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## Heritable epigenetic changes at single genes: challenges and opportunities in *C. elegans*

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### Abstract

Organisms rely on stereotyped patterns of gene expression for similar form and function in every generation. The analysis of epigenetic changes in the expression of different genes across generations can provide the rationale for measured actions in one generation that consider impact on future generations.

### Keywords

RNA interference (RNAi); transgenerational epigenetic inheritance (TEI); regulatory networks

Organisms develop in each generation using heritable information stored in gene sequences, gene regulators, and their arrangements [1]. Regulators can interact to form different networks that have different architectures. Faithful recreation of such regulatory architectures, the interacting regulators, and the genome are all required for the preservation of form and function across generations in a given environment.

Changes that do not alter the sequence information in genomes but are nevertheless transmitted across generations are broadly considered **heritable epigenetic changes** (see glossary) and are poorly understood. Epigenetic changes can be driven by changes in the environment and occur within the context of a complex network of interacting molecules. Cases that alter either physical/chemical properties of the molecules or only alter interactions have been documented [2,3]. However, excluding inadvertent selection of a pre-existing genetic difference, constructing explanations that account for all aspects of the heritable effect, and developing models that can predict heritable epigenetic effects remain challenges.

To begin addressing these challenges, epigenetic changes associated with the expression of single genes could be analyzed across generations with the ultimate goal of developing

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an aggregate model that can predict heritable effects at any gene. Providing molecular explanations for heritable epigenetic effects requires answers to three key questions:

1. *How many generations does the effect last?* Heritable changes can be classified into three broad categories based on their duration: parental or grandparental effects, which last one or two generations, respectively, and can be the result of passive mechanisms in parents enabling perdurance of regulatory molecules or active mechanisms in descendants that restrict further transmission; unstable heritable effects, which last for a few generations, before opposing mechanisms or dilution of the change cause return to initial states; and stable heritable effects, which can potentially last forever.
2. *What regulatory molecules are required for the observed effects in each generation?* Molecules can change in abundance (e.g., decrease in mRNA levels), in chemical composition (e.g., methylation of histones), or physical structure (e.g., folding of prions) for the duration of a heritable epigenetic effect, making them either essential components or byproducts of the mechanism transmitting the change across generations.
3. *Which regulatory interactions explain the nature and the duration of the effect?* Regulatory architectures created by a network of interacting molecules can persist, despite turnover of individual molecules, if the information necessary for all interactions is preserved in the remainder of the network. Therefore, explaining the origin and eventual loss or permanent gain of a new regulatory architecture is needed to account for the nature and duration of a heritable epigenetic effect.

The nematode *C. elegans* is a well-characterized animal with a generation time of three days, making it useful for analyzing effects that can last for many generations. Here, we highlight advances that have been made towards the explanation of heritable epigenetic changes in the expression of single genes in *C. elegans*, and outline the opportunities and challenges that remain.

## Expressed genes can become stably silenced

Recurrent expression of a gene in every generation requires the information for driving such expression to be reliably inherited from parent to progeny. Changing this heritable epigenetic information can alter the expression of the gene for many generations. Here, we highlight five different ways of causing stable RNA silencing of single genes in *C. elegans* – two use molecularly defined RNAs to initiate silencing and three rely on, as yet, undefined molecular initiators.

### Stable RNA silencing induced by well-defined initiators.

Silencing that can last for multiple generations has been observed in response to double-stranded RNA (dsRNA) or **piwi-interacting RNA (piRNA)** (Figure 1A, [4,5]). Gene silencing caused by dsRNA or piRNA is associated with changes in mRNA, production of RNA intermediates, and addition of chromatin modifications. This pathway for gene

silencing provides several candidate molecules (e.g., modified mRNA fragments [6], amplified small RNAs [7], chromatin modifications [8]) and interactions (e.g., between nascent RNA and small RNAs) that can act as heritable silencing signal(s). In principle, such signal(s) have three characteristics: they are selectively associated with specific gene sequences, can initiate gene silencing, and ensure transmission of a signal with the same capabilities to the next generation. Despite the diversity of candidates for heritable silencing signal(s), an unexplained feature of silencing by dsRNA or piRNA is that exposure to the same dsRNA or piRNA does not cause similarly persistent RNA silencing of all genes with matching sequences (e.g., [9]).

### **Stable RNA silencing induced by poorly defined initiators.**

Spontaneous silencing, *trans* silencing, and mating-induced silencing require some of the same proteins and RNAs required for dsRNA- or piRNA-initiated silencing, but for which the factors that initiate the silencing and that dictate its duration are both unknown. Some single-copy transgenes are spontaneously silenced (Figure 1B, *left*, [10]) through a mechanism that requires complementary piRNA [4,5]. Understanding this form of silencing potentially requires the analysis of events that occur between injection of DNA into the germline and eventual integration of injected DNA into the genome. A gene that shows stable RNA silencing is associated with signal(s) that can silence other homologous sequences in *trans* (Figure 1B, *middle*, [11]). This *trans* silencing can last for many generations between some sequences with perfect homology (e.g., [9]), reflecting continuous production of sequence-specific silencing signal(s) from the stably silenced gene. Mating can cause stable RNA silencing of some transgenes when males expressing the transgene are mated with hermaphrodites lacking that transgene or homologous sequences (Figure 1B, *right*, [9]). The persistence of such mating-induced silencing relies on RNA-based regulation and positive feedback loops.

### **Silenced genes can become stably expressed**

Gain of new epigenetic changes or loss of previous epigenetic changes (i.e., epigenetic recovery) can cause a silenced gene to become expressed. Here we highlight three such gene expression phenomena where transitions from silenced to expressed states have been observed, but the underlying causal mechanisms are unknown.

A gene that has been silenced for multiple generations can spontaneously regain expression (Figure 1C, *left*, e.g., [9]). Such recovery from silencing could occur because of the loss of heritable silencing signal(s) and/or the gain of opposing epigenetic changes. A gene that was once silenced in *trans* by a stably silenced gene can recover expression and then remain expressed despite the presence of the stably silenced gene in the same nucleus (Figure 1C, *middle*, e.g., [9]). Such resistance to *trans* silencing could occur because of changes in the regulation of the newly silenced gene or the stably silenced gene. An expressed gene can activate the expression of a homologous gene that has remained stably silenced for many generations when the two genes are brought together through a genetic cross (Figure 1C, *right*, [11]). Such reactivation is likely driven by sequence-specific signal(s) from the expressed gene.

## Heritable epigenetic changes persist as part of regulatory architectures that include loops

The indefinite persistence of any process (e.g., metabolism) requires at least one closed loop of mutual production [2, 12–14] (Figure 1D, *top*). This requirement implies that the positive regulators of a gene that is expressed in every generation need to form at least one closed loop. Gene silencing phenomena such as **paramutation** [15], **RNAe** [4,5], mating-induced silencing [9], etc., that appear capable of persisting forever reveal that the negative regulators of the silenced gene also form at least one closed loop. Alternatively, both positive and negative regulators could be part of the same closed loop.

A transient change in the amount of one regulator could be propagated to all other regulators in a closed loop if the induced change in the amount of every regulator is greater than that required to change the next regulator in the loop [2]. Such an overall increase in the activity of the loop(s) formed by positive or negative regulators will result in the permanence of new expression or silencing, respectively (Figure 1D, *bottom*). When a transient change in an interaction between the loops is propagated to all other regulators in one of the closed loops (positive or negative), the activity of that loop could permanently change relative to the other loop (dotted lines in Figure 1D, *bottom*). Finally, transient changes in the inputs regulating the gene (arrow or bar to the gene in Figure 1D, *bottom*) can only be sustained if that regulator is part of a closed loop (black in Figure 1D, *top*) and not if it is one of the regulators emanating from the loop (grey in Figure 1D, *top*).

## Concluding Remarks

Examining changes in the expression of genes across generations holds promise for making rapid progress towards understanding heritable epigenetic effects. In *C. elegans*, RNA-mediated changes in gene expression have enabled such analyses at single-gene resolution. Given the incomplete knowledge of molecular changes underlying any heritable effect, preserving information about how the effect arose is necessary for effective analysis. Models that successfully explain both the nature and the duration of a heritable epigenetic effect will include loss or gain of a heritable regulatory architecture. Arriving at such molecular explanations for diverse heritable epigenetic effects at any gene is needed for equal understanding of genetic predisposition and epigenetic predisposition.

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## Glossary

### Heritable epigenetic changes

changes that occur in biological molecules and/or their arrangements that are transmitted across generations without altering the genome sequence. Past definitions have been

similarly broad or narrower with a focus on particular chemical modifications (e.g., DNA methylation) or changes in regulators (e.g., small RNAs). The concepts outlined here apply to both types of definitions

### **Paramutation**

a gene silencing phenomenon, initially identified in maize, whereby one allele can induce heritable changes in another allele at the same locus

### **Piwi-interacting RNA (piRNA)**

a class of small RNA that is processed from RNA transcribed within the germline, binds the Piwi subfamily of Argonaute proteins, and base pairs with complementary mRNAs, typically resulting in gene silencing

### **RNAe**

RNA-induced epigenetic silencing that can last for many generations. This term was initially used to describe the silencing of single-copy transgenes within the *C. elegans* germline through a mechanism that requires piRNA binding. Discovery of distinct phenomena that share some aspects of piRNA-mediated silencing suggest a diversity of underlying mechanisms

