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Review

Paramutation phenomena in non-vertebrate animals

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ABSTRACT

Paramutation was initially described in maize and was defined as an epigenetic interaction between two alleles of a locus, through which one allele induces a heritable modification of the other allele without modifying the DNA sequence [1,2]. Thus it implies that the paramutated allele conserves its new properties on the long term over generations even in the absence of the paramutagenic allele and that it turns paramutagenic itself, without undergoing any changes in the DNA sequence. Some epigenetic interactions have been described in two non-vertebrate animal models, which appear to exhibit similar properties. Both systems are linked to *trans*-generational transmission of non-coding small RNAs. In *Drosophila melanogaster*, paramutation is correlated with transmission of PIWI-Interacting RNAs (piRNAs), a class of small non-coding RNAs that repress mobile DNA in the germline. A tandem repeated transgenic locus producing abundant ovarian piRNAs can activate piRNA production and associated homology-dependent silencing at a locus that was previously stably devoid of such capacities. The newly converted locus is then perfectly stable in absence of the inducer locus (>100 generations) and becomes fully paramutagenic. In *Caenorhabditis elegans*, paramutation is correlated with transmission of siRNAs, which are produced by transgenes targeted by piRNAs in the germline. Indeed, a transgenic locus, targeted by the piRNA machinery, produces siRNAs that can induce silencing of homologous transgenes, which can be further transmitted in a repressed state over generations despite the absence of the inducer transgenic locus. As in fly, the paramutated locus can become fully paramutagenic, and paramutation can be mediated by cytoplasmic inheritance without transmission of the paramutagenic locus itself. Nevertheless, in contrast to flies where the induction is only maternally inherited, both parents can transmit it in worms. In addition, a reciprocal phenomenon – (from *off* toward *on*) – appears to be also possible in worms as some activated transgenes can reactivate silent transgenes in the germline, and this modification can also be transmitted to next generations, even so it appears to be only partially stable. Thus, in a given system, opposite paramutation-like phenomena could exist, mediated by antagonist active pathways. As in plants, paramutation in flies and worms correlates with chromatin structure modification of the paramutated locus. In flies, inheritance of small RNAs from one generation to the next transmits a memory mainly targeting loci for repression whereas in worms, small RNAs can target loci either for repression or expression. Nevertheless, in the two species, paramutation can play an important role in the epigenome establishment.

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1. Introduction

Various strategies are used to recognize and repress mobile or foreign DNAs in genomes and to epigenetically transfer silencing memory over generations. In fly, transgenerational information seems to ensure predominantly repression of mobile DNA. Indeed, systems based on the principle of genomic traps have been established in order to identify and repress Transposable Elements (TEs). The *Drosophila melanogaster* genome contains about 140 discrete loci composed of TE fragments which undergo non-canonical transcription. This results in the inactivation of the transcript of the locus itself and of all the homologous transcripts produced by the genome [3–6]. Therefore, this system based on the PIWI-Interacting RNAs (piRNAs) results in repression of all TE copies in the genome if at least one copy has inserted into a piRNA-producing locus. Euchromatic TE repression occurs at both the transcriptional and post-transcriptional levels [7–10]. Thus, when a TE copy is active, it will move and insert into a piRNA-producing locus and this "piRNA locus" copy will progressively establish repression of the entire TE family: the family gets trapped. An important functional aspect is that production of piRNAs by piRNA loci requires maternal transmission of piRNAs both for repressing TE activity in the embryo germline and for stimulating piRNA production by the piRNA loci in the adult gonads [11,12]. Thus, in flies, a catalog of potentially dangerous sequences is transmitted from one generation to the other *via* cytoplasmic inheritance of piRNAs within the embryo. Worms also have a piRNA machinery but the strategy of transgenerational inheritance appears to be different. Indeed, two large clusters of piRNA loci produce thousands of different piRNAs that associate with the PIWI protein PRG-1 and target many sequences, as mismatches between piRNAs and their targets are tolerated [13,14]. When a sequence is targeted by piRNAs, the signal is amplified by an RNA-dependent RNA Polymerase (RdRP) and further relayed to a small interfering RNA pathway based on double strand RNA slicing performed by WAGO proteins [14–18]. Once this silencing is established, the piRNA machinery is no longer necessary and the information can be maintained in further generations solely by the WAGO protein pathway. The piRNA machinery has thus a crucial role for the establishment of silencing but not for its maintenance. To avoid too extensive, unspecific attack of transcripts by piRNAs, an active pathway exists, linked to production of small RNAs associated with a protein called CSR-1, which counteracts the effect of the piRNAs and consequently licenses transcripts for expression [16,19]. This CSR-1-associated RNAs are also transmitted from one generation to the other and allow transgenerational information of transcripts that have to be protected from degradation by piRNAs loaded onto PRG-1 [19].

