Trends in Genetics

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Stable Intronic Sequence RNAs Engage in Feedback Loops

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Stable intronic sequence RNAs (sisRNAs) are conserved in various organisms. Recent observations in *Drosophila* suggest that sisRNAs often engage in regulatory feedback loops to control the expression of their parental genes. The use of sisRNAs as mediators for local feedback control may be a general phenomenon.

When one asks a group of biologists about the functions of introns, the responses may range from introns playing a role in enhancer function, nonsense-mediated decay, alternative splicing, recombination, exon shuffling, or that they are merely useless sequences that populate the genome [1]. Once in a while a molecular biologist may appreciate the fact that introns give rise to long noncoding RNAs (ncRNAs) such as small nucleolar RNAs (snoRNAs) and small Cajal body-specific RNAs (scaRNAs) that facilitate modifications of diverse species of RNAs in the cell [2]. A typical human gene has an average of eight introns, and each intron can be 10–100-fold longer than an exon, indicating that intronic RNAs account for massive amounts of genetic material produced during gene expression.

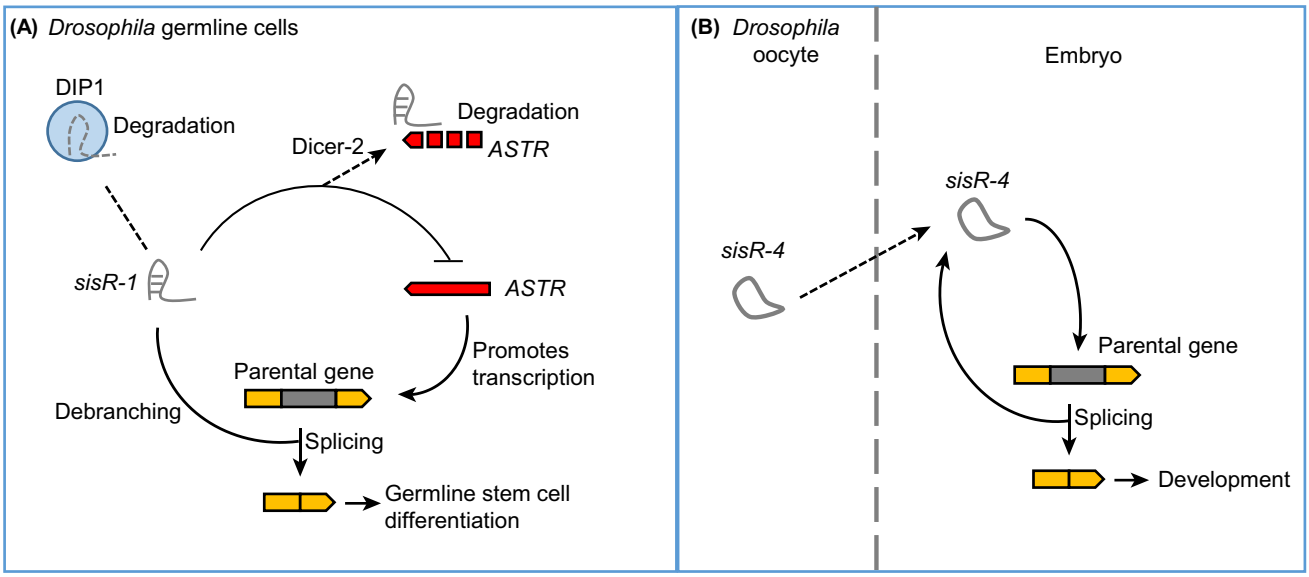
More than two decades ago John Mattick proposed that intronic RNAs can serve as regulators of gene expression [3], building on previous ideas from Roy Britten and Eric Davidson that nuclear RNAs can activate gene expression via sequence-specific complementary base-pairing [4]. Although revolutionary, these ideas received limited support from experimental evidence. This is because of the commonly held assumption that most intronic RNAs are extremely unstable, except for a few rare cases that were considered to be the exceptions rather than the norm [5,6].

While looking for nascent transcripts in the *Xenopus tropicalis* oocyte nucleus, the laboratory of Joseph Gall laboratory serendipitously found genome-wide occurrence of sisRNAs that are stable for months during oogenesis before being deposited into the embryo [7]. In the same year a similar phenomenon was also reported in the mouse [8]. No doubt, the genome-wide occurrence of sisRNAs immediately begged the question of whether these sisRNAs are functional. By function, I refer to both the molecular roles and the biological processes that they regulate. Within a short period of 5 years, we now find sisRNAs in many organisms [5]. Recent studies in *Drosophila* suggest that sisRNAs frequently engage in both positive and negative feedback loops to regulate the expression of their parental genes.

Drosophila early embryos and unfertilized eggs are excellent systems to identify sisRNAs because they contain mature and stable RNAs without any contamination from pre-mRNAs. *sisR-1* was the first sisRNA to be characterized from a screen for sisRNAs in the early embryos [9]. It is transcribed from the second intron of the *regena* (*rga*) locus, and is further processed after debranching into a mature linear sisRNA in the nucleus. The *rga* locus is complex because an antisense RNA (*cis*-natural antisense transcript, or cis-NAT) is highly transcribed during early embryogenesis and base-pairs with *sisR-1* over a 76 nt sequence located at the 3′ ends of both transcripts. This cis-NAT (called *ASTR*) promotes the expression of *rga*. *sisR-1* functions to negatively regulate *rga* by repressing *ASTR* (Figure 1A). We can see this feedback mechanism as an autoregulatory loop whereby *ASTR* first promotes robust *rga* transcription. During transcription, *sisR-1* is generated simultaneously with the *rga* mRNAs. Over time, *sisR-1* levels build up in the nucleus and eventually shut down *ASTR,* reducing *rga* expression back to normal.

