

Fig. 1. (M. Sentmanat et al.) Position-effect variegation in *Drosophila melanogaster*. Schematic depiction of the chromosomal inversion (X chromosome) generating the white-mottled four line ($ln(1)w^{m4}$) recovered by Muller [14]. The inversion places the euchromatic *white* gene (coding for a transporter protein required for red eye pigment deposition) adjacent to pericentric heterochromatin. The light red bar represents heterochromatin, while the light green bar represents a euchromatic chromatin state. The chromosomal inversion results in gene silencing in some cells (white) due to heterochromatin spreading over the *w* gene in a stochastic process that allows expression to be maintained in other cells (red).

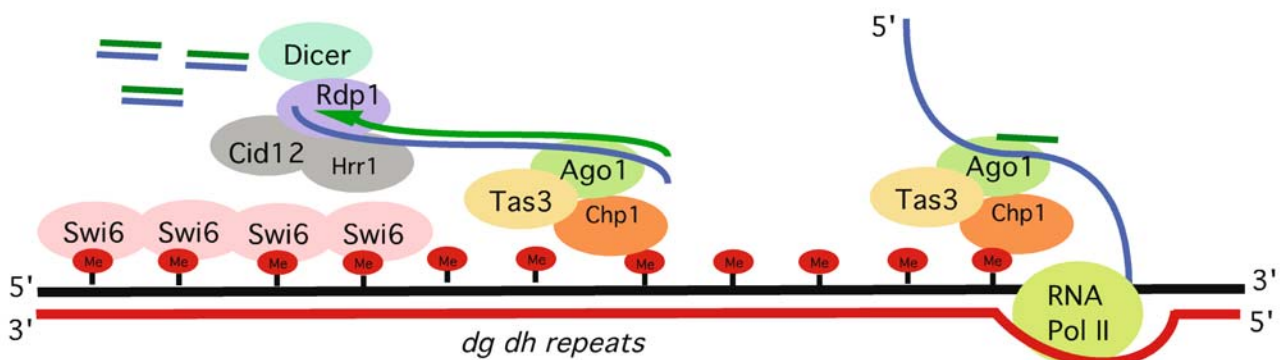


Fig. 2. (M. Sentmanat et al.) RNAi-transcriptional silencing in *S. pombe*. Transcripts of *dg/dh* pericentric repeats are targeted by the RNA-induced transcriptional silencing complex (RITS). RITS consists of the chromo domain protein Chp1, Tas3 and the small RNA-associated protein Ago1. A second complex, the RNA-directed RNA polymerase complex (RDRC) consists of the RNA-directed RNA polymerase 1 (Rdp1), a putative polyA polymerase Cid12, and helicase Hrr1. RDRC is recruited to *dh/dg* repeats by a physical interaction with RITS to synthesize double stranded RNAs; these are targeted by Dicer to make additional siRNAs to reinforce RITS recruitment. See Slotkin and Martiensson [19] and Kloc and Martiensson [20] for review of the supporting evidence.

