REPORT

EPIGENETIC INHERITANCE

Transgenerational transmission of environmental information in *C. elegans*

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The environment experienced by an animal can sometimes influence gene expression for one or a few subsequent generations. Here, we report the observation that a temperature-induced change in expression from a *Caenorhabditis elegans* heterochromatic gene array can endure for at least 14 generations. Inheritance is primarily in cis with the locus, occurs through both oocytes and sperm, and is associated with altered trimethylation of histone H3 lysine 9 (H3K9me3) before the onset of zygotic transcription. Expression profiling reveals that temperature-induced expression from endogenous repressed repeats can also be inherited for multiple generations. Long-lasting epigenetic memory of environmental change is therefore possible in this animal.

Resident animals are not the only ones subject to their environment; their progeny can also be affected (*1*–*11*). For example, starvation or exposure to high temperature in *Caenorhabditis elegans* can lead to altered small RNA transmission and putative target mRNA expression for up to three generations (*12*, *13*), and a few temperature-induced expression changes have been detected for two generations in animals with an inactive nuclear RNA interference (RNAi) pathway (*14*). In contrast, gene silencing initiated by exogenous double-stranded RNA (dsRNA) or piwi-interacting RNAs (piRNAs) can sometimes be stably inherited between generations (*15*–*19*).

When we subjected *C. elegans* to high temperature (25°C), expression from *daf-21 (Hsp90)* promoter::fluorescent protein constructs was strongly elevated (fig. S1). Expression from a single-copy transgene was still elevated in the progeny of animals transferred to 20°C after five generations at 25°C but not in their descendants (fig. S1A). In contrast, expression from an integrated multicopy array took 14 generations to return to basal levels after the temperature was reduced after 5 generations at 25°C (Fig. 1A and fig. S1). A single generation of growth at 25°C was sufficient to generate a seven-generation memory of increased expression (fig. S1C). Multigeneration inheritance of temperature-induced expression and a transgene-dependent phenotype was also observed with other high-copy arrays (table S1).

mRNA transcribed from a *daf-21* promoter array is first detected in wild-type (WT) worms at the 16-cell stage of development; this confirms no maternal supply of mRNA to the embryo (fig. S2) (*20*). Expression differences inherited from parents reared at different temperatures or sorted according to their expression were apparent from the onset of zygotic transcription (Fig. 1B and fig. S3), and genetic crosses demonstrated inheritance through both oocytes (Fig. 1C) and sperm (Fig. 1D). The array is therefore inherited in an inactive state but poised for a specific level of activation that reflects expression in the previous generation.

To distinguish whether inheritance occurs in cis with the DNA locus or in trans—for example, in the cytoplasm—we crossed worms with high and low expression to each other and then crossed the resulting F_1 male progeny to WT hermaphrodites (fig. S4) (*20*). The bimodal distribution of expression in the F_2 progeny indicates that the major mode of inheritance is in cis with the locus (Fig. 1E) (*21*).

To investigate chromatin modifications as potential mediators of this inheritance, we quantified histone modifications on the array in early embryos developing at 20°C whose grandparents had developed at either 16° or 25°C (Fig. 2A). Embryos whose grandparents developed at 25°C had less of the repressive histone modification H3K9me3 on the array than embryos whose grandparents developed at 16°C (Fig. 2, A and B). This difference was apparent in early embryos before the onset of zygotic transcription, indicating that the altered chromatin is not a secondary response to altered transcription in the embryo (Fig. 2, A and B). No differences were observed in the Polycomb-associated repressive modification trimethylated histone 3 lysine 27 (H3K27me3) or in H3K36me3 and H3K4me2, two modifications associated with active chromatin (Fig. 2B and fig. S5). The differences in H3K9me3 were maintained in late embryos after the onset of transcription (fig. S6).

No mRNA expression from the array was detected in the adult germ line (fig. S7). However, H3K9me3 was reduced on the array in the germline nuclei of adults that had been transferred from 16° to 25°C as embryos (Fig. 2, C and D, and fig. S8). Therefore, high temperature during germline development results in depletion of H3K9me3 from the array, even though there is no production of stable transcripts in this tissue.

The putative histone methyltransferase, SET-25, is responsible for all detectable H3K9me3 in *C. elegans* embryos (*22*) (fig. S5B), colocalizes with H3K9me3-enriched transgenic arrays within embryonic nuclei (*22*), and is required for the maintenance of piRNA-initiated stable gene silencing (*15*). Inactivating *set-25* increased expression from the array, with no difference in expression between animals maintained at 20° or 25°C (Fig. 3, A and B). Hence, the repression of the array at low temperature requires SET-25. Moreover, no differences in expression were observed between the F_1 offspring of *set-25* hermaphrodites mated with male animals transmitting an array with either high or low expression (Fig. 3B). In contrast, the inactivation of seven other small RNA pathway or chromatin components (including a Polycomb mutant *mes-2*) showed no obvious defects in the transmission of the expression memory (fig. S9). Even after >20 generations of growth at a constant temperature, substantial variation in transgene expression is observed in both WT and *set-25* mutant populations (Fig. 3C). In WT animals, these differences are transmitted to the next generation (Fig. 3C), but this is not the case in *set-25* mutants (Fig. 3C).

Our results suggest a simple model for how the transgene array shows memory of high-temperature exposure that endures for many generations (fig. S10). High temperature inhibits SET-25–mediated repression in the germ line, causing loss of H3K9me3 from the array. This derepressed chromatin is transmitted to subsequent generations, resulting in increased expression when transcription initiates in somatic lineages. Over multiple generations of growth at low temperature, repression is gradually restored by heterochromatin remodeling in each germline cycle. This is consistent with previously reported gradual quantitative intergenerational changes in H3K9me3 following a temperature change at some loci (*14*).

We tested whether this model predicts the behavior of endogenous loci in the genome by sequencing RNA from *set-25* mutants and WT animals at 20° and 25°C and from WT animals three generations after a change from 25° to 20°C. For protein-coding genes, derepression in *set-25* mutants provided weak prediction of increased

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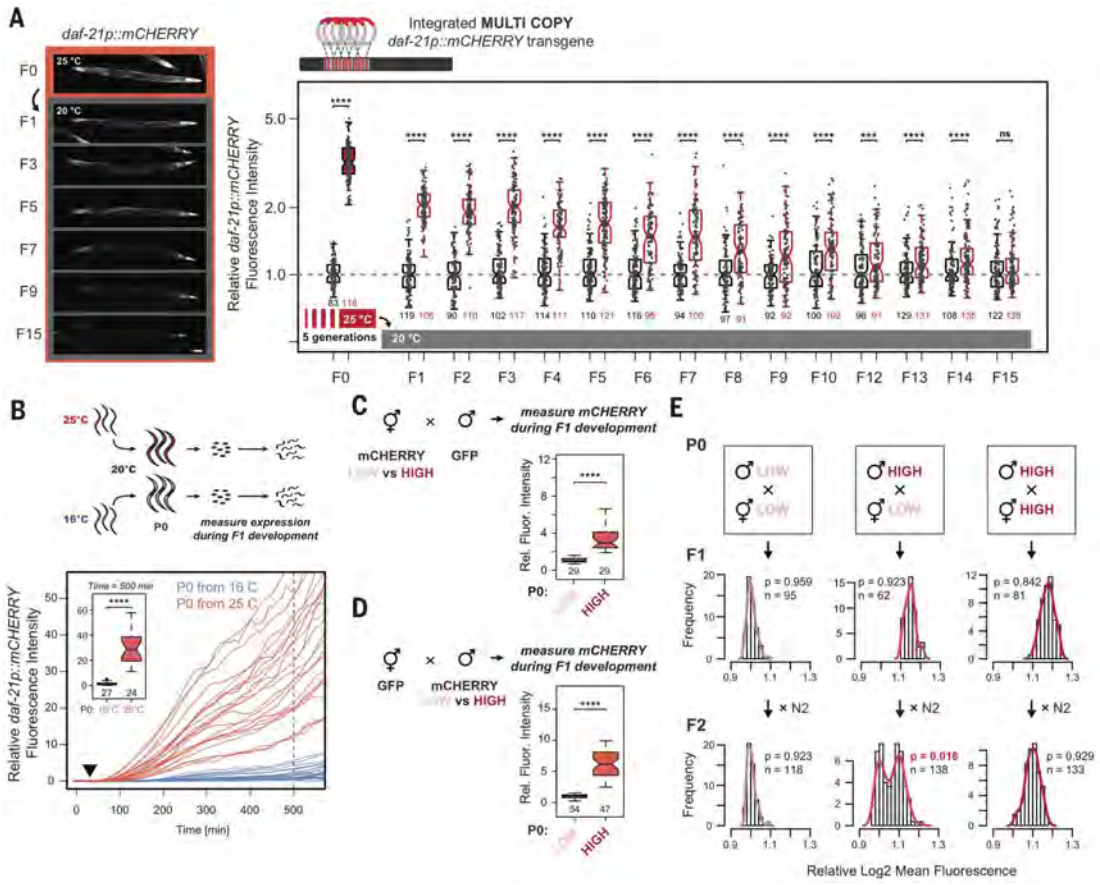

Fig. 1. Fourteen-generation memory of high temperature. (**A**) Adult expression of a *daf-21p::mCHERRY* integrated multicopy transgene at 20°C after five generations at 25°C. Scale bar, 0.1 mm. Stage-matched worms at 20°C are used as a reference for normalization (black). False-discovery rates (FDR) *q* values: ****$q < 0.0001$; ***$q < 0.001$; ns, $q > 0.05$ (Wilcoxon test). Sample size indicated. (**B**) Expression in embryos from animals transferred to 20°C at the L4 larval stage (inset: quantification at 500 min). Arrowhead indicates start of zygotic transcription of the transgene. Transmission occurs through oocytes (**C**) and sperm (**D**) and in cis with the locus (**E**). See fig. S4 for experimental design, intensities normalized to the "low" (low-expression) population; sample size and *P* value for Hartigans' dip test for unimodality. (B) (inset), (C), and (D), ****$P < 0.0001$.

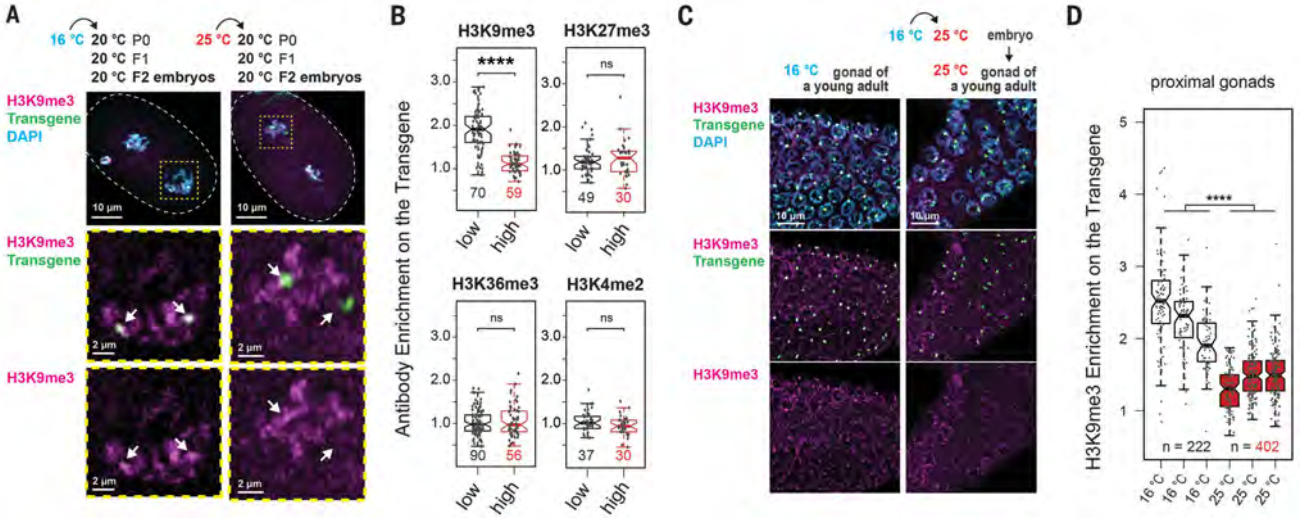

Fig. 2. Changes in H3K9me3. (**A** and **B**) H3K9me3 is depleted from the transgene locus in the F_2 descendants of animals grown at 25°C. L4 larvae from populations grown at 16° or 25°C were transferred to 20°C and cultivated until the following F_1 generation reached adulthood. The F_2 embryos were extracted and histone modifications quantified on the array by immunofluorescence combined with DNA fluorescence in situ hybridization (DNA FISH). (A) Representative two-cell–stage embryos stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue), antibody against H3K9me3 (anti-H3K9me3) (pink), and a DNA FISH probe complementary to mCHERRY (green). Arrows indicate transgene loci. See fig. S5. (B) Quantification of histone modifications in early embryos. (**C** and **D**) Development at high temperature from embryo to adult results in reduced H3K9me3 on the array in the germline nuclei of adults. Gonads were extracted from adult worms shifted from 16° to 25°C during embryonic development, fixed, stained, and compared with those from animals kept constantly at 16°C. (C) Representative gonads stained with DAPI (blue), anti-H3K9me3 (pink), and a DNA FISH probe complementary to mCHERRY (green). (D) Each boxplot quantifies the nuclei of a single gonad (see also fig. S8). (B) and (D), ****$P < 0.0001$; (B) ns, not significant.

