RNA Interference: Endogenous siRNAs Derived from Transposable Elements

The Piwi-interacting RNA interference pathway plays an important role in suppressing transposable elements in the *Drosophila* germline. Now, deep sequencing of short RNAs from somatic tissue and cell culture has identified a novel class of endogenous siRNAs that may have a similar role in the soma.

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Transposable elements (TEs) are stretches of DNA that are able to move around the genome by mechanisms that lead to duplication of the element concerned, resulting in evolutionarily rapid changes in genome size and a potentially high copy number for TEs [1]. This will happen, and TEs will persist and spread within a population, even if they are costly to their host [2,3].

Although TE insertion can occasionally be beneficial and may indeed have been important in several key evolutionary innovations [1], the vast majority of insertions are likely to be deleterious or neutral [2,3], imposing a fitness cost on the rest of the genome. Costs arise directly when an insertion disrupts functional sequences, or indirectly by increasing rates of ectopic recombination between distant sites. In either case, there is a conflict of interest between TEs, which need to transpose to survive, and the rest of the genome, which would be better off if these elements did not transpose [2,3]. The situation is different in somatic tissues. Here transposition cannot benefit the TE by increasing its copy number in subsequent generations but may be harmful to the host nevertheless. As a result, the interests of the host and its TEs should be aligned, with both benefiting from reduced transposition [3]. In principle, this might even lead to the evolution of different mechanisms for regulating TEs in different tissue types, reflecting conflict in the germline and cooperation in the soma.

Suppression of TE activity in the germline is mediated, at least in part, by RNA interference (RNAi) and in the animal germline depends on the Piwi family of Argonaute proteins and a class of single-stranded 23–29 nucleotide (nt) Piwi-interacting RNAs (piRNAs) [4]. *Drosophila* has two other RNAi mechanisms (Figure 1) — the micro-RNA (miRNA) pathway, which plays an essential role in controlling gene expression [5], and the siRNA pathway, which is best understood as an antiviral defence mechanism [6]. Although each of these pathways utilizes short RNAs complexed with an Argonaute-family protein, they differ in several ways. In summary, miRNAs are about 22 nt long, are derived from host-expressed fold-back structures, and associate primarily with Ago1 [5]. Conversely, antiviral siRNAs (viRNAs), are about 21 nt, are derived from double-stranded viral RNA, and associate with Ago2 [6]. Both miRNAs and viRNAs are produced by cleavage of double-stranded RNA by a Dicer-family protein: Dcr1 for miRNAs and Dcr2 for viRNAs. In contrast, piRNAs appear to be Dicer-independent and are generated by Piwi-mediated cleavage of single-stranded sense and antisense TE transcripts, a process initiated in the zygote by maternally derived piRNAs [4]. There are also differences in short-RNA structure between pathways. In *Drosophila*, the 3′ end of both piRNAs and siRNAs, but not miRNAs, is 2′-*O*-methylated [7], while piRNAs are preferentially antisense and display a bias toward uridine at the 5′ end [4].

Plants [8] and nematodes [9] have a wider range of siRNAs than have so far been found in *Drosophila*, which, in this regard, has appeared to be something of a ‘poor relation’. Moreover, the different RNAi pathways in *Drosophila* originally appeared quite separate (e.g., [10]) in sharp contrast to plants where there is appreciable overlap between pathways and gene functions [8]. These views are now challenged by Chung *et al.* [11], in a recent issue of *Current Biology*, and by other groups [12–15] in studies that identify a novel class of endogenous *Drosophila* siRNAs derived from TEs and host transcripts.

By deep sequencing the short-RNA populations derived from adult flies and tissue culture cells, these groups have revealed a class of 3′-methylated endogenous 21 nt RNAs derived from TEs and several other loci, including natural antisense transcripts and long fold-back structures [11–15]. These endogenous siRNAs depend on *Dcr2* and *Ago2* of the antiviral siRNA pathway but not *Dcr1* or *Ago1* of the miRNA pathway or on components of the piRNA pathway. Moreover, the endogenous siRNAs show a much lower strand bias than is seen in piRNAs and they do not have a strong U bias in the 5′ base. Although there had been several previous reports hinting at the involvement of the siRNA pathway in TE suppression in *Drosophila* somatic tissue (e.g., [16,17]), this is the first time that comprehensive studies have quantified the number and origin of these endogenous short RNAs. The results identify TE-derived siRNAs as a distinct class of small RNAs and, because reduction in expression of *Ago2* or *Dcr2* is associated both with decreased numbers of siRNAs derived from TEs and with increased levels of TE transcripts, it suggests that this siRNA pathway may actively regulate TE transcript levels.

The discovery of TE-derived endogenous siRNAs in many tissues, including the germline, raises as many questions as it answers. The dependence of endogenous siRNAs on *Dcr2* and their reduced strand-bias suggests that they are derived from a long dsRNA precursor, but how is the antisense transcript generated? Is it through active host promoters, for example, the loci that give rise to piRNA-generating transcripts — Czech *et al.* [13] suggest ‘yes’, Chung *et al.* ‘no’ [11]) — or are they the result of incidental ‘background’ transcription? Ghildiyal *et al.* [12] observe that there is at least some overlap between piRNA and TE-siRNA generating, suggesting that both may be involved.