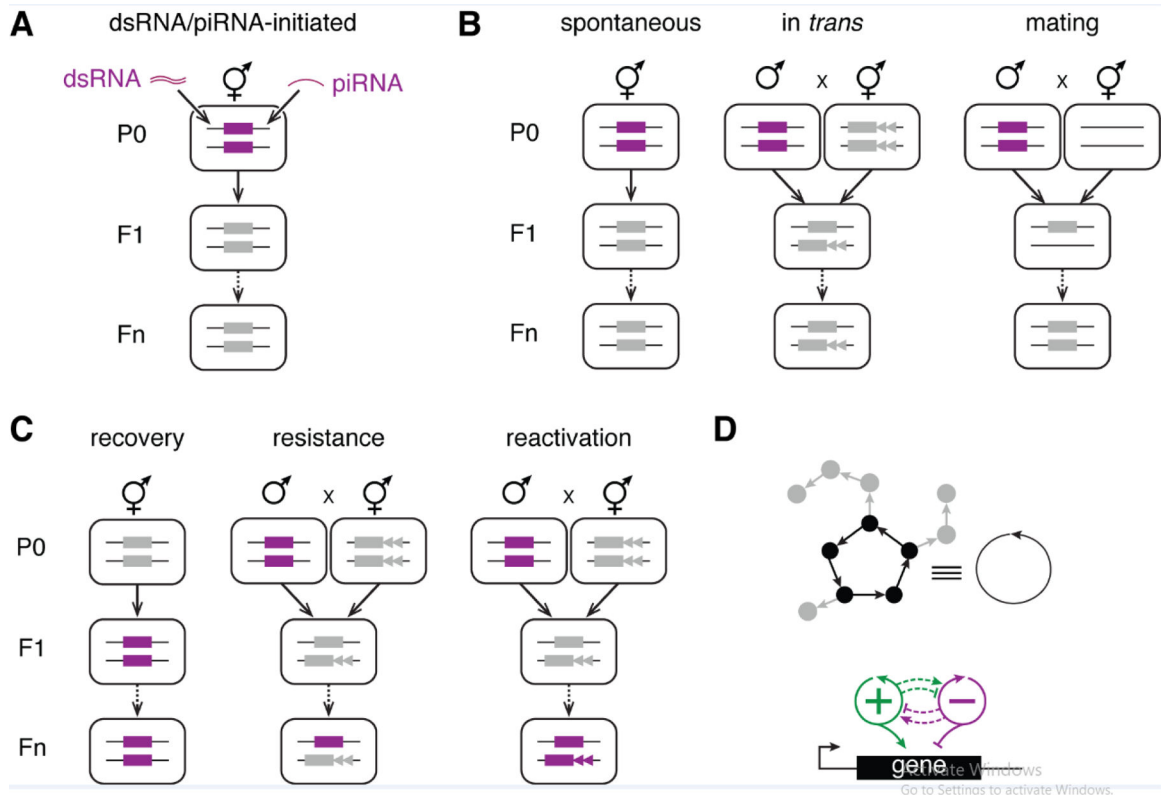
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**Box 1.****Analysis of heritable epigenetic changes requires nomenclature that preserves history**

Mutations and epimutations are used as descriptors for genetic and epigenetic change, respectively. However, while genetic changes are precisely described as altered genome sequences (e.g., A to C, deletion of 100 bp, etc.), epigenetic changes are incompletely described at the molecular level. For example, a variety of different molecular changes that result in reduced expression could be described as 'silencing'. Since epigenetic changes can be distributed among many molecules and their arrangements [2], technical limitations typically preclude their complete description. Furthermore, phenomena that can be distinguished by their duration nevertheless can depend on some of the same molecules [9]. Thus, effective analysis requires the silenced or expressed states generated through different means to be at least provisionally considered as distinct. For example, for analyzing a *C. elegans* gene, the nomenclature could be *gene(lab#1){Epi-gene(lab#2)}*, where *lab* designates the laboratory of origin, *#1* is a number indicating the genetic state and *#2* is a number indicating both the epigenetic state *and* the sequence of events that led to it. This conservative assumption of differences between epigenetic states despite shared requirements for a few regulators aids the discovery of causal explanations.



**Figure 1. Heritable epigenetic changes in *C. elegans* and the regulatory architectures that support them.**

(A) Heritable gene silencing caused by known molecular initiators. Some expressed genes (magenta box) can be silenced (grey box) for many generations (F1 through Fn) using dsRNA of matching sequence or piRNA of complementary sequence. (B and C) Heritable epigenetic changes caused by unknown molecular initiators. Chromosomes without a transgene (line) and with expressed (magenta) or silenced (grey) transgenes that have unique (arrowheads) and/or shared (box) sequences when compared with other homologous transgenes are indicated. (B) Heritable gene silencing. *Left*, Spontaneous silencing. *Middle*, *Trans* silencing. *Right*, Mating-induced silencing. (C) Heritable gene expression. *Left*, Recovery of expression. *Middle*, Resistance to silencing. *Right*, Reactivation of expression. In every case, the epigenetic changes can persist for many generations (Fn). See text for details. (D) Regulatory architectures needed to explain heritable epigenetic changes that can persist forever. *Top*, At least one closed loop of mutual production (black circles and arrows) is needed for the indefinite persistence of regulators (black or grey circles) and their interactions. Depiction of multiple regulators (circles) and their interactions (arrows), including a closed loop (black), and the equivalent ( $\equiv$ ) simplification. *Bottom*, Schematic depicting regulatory interactions that control heritable epigenetic changes. Evidence suggests that positive regulators (green, +) and negative regulators (magenta, -) minimally form two separate closed loops. Additional interactions (dotted lines) could connect both loops (positive, arrow; negative, bar) into a single heritable network.