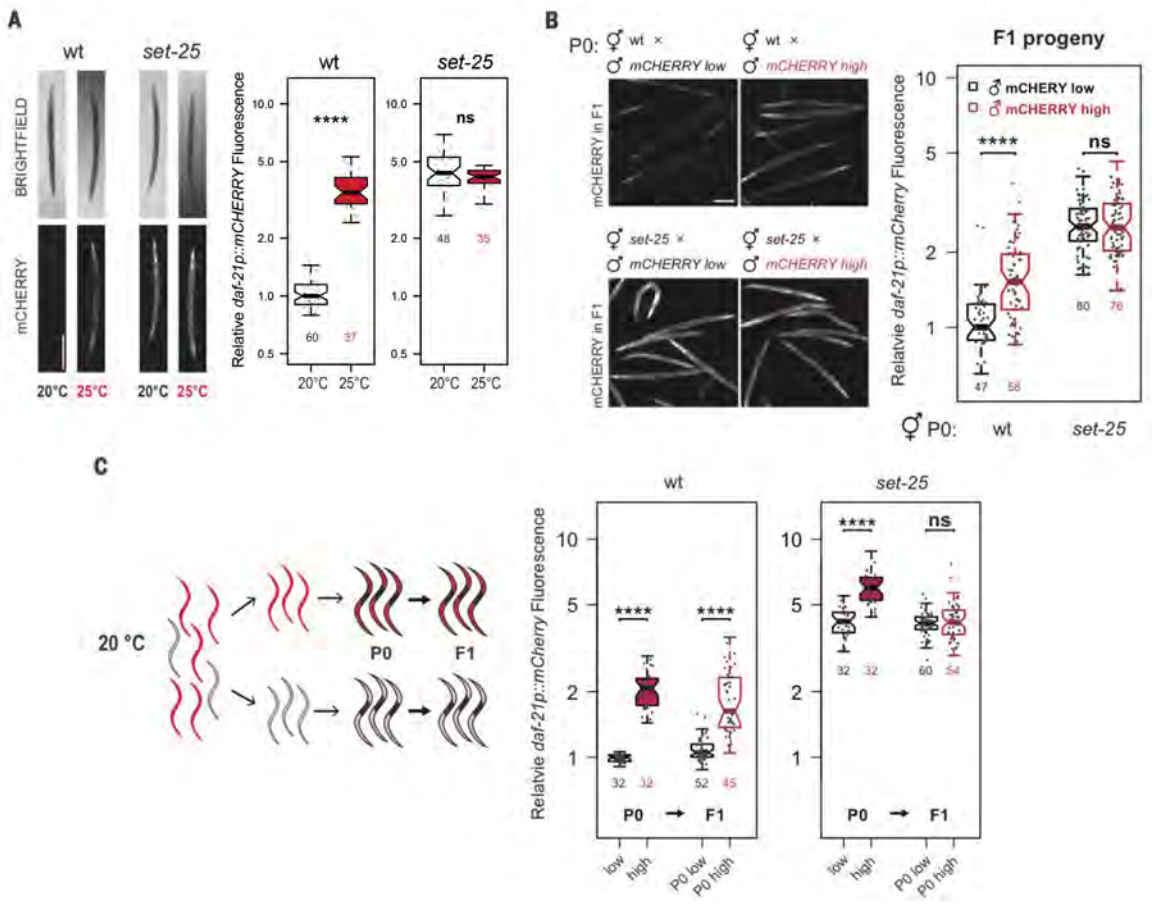

Fig. 3. Requirement for SET-25. (**A**) Quantification of *daf-21p::mCHERRY* expression in L4 larvae at 20° and 25°C in WT and *set-25* mutants. (**B**) Expression of a paternally derived transgene in the adult progeny of WT and *set-25* mutant mothers. A common batch of low- and (temperature-induced) high-expressing males was used. (**C**) Quantification of *daf-21p::mCHERRY* expression in the self-progeny (F_1) of parental (P0) animals sorted into high and low groups based on transgene expression at the L4 stage in WT and *set-25* mutants. ****$P < 0.0001$; ns, not significant. (A) and (B) Scale bars, 0.2 mm.