Strikingly, both Czech *et al.* [13] and Chung *et al.* [11] show that many endogenous siRNAs depend on the RNA-binding protein Loquacious (Loqs), the canonical partner of Dcr1 in the miRNA pathway [18]. Loqs has not previously been found to associate with Dcr2, which in the antiviral siRNA pathway is thought to act with the Loqs

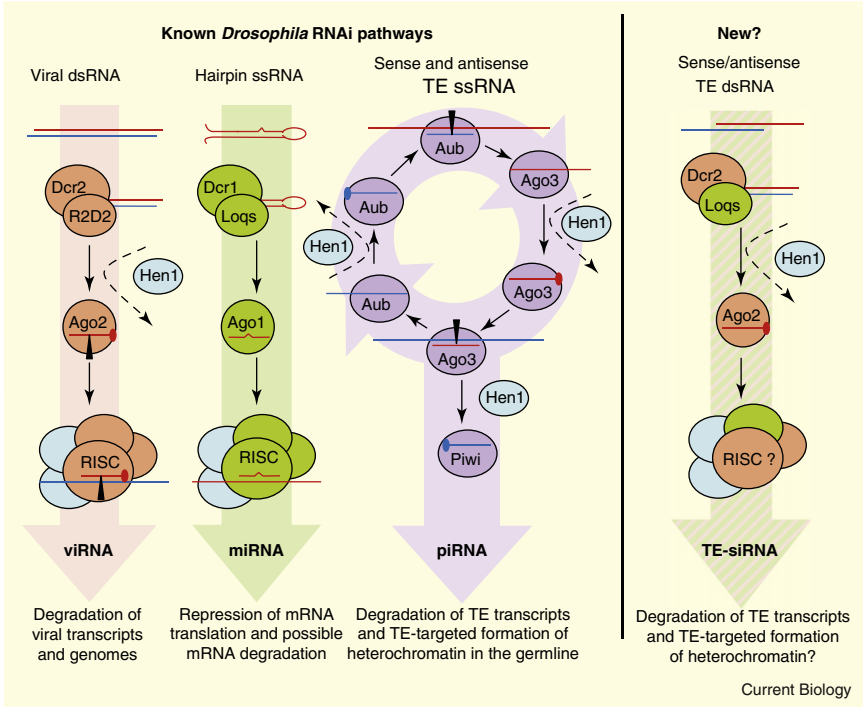

Figure 1. The three well-studied RNAi pathways in *Drosophila*.

The viRNA pathway processes dsRNA derived from viruses and viral-replication intermediates into ~21 nt, 3′-methylated viRNAs that are used to recognise and bind viral targets for cleavage by the RNA-induced silencing complex (RISC). This processing is mediated by Dicer-2 (Dcr2), Argonaute2 (Ago2) and R2D2, and viRNAs are methylated by Hen1. The miRNA pathway processes host-encoded fold-back structures (primary miRNAs) into short hairpins (pre-miRNAs) and then into ~22 nt un-methylated miRNAs. This processing is mediated by Dicer-1 (Dcr1), Argonaute-1 (Ago1) and Loquacious (Loqs). In *Drosophila*, miRNAs generally include mismatches to their targets, which are usually 3′ UTRs, and induce transcriptional repression. The piRNA pathway is functional only in the germline and is thought to alternately cleave sense (red) and antisense (blue) TE transcripts, guided by a piRNA in complex with one of the Piwi family of Argonaute proteins — Aubergine (Aub), Argonaute-3 (Ago3), or Piwi. It has been proposed that cleavage not only reduces transcript levels but also generates the 5′ end of a new piRNA (the fragment is then bound by a partner Piwi-family member, shortened by an exonuclease, and methylated by Hen1). This feedback loop may be initiated by maternally generated piRNAs. The new pathway that has been identified in the recent studies comprises components of both the viRNA and miRNA pathways and processes endogenous dsRNA (as opposed to exogenous dsRNA, such as that of viral replication intermediates) into 21 nt methylated siRNAs. These derive from (and presumably target) transposable elements, overlapping gene transcripts (especially 3′ UTRs) and larger fold-back structures.

homologue R2D2 [6,19]. Does this hint at distinct mechanisms for the biogenesis of TE siRNAs and viral siRNAs? In a further twist, Kawamura *et al.* [14] observe signs of RNA editing in their siRNAs, and this can also be seen in the data of Chung *et al.* [11], reinforcing the idea that RNA editing and RNAi pathways may interact [20].