It appears that in fly, one generation epigenetically transmits to the next a list of sequences to be repressed, whereas worms transmit information about both, correct repression or expression [20]. In both species, these systems were investigated using transgenes, and transgenic loci can exist at a given locus in opposite epigenetic states. In fly, clusters of transgenes can be stably maintained over generations, in either a quiescent or active state for production of abundant amounts of transgene-homologous piRNAs that establish *trans*-silencing of homologous transgene in the germline [21,22]. In worm, the same transgene can exist in an active or repressed state for their expression depending on the line [16]. The key point is that in both species, epigenetic conversion processes can occur in the germline between repressed and active loci, which can further be transmitted to the next generations in the absence of the converting locus. It results in a paramutation like-phenomenon, resembling this classical epigenetic conversion as observed in maize [1,2,23,24]. This review will describe properties of these epigenetic conversion processes and highlight the parallels and differences between the two models.

2. *Drosophila* paramutation-like conversion

The piRNA pathway was discovered in fly in 2006–2007 [3,4,25] and functionally validated using genomic sites capable of transposon repression [11]. Indeed, two TEs invaded the genome of natural *D. melanogaster* populations in the 20th century, the *P* element (a DNA transposon) [26] and the *I* factor (a LINE retroelement) [27]. For the two TEs, it was shown that the cross of females devoid of TE copies (from lines collected before the invasion) with males carrying numerous copies of the TE (from lines collected after the invasion) produces progeny showing a syndrome of genetic abnormalities in the germline called hybrid dysgenesis (high mutation rate, chromosomal breakages, thermo-sensitive sterility) [28,29]. Cytoplasmic inheritance was shown to play a key role as reciprocal crosses (TE-bearing females × TE-devoid males) produced progeny without dysgenesis [29–32]. Thus cytoplasm from females devoid of *P* or *I* elements was therefore missing "something" [33–35], later shown to be *P*- or *I*-homologous piRNAs [11,36]. For the *P* element, a master locus for repressing hybrid dysgenesis was identified at the telomere of the *X* chromosome [37–40], within subtelomeric heterochromatin called Telomeric Associated Sequences (TAS) [41]. Such telomeric heterochromatin was shown to produce abundant ovarian piRNAs [3]. In addition, *P* copies inserted in TAS were also shown to produce abundant ovarian piRNAs [11,21,42]. A transgenic system based on *P*-derived sequences located in TAS was developed and used to study the phenotypic and genetic properties of piRNA-mediated repression in the germline. In this system, called *Trans*-Silencing Effect (TSE), a *P*-transgene located in TAS can repress a homologous transgene in the female germline [12,43,44]. Inheritance of this repressive capacity shows both a maternal effect and a partial persistence of the maternal effect over up to 6 generations [12]. TSE studies and a mutant approach allowed to confirm that all the piRNA genes tested were necessary for the *trans*-silencing capacities of telomeric *P* insertions [12,43,45]. Finally, the use of TSE also allowed to discover that some other structures in the genome can establish piRNA-mediated repression [22]. Such studies revealed puzzling situations as the two lines *T-1* and *BX2* carrying a similar cluster of *P-lacZ-white* transgenes exhibit different properties. Indeed the *T-1* induces a strong TSE whereas *BX2* showed no repression capacity [22]. These *P-lacZ-white* clusters show stochastic *on-off* repression of the *white* marker in the eye (called variegation). This variegation results from Repeat Induced Gene Silencing (RIGS) [46,47] and is associated with the local binding of Heterochromatin Protein 1 (HP1) at the cluster, as tested by immuno-staining of salivary gland polytene chromosomes [48]. *T-1* has the same cluster than *BX2* but carries chromosomal inversions and translocations induced by X-ray treatment. The *trans*-silencing capacities of *T-1* and *BX2* appeared stable over more than one decade for the two lines. This allowed investigating possible factors that could activate piRNA production by the quiescent *BX2* locus.

It was then tested if maternal inheritance of *P-lacZ-white* homologous piRNAs could *de novo* activate the production of piRNAs from an initially quiescent *BX2* locus [21,49]. *T-1* heterozygous females were crossed with *BX2* males. Female progeny having inherited the *BX2* locus and a *T-1* cytoplasm, but not the *T-1* locus, produced ovarian *P-lacZ-white* piRNAs and induced a complete *trans*-silencing (Fig. 1). The phenomenon showed complete penetrance in each experiment. These paramutated *BX2* females were called *BX2**, by contrast to the initial "*BX2* naïve" (non-paramutated) flies producing no *P-lacZ-white* piRNAs. *BX2** lines were established and further studied for their capacity to induce TSE and produce piRNAs. Again all the tested lines (more than 20) exhibited stable repression. One line was investigated for very long terms: it still induced complete TSE after more than 120 generations, and ovarian *P-lacZ-white* piRNA production was confirmed at G_{42} and G_{83}. It was also tested if the *BX2** line is paramutagenic by crossing *BX2** females