understanding how the specific structure of this chromosome reflects its functions in normal and pathological conditions.

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