There are also questions of adaptive significance. It seems clear that TEs do indeed feed into an endogenous siRNA pathway in both somatic and germline tissue and that this affects their transcript levels [11–14], but whether or not this is a 'defence' mechanism is far from clear. As we point out above, the interests of the TE and the rest of the genome should be aligned in somatic tissues, with both benefiting from reduced transposition. It is not clear that the endogenous siRNA pathway affects transposition *per se*, only that it affects transcript levels, and Kawamura *et al.* [14] suggest that siRNAs may be involved in the somatic maintenance of heterochromatin: perhaps both are affected. Why flies should employ two different RNAi pathways to suppress TE activity, especially given that both are active in the germline [13], is also not clear. Do these pathways feed into each other, or is one — the TE-siRNA pathway — of mutual benefit, perhaps being derived from a system regulating host genes, while the other — the piRNA pathway — is solely directed towards suppressing transposition? We still have much to learn.

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Phagocytosis: Dynamin's Dual Role in Phagosome Biogenesis

Dynamins have a well-established role in the fission of vesicles at sites of endocytosis. In phagocytosis, however, a role for certain dynamin isoforms has been reported in the full extension of pseudopods during phagosome formation, not in fission of the phagocytic vacuole. Recent studies in *Caenorhabditis elegans* have now uncovered a new function of dynamin in phagosome maturation.

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Phagocytosis is an integral part of our innate immunity, providing an early line of defense against invading microorganisms. In addition, phagocytosis plays a key role in wound healing, tissue morphogenesis and development by removing necrotic and apoptotic cells. Particle intake is initiated by phagocytic receptors that recognize ligands on the surface of the target and unleash a signaling cascade that culminates in actin-driven extension of pseudopods that ultimately encircle and engulf the particle (Figure 1). However, additional steps are required to complete the elimination of the phagocytic targets. Full clearance requires the degradation of the internalized particles and the disposal of their breakdown products, which occur as the phagosomes acquire degradative properties akin to those of lysosomes by a process known as phagosomal maturation.

Despite its critical importance to the overall process, remarkably little is known about phagosome maturation. It has been studied much less intensively than phagosome formation, perhaps because the latter is much easier to visualize and quantify. However, this gap is likely to narrow in the future through studies like the recent work by Kinchen *et al.* [1]. These authors took advantage of the fact that in *Caenorhabditis elegans* phagosomes containing apoptotic cells are identifiable under differential interference microscopy by their refractile appearance and affinity for nuclear dyes. Unusual persistence of such refractile structures is indicative of defective maturation and, when combined with gene manipulation, this analysis can provide valuable information about proteins involved in the maturation sequence. Indeed, because the genome sequence of *C. elegans* is known and the worms are genetically tractable, it should now be possible to investigate systematically the role of individual proteins in phagosome maturation. This analysis will be facilitated by the spontaneous occurrence of multiple phagocytic events because, during the course of *C. elegans* development, over 600 cells undergo programmed death and are ingested by neighboring cells.

In the new work, Kinchen *et al.* [1] used a convenient and effective means of silencing selected genes in *C. elegans* to investigate their role in the maturation of phagosomes containing apoptotic cells. They fed to the larvae bacteria transformed with double-stranded siRNA to manipulate the expression levels of Rab5, Rab7, EEA1, and the VpsC/HOPS complex. These proteins were known to regulate vesicular traffic in the endocytic and phagocytic pathways, controlling the transition between early and late stages. By these means, the authors were able to confirm the requirement for Rab5 and Rab7 in the early stages of the maturation process, as established in previous work in mammalian phagocytes [2]. They also documented a role for the VpsC/HOPS complex in phagosome maturation. This multi-subunit complex had been proposed to bridge the transition from Rab5- to Rab7-positive vesicles during endosome progression [3], but its involvement in phagosome maturation had not been explored. Kinchen *et al.* [1] systematically silenced the expression of individual components of this complex and found that depletion of the Vps11, Vps16, Vps18, Vps33, or Vps44 subunits arrested phagosome maturation at the Rab7-positive stage. These data imply that, while the VpsC/HOPS complex is essential for phagolysosome biogenesis, the complex is seemingly not needed for Rab7 recruitment and may instead regulate vesicle fusion and traffic, as has been suggested for the endocytic pathway [4–7]. A surprising observation was that elimination of EEA1 had little or no effect on the ability of *C. elegans* to clear apoptotic corpses. This contrasts with the results reported using mammalian macrophages, which were unable to undergo maturation following inactivation of EEA1 [8]. This discrepancy may indicate that not all phagosomes are created or processed equally and that the nature of the particular phagocytic receptor engaged may dictate the pathway used for maturation.

The salient findings in the paper by Kinchen *et al.* [1], however, refer to the role of dynamin in the early stages of phagosome maturation. The involvement of dynamin in phagosome formation had been explored previously in professional phagocytes, but its role in maturation was unsuspected and therefore unexplored. Unlike *C. elegans*, which has a single form of dynamin, mammals express three distinct isoforms: dynamin-1, -2, and -3 [9]. Dynamin-2 is expressed ubiquitously while dynamin-1 is present exclusively in the brain and dynamin-3 in the testes, lungs and neurons [10]. It is noteworthy that dynamin-2 is present both in endomembranes and in the plasma membrane, while dynamin-1 is predominantly