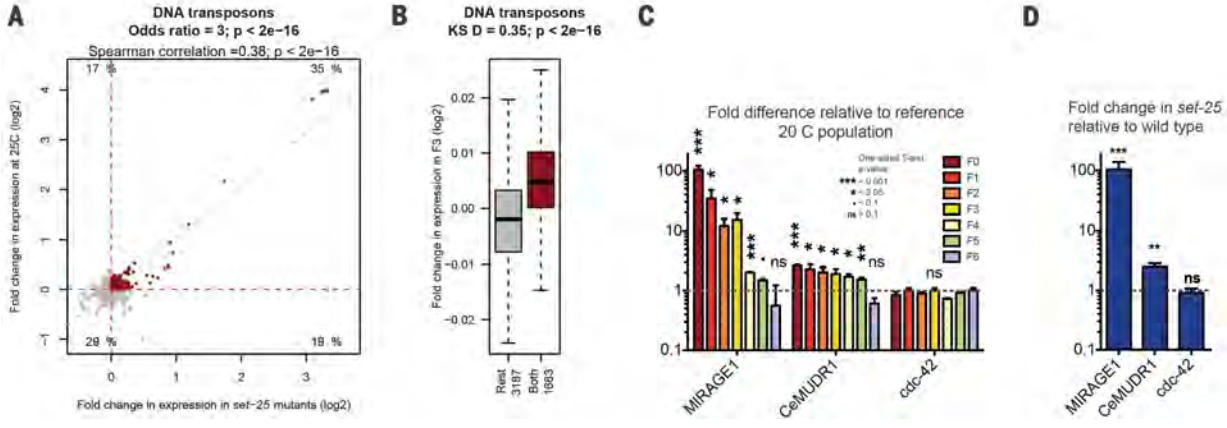

Fig. 4. Epigenetic expression memory of endogenous loci repressed by SET-25. (**A**) DNA transposon expression change in *set-25* mutants and at high temperature. Odds ratio quantifies the overlap (red loci, "both") between $\log_2$ fold change (FC) > 0. (**B**) FC expression three generations after a reduction in temperature from 25° to 20°C. Kolmogorov–Smirnov (KS) test statistic and *P* value are shown. See figs. S11 to S14 and table S4 for other repeats, protein coding genes, and analysis methods. (**C**) Expression of two DNA transposons at 25°C (F_0) and for six generations after decreasing the temperature to 20°C determined by quantitative PCR (table S3). *cdc-42* is a housekeeping gene as control. Expression is relative to animals grown at 20°C in parallel. (**D**) Expression of the same DNA transposons is increased in *set-25* mutants. **$P < 0.01$; ***$P < 0.001$; ns, not significant.

expression at high temperature (fig. S11), consistent with a larger contribution from other regulators, such as specific transcription factors. Derepression in *set-25* mutants was, however, a better predictor of increased expression at high temperature for multiple classes of repetitive elements and also for pseudogenes (Fig. 4A and figs. S11 to S14), consistent with impaired SET-25 activity's making an important contribution to the increased expression of many loci at high temperature.

Moreover, the increased expression of loci repressed by SET-25 with increased expression at high temperature was, although small, still detectable three generations after a return to low temperature (Fig. 4, A and B, and figs. S11 to

S14). Quantifying the expression of two DNA transposons by quantitative real-time polymerase chain reaction (PCR) in independent samples confirmed that their expression remained elevated for four and five generations after a return to 20°C (Fig. 4C and fig. S15). Their expression was also confirmed as SET-25–dependent (Fig. 4D).

Taken together, these results support the mechanistic model: At high temperature, SET-25 pathway activity is reduced, resulting in the derepression of many loci in the genome. After a return to low temperature, SET-25 activity is restored, but it takes multiple generations for repression to be completely reestablished. Expression from SET-25–repressed repeats therefore transmits information about a prior environmental exposure in this species.

In mammals, repressed repetitive elements can also escape epigenetic reprogramming (*23*, *24*) with variation in the expression of both individual repeats (*25*) and multicopy heterochromatic transgenes (*26*) being transmitted between generations. In flies, diet- (*6*) and stress-induced (*5*) changes in heterochromatin can also be transmitted for at least one generation. It is possible, therefore, that environmentally triggered changes in heterochromatin may provide a general mechanism for the epigenetic transmission of information between generations. It is interesting to speculate that the inheritance of environmentally triggered changes in expression from repressed chromatin may have been coopted to provide adaptive benefits to an organism.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6335/320/suppl/DC1
Materials and Methods
Figs. S1 to S15
Tables S1 to S4
References (*27*–*35*)

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