Subsequent studies have shown that the *sisR-1* negative feedback loop plays a role in ensuring germline stem cell (GSC) homeostasis in the *Drosophila* ovaries [10]. The *rga* gene encodes the NOT2 protein, a component of the CCR4–NOT deadenylase complex. High expression of Rga in the GSCs promotes differentiation while low levels inhibit, indicating that fine-tuning of Rga expression achieves GSC homeostasis. While *sisR-1* mediates the negative feedback loop to control *rga* expression, the unusual stability of *sisR-1* can lead to overaccumulation over time, which results in prolong dampening of *rga*. This will in turn result in the overproduction of GSCs. The excessive production of *sisR-1* is counteracted by a double-stranded RNA binding protein called Disco-interacting protein 1 (DIP1). DIP1 binds to and promotes the decay of *sisR-1* in a post-transcriptional manner in the nucleus. Such a feedback mechanism ensures that the *sisR-1/ASTR/rga* feedback loop is fine-tuned in the GSCs to maintain a steady-state number of stem cells in the ovarian niche.

Following the first observation of maternally deposited sisRNAs in the *Xenopus tropicalis* oocytes, it had been suggested that sisRNAs may play regulatory roles during early embryonic development [7]. Similarly to *Xenopus*, *Drosophila* oocytes also contain a pool of maternally deposited sisRNAs, both in linear and circular conformations [9,11]. *sisR-4* is a circular sisRNA that is generated from the second



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Figure 1. *Drosophila* Stable Intronic Sequence RNAs (sisRNAs) Engage in Feedback Loops. (A) *sisR-1* negatively regulates *rga* transcription by repressing *ASTR* in *Drosophila* germline cells. DIP1 controls *sisR-1* by binding to and promoting the degradation of *sisR-1*. The regulation of *rga* is required for germline stem cell (GSC) homeostasis. (B) In *Drosophila*, maternally deposited circular *sisR-4* promotes *dpn* expression during early development. *sisR-4* acts via an intronic enhancer, and this regulation is important for development.

intron of the *deadpan* (*dpn*) locus. Maternally deposited *sisR-4* promotes the transcription of *dpn* during early development via an intronic enhancer where *sisR-4* is encoded [11] (Figure 1B). Because *dpn* is expressed in the embryonic neuroblasts (neural precursor cells), failure of *sisR-4* to activate the positive feedback loop leads to lower *dpn* expression and subsequently to lethality during embryonic development.

Do other maternal sisRNAs also play a role in parental gene activation during early development? I suspect that they do. About half of the sisRNAs identified in *Drosophila* early embryos map to sites of open chromatin within the corresponding introns [9,11], suggesting that they may facilitate the establishment of chromatin structure that is permissive for transcription. Conceptually, these sisRNAs can be considered as a subclass of enhancer RNAs that are derived from introns.

While the roles of sisRNAs in regulatory feedback loops may have started to become clearer in *Drosophila*, studies have suggested that such a mechanism may also be conserved in human cells, including cancer [5]. For example, in human colon cancer cells and mouse models, sisRNAs have been found to negatively regulate the parental gene *SMYD3*, which encodes a H3K4 methyltransferase involved in cell division and viability [5]. These sisRNAs act by guiding polycomb repressive complex 2 to the local chromatin. By contrast, in breast cancer cells, sisRNAs (called *Eleanors*) from the estrogen receptor gene *ESR1* modulate a positive feedback loop to induce *ESR1* expression via an unknown mechanism [12]. Thus, as in *Drosophila*, sisRNAs also engage in regulatory feedback loops in cancer.

Because sisRNAs and fundamental molecular mechanisms of ncRNAs are generally well-conserved, it is likely that similar regulatory relationships operate in other organisms. SisRNAs have characteristics that make them suitable for relaying feedback loops. Because introns are cotranscribed with the exons, sisRNAs are a direct readout of the transcriptional activity of a locus. Because sisRNAs are produced in close proximity to their sites of action, they provide a local concentration at the parental locus for rapid and robust actions. From the perspective of evolution, the regulatory relationships between sisRNAs and their targets (complementary DNA or cis-NATs from the same parental loci) are less sensitive to point mutations, and tend to coevolve together.

However, the biological significance of sisRNA-mediated feedback loops is still lacking. In addition, it is still a mystery why sisRNAs are unusually stable relative to other intronic transcripts, and if this is important for their function. So far, sisRNAs have been shown to regulate stem

cell homeostasis and development in *Drosophila* [10,11]. To study the biological functions of sisRNAs, animal models such as *Drosophila* provide excellent opportunities because of the available genetic tools and simple tissue architectures in a multicellular organism. Because sisRNAs often engage with their parental loci at the DNA level or with overlapping cis-NATs, the use of deletion mutants of sisRNA sequences, which also remove the corresponding DNA sequences or cis-NATs, may not be informative at the phenotypic level. Therefore, development of techniques that specifically manipulate sisRNA activity *in vivo* will be particularly useful in the future.

In conclusion, recent work in *Drosophila* has highlighted the importance of sisRNAs as mediators for both positive and negative feedback loops on their parental genes. Because such feedback loops are often involved in signal amplification or fine-tuning, sisRNAs may have diverse functions in cellular differentiation and stress responses, as well as in tissue homeostasis and robustness. Misregulation of any of these processes is likely to contribute to diseases.

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