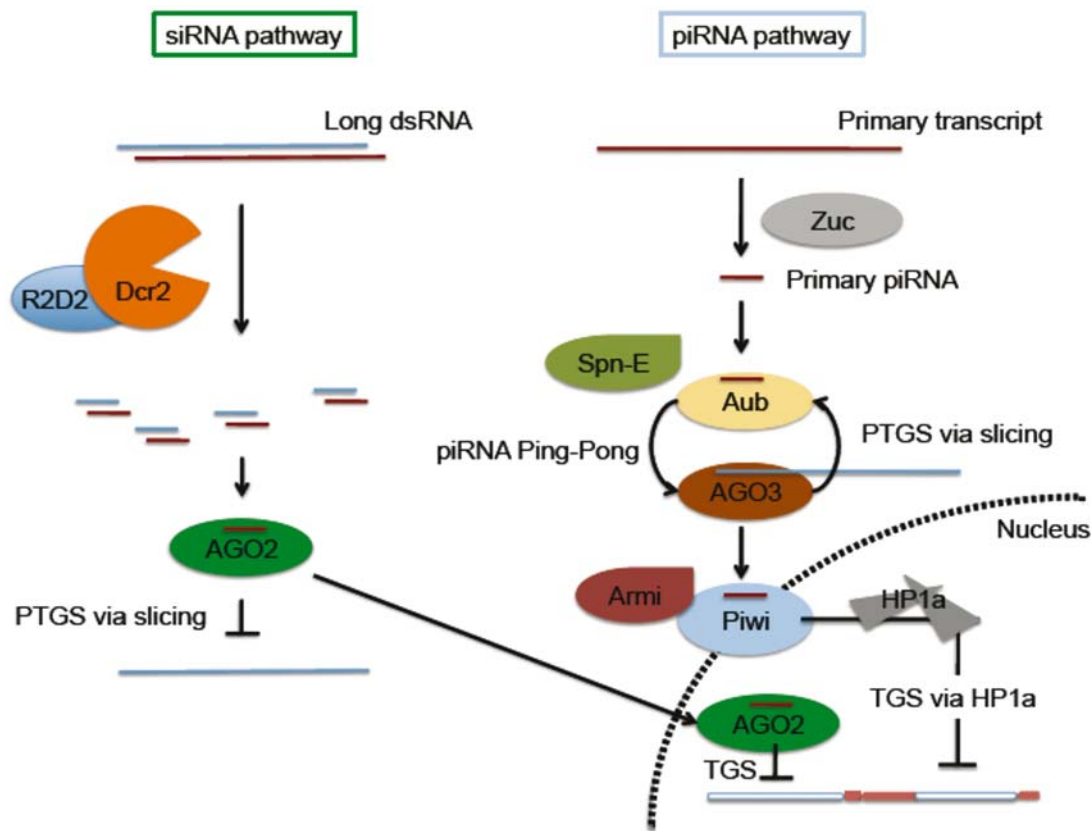


Fig. 3. (M. Sentmanat et al.) Small RNA-mediated silencing in *D. melanogaster*. Only siRNA and piRNA pathways are illustrated. Note that while the piRNA pathway is most active in the reproductive system, the siRNA pathway has a broader distribution. Both pathways have been implicated in a small RNA-mediated heterochromatin targeting process. In the siRNA pathway, small RNA generated by Dcr2 is loaded on to AGO2 RISC. The AGO2 complex can suppress expression through either of two mechanisms: slicing target mRNA in the cytoplasm through a well-characterized post-transcriptional gene silencing (PTGS) mechanism, or utilizing a yet to be characterized chromatin-based transcriptional silencing mechanism (TGS) in the nucleus. In the piRNA pathway, primary piRNA generated by a process involving Zuc is fed into the Ping-Pong cycle involving Aub and AGO3 to generate secondary piRNA. This process is proposed to function simultaneously to amplify anti-sense secondary piRNAs and suppress transposon expression via slicing. *Spn-E* is required for secondary piRNA production, although the detailed mechanism is unclear. Secondary piRNAs loaded onto Piwi, likely by Armitage, allow nuclear localization of Piwi and downstream recruitment of HP1a to induce heterochromatin silencing of a subset of TEs. See text for pertinent citations; reviewed in Huisinga and Elgin [22] and Dai et al. [23].

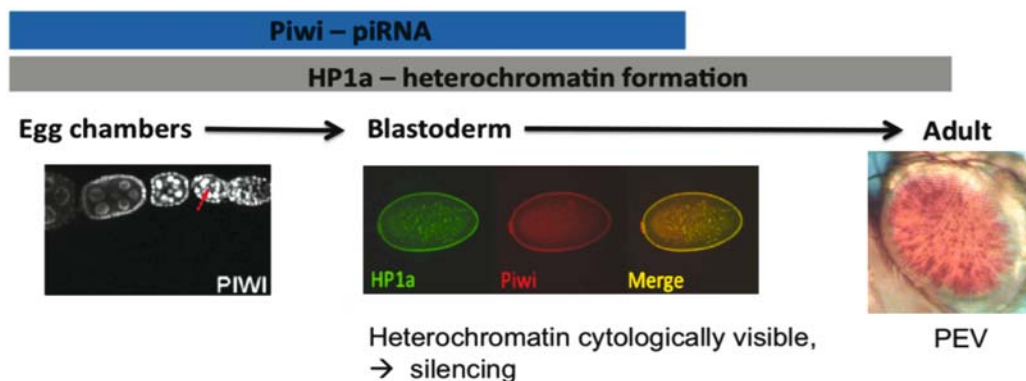


Fig. 4. (M. Sentmanat et al.) Model for piRNA-directed silencing in *Drosophila*: Piwi and HP1a are both present as nuclear proteins during blastoderm, when heterochromatin becomes visible at 1.5 h followed by silencing at 2.5 h development. Piwi and HP1a are both required for silencing of a subset of TEs during oogenesis. Piwi is loaded into the oocyte and co-localizes with HP1a during blastoderm. This is a critical time point for heterochromatin assembly and silencing; the impact of perturbations at this stage can be seen at PEV loci in adults [4]. Illustrations: left, nuclear localization of Piwi in female germ line [11]; middle, co-localization of Piwi and HP1a in blastoderm nuclei; right, PEV from a transgene reporter, scored in the adult.