eLife digest Genetic material is contained within molecules of DNA. In plants and many other organisms, these DNA molecules are packaged around proteins called histones to make a structure known as chromatin. Altering how the DNA is packaged in chromatin can control the activity of genes. For example, a group of proteins called the Polycomb Repressive Complex 2 (PRC2) adds methyl tags to histones, which can alter the packaging of chromatin to lower the activity of particular genes.

When a cell divides, it is sometimes important that genes in the daughter cells have similar levels of activity as the parent cell. This allows individual cells to 'remember' past events, such as exposure to cold temperatures or other environmental conditions. The pattern of methyl tags on histones can be passed onto the daughter cells, but it is not clear if this is actually responsible for providing the memory.

One gene that PRC2 regulates is called *FLC*, which influences when a plant called *Arabidopsis* produces flowers. If the plants are exposed to cold temperatures, the activity of *FLC* is repressed. *FLC* activity remains low after the period of cold has ended to ensure that the plants produce flowers at an appropriate time. If this 'memory of cold' is held locally in the chromatin of the *FLC* gene, then it should be possible for two copies of the *FLC* gene in the same cell to show different gene activities. However, if the memory is stored more globally inside each cell by other proteins, then the two copies should have identical gene activities.

To distinguish between these two possibilities, Berry et al. added different fluorescent tags to two copies of the *FLC* gene in *Arabidopsis* plants, which allowed the activity of each gene copy to be tracked in individual cells under a microscope. The experiments show that one copy of *FLC* may be switched off, while the other remains switched on inside the same cell. Furthermore, it was found

in the absence of functional PRC2 (Gaydos et al., 2014). Other studies have suggested that Polycomb proteins themselves are maintained locally through DNA replication (Petruk et al., 2012; Francis et al., 2009).

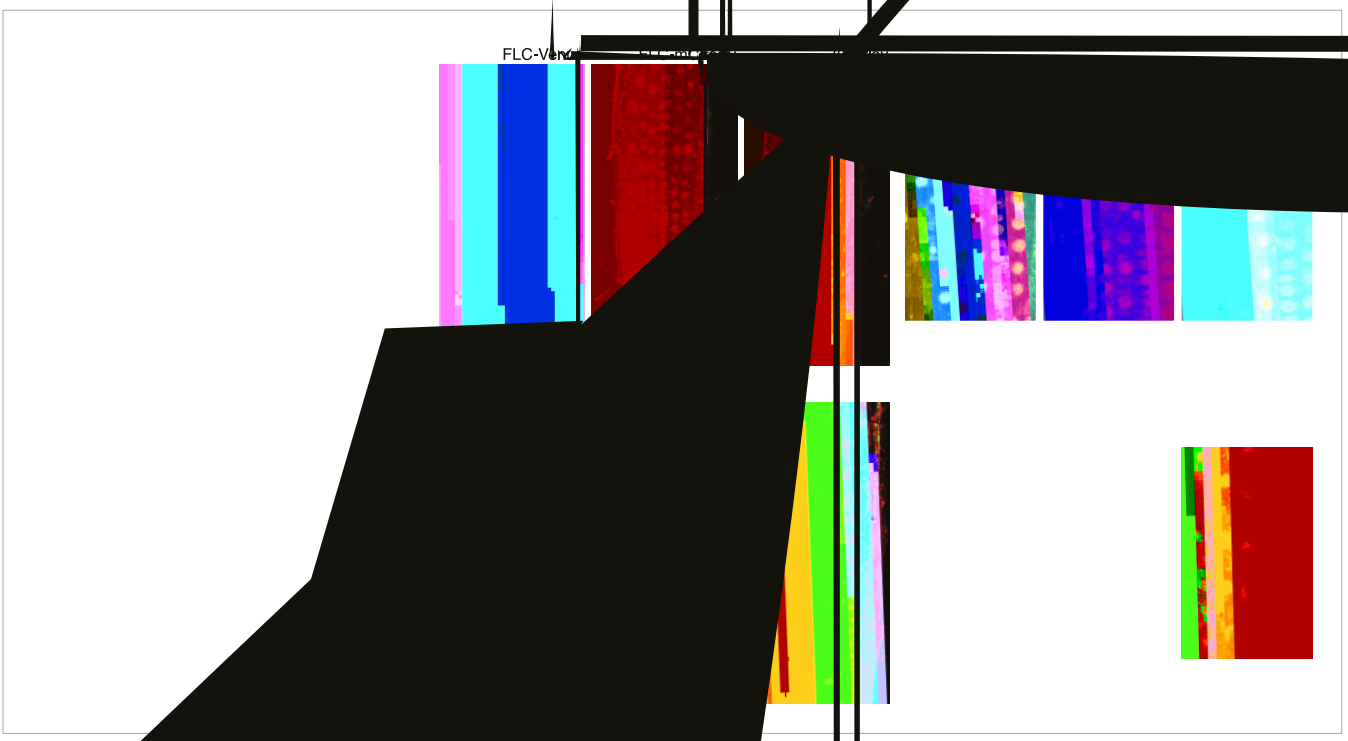
These studies provide valuable mechanistic information about how a Polycomb system store the memory of transcriptional states of individual genes in cis. However, to definitively demonstrate that memory stored in cis, it is necessary to show that two copies of the same DNA sequence can be independently maintained in different transcriptional states in the same nucleus (Bonasio et al., 2010). This has been observed in genomic imprinting and in random X-chromosome inactivation. Since imprinting involves DNA methylation (Ferguson-Smith, 2011), and X-chromosome inactivation involves chromosome-wide changes in chromatin structure and nuclear positioning (Gendrel et al., 2014), it remains an open question whether Polycomb-repressed chromatin at a single gene can store epigenetic memory. Cis memory has also been implicated in random monoallelic expression. However, studies so far have been limited to genes with naturally occurring genetic polymorphisms (Eckersley-Maslin et al., 2014; Deng et al., 2014) or are performed on fixed tissues limiting conclusions regarding heritability (Gendrel et al., 2014). Importantly, a requirement for chromatin-modifying factors in epigenetic memory does not necessarily imply that memory is itself stored in chromatin.

To address the question of whether Polycomb-repressed chromatin at a single gene can instruct its own inheritance and therefore constitutes a cis memory system, we exploited the classic epigenetic process of vernalization in *A* . Vernalization is the acceleration of flowering following prolonged cold exposure and is mediated by cold-induced epigenetic repression of the Polycomb target gene and floral repressor *FLC*. *FLC* repression requires PRC2 but is independent of DNA methylation (Finnegan et al., 2005), making it a useful system for studying the cis-memory storage capability of Polycomb at a single gene. In vernalized plants, *FLC* expression is bistable, with the

number of cells in which *FLC*

cells in the *FLC*- /*FLC*- *C* ON/OFF and OFF/ON expression states (Figure 3—figure supplement 2), we consider it unlikely that differences in genomic location or fluorophore sequence between the transgenes causes one of the copies to be preferentially repressed over the other.

Our findings demonstrate that the molecular changes to the chromatin environment of *FLC* induced by prolonged cold exposure are sufficient to instruct epigenetic inheritance of the Polycomb-repressed transcriptional state. However, Polycomb complex binding and H3K27me may not be the only factors that constitute this locally encoded epigenetic state. Specific nucleic acid structures (Klose et al., 2013), non-coding RNAs (Herzog et al., 2014



nts, plants were transferred from plates to soil 5 days after vernalization. grown almost vertically on MS plates supplemented with 1% (wt/vol) tagel (Sigma–Aldrich, St. Louis, MO, P8169).

trogressed active *FRIGIDA* allele (*FRI*-sf2), and was previously described the *f -2* mutant in Col-FRI (Michaels et al., 1999). *FLC*- and fusions were generated in a similar way to a previously published w et al., 2004). Specifically, either the (Nagai et al., 2002) or 2004) coding sequence was inserted into the NheI site of *FLC* exon 6. XhoI genomic fragments were transferred into pSLJ-755I6 (Jones et al., 1992) *-2 FRI*-sf2 plants using *A f* . Many (>30) independent analysed rescued the early-flowering phenotype of parental *f -2 FRI*-sf2 plants flowering in response to cold exposure. Transgenic *FLC* lines were selected for n similarity between expression level of the transgene and that of endogenous single-copy transgene were identified using a qPCR-based assay adapted for viously described method (Bartlett et al., 2008) (performed by IDna Genetics, UK). quences are listed here: Bar-F: ggccgagtcgaccgtgta; Bar-R: ttgggcagcccgatga; caccagcggacggga-TAMRA; AtCO-F: gtccgggtctgcgagtca; AtCO-R: gctgtgca- tCO-Probe: VIC-tgctccggctgctttttgtgtgag-TAMRA. Single-copy *FLC*- and

Consecutive z-stack images were separated by 3 μm and each root typically contained 14–18 images, which encompassed approximately the top third of the root in the meristematic and elongation zones. It was observed that FLC-Venus intensity decreased with depth in the image stack (Figure 2—figure supplement 5B

Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. 2002. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. *Nature Biotechnology* 20:87–90. doi: 10.1038/nbt0102-87.
Novick A, Weiner M. 1957. Enzyme induction as an all-or-none phenomenon. *Proceedings of the National Academy of Sciences of USA* 43:553–566.