

Silencing by Throwing Away: A Role for Chromatin Diminution

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Chromatin diminution during development generates cells with varying genetic content within the same organism. Two recent papers demonstrate that in two different systems chromatin diminution removes a considerable number of genes from somatic cells, thereby restricting their expression to the germline.

In multicellular organisms, all cells typically contain identical genetic information. As a consequence, every cell carries all the genetic information necessary to build an entire individual. However, there are exceptions to this rule. The purposeful elimination of entire chromosomes (chromosome elimination) or parts of chromosomes (chromatin diminution) from certain cells during development occurs in a relatively small number of species but over a wide phylogenetic range (Tobler and Muller, 2001). The first and still best-known case of chromatin diminution was originally described by Boveri (1887) in early embryos of giant round worms. Although the phenomenon has been known for more than 120 years, the exact nature of the eliminated chromatin, the mechanism of elimination, and the biological role of chromatin diminution has remained largely elusive in any species employing chromatin diminution. A few eliminated loci and chromosome breakpoints have been identified and characterized in the past, but until very recently it was impossible to address these questions comprehensively because suitable methodology was not available. Two recent papers, one in this issue of *Developmental Cell* (Wang et al., 2012) and one recently published in *Current Biology* (Smith et al., 2012), demonstrate how two unrelated species employ chromatin diminution to remove large numbers of genes from somatic tissues, thereby restricting their expression to the germline and the early embryo.

In *Ascaris suum* and closely related nematodes, chromatin diminution occurs in multiple somatic blastomeres and creates a genetic difference between the germline and the soma (Tobler and Muller,

2001). It has been shown that a large portion of the eliminated material consists of noncoding repetitive sequences. A very small number of genes were also known to undergo chromatin diminution (Etter et al., 1994), but it was unclear whether this was biologically significant or just a tolerated side effect. These findings did not allow the determination of whether chromatin diminution serves merely as a way of disposing of “junk DNA” or whether it is also used to deliberately silence genes in somatic cells.

In a landmark paper in this issue of *Developmental Cell*, Wang et al. (2012) compared the genomes of the germline and multiple somatic lineages of a single male and a single female *A. suum*. The authors found that about 13% of the 334 Mb present in the germline was absent from somatic tissues. Although the exact location of the breakpoints varied slightly (within several hundred to a few thousand base pairs), the same sequences were eliminated from the different somatic cell lineages of both individuals. In contrast to *Strongyloides papillosus*, a nematode in which chromatin diminution is restricted to males and removes only one of the two copies of affected genes (Nemetschke et al., 2010), in *A. suum* chromatin diminution always removed both gene copies, leading to the complete absence of the corresponding genetic information from the soma of both sexes. The authors identified 52 DNA breakpoints. In no case was there indication of rejoining of ends after the elimination of an internal fragment, but rather the stabilization of the new chromosome ends appeared to occur exclusively through the addition of new telomeres, as had been demonstrated earlier for a few breakpoints (Müller et al., 1991).

The molecular mechanisms that define the genomic regions to be eliminated or maintained remain completely mysterious. The regions around the breakpoints show no obvious common sequence features, and the authors also present evidence that an RNA-guided mechanism similar to the one involved in DNA elimination during ciliate macronucleus formation (Chalker and Yao, 2011) is unlikely to be at work in *A. suum*.

About 70% of the eliminated chromatin in *A. suum* consists of a 121 bp satellite repeat sequence, but the remaining 12.7 Mb are nonrepetitive and contain almost 700 genes. Considering the total number of genes present in *A. suum*, estimated to be about 15,500 by the authors and about 18,500 by an earlier publication (Jex et al., 2011), this means that somatic cells lack about 4% of the genes present in the germline. By high-throughput RNA sequencing, the authors demonstrated that the expression of these genes is indeed limited to the germline and the early embryo, with more than half of them specifically expressed in the testis. It is noteworthy that not all genes with germline-specific expression are eliminated from the soma. Another striking point is that a large portion of the eliminated genes code for components of basic cellular machineries, in particular, translation. About half of the eliminated genes have paralogs, homologous genes derived from gene duplication events, and these paralogs are not affected by chromatin diminution; perhaps the germline-specific duplicates and the ones retained in the soma have evolved different or more-specialized functions.

These findings strongly suggest that at least one of the roles of chromatin diminution is to remove from the soma

germline-specialized paralogs and other germline-specific genes.

This function of chromatin diminution may be more widespread. In an evolutionarily independent case of chromatin diminution, about 20% of the DNA present in germ cells of lampreys (*Petromyzon marinus*) is removed from the soma during early embryogenesis (Smith et al., 2009). In a recent paper in *Current Biology*, Smith et al. (2012) used hybridization-based assays and low-coverage sequencing to survey about 10% of the germline genome. Although not as comprehensive as the analysis of *A. suum* described above, this study clearly demonstrates that hundreds to thousands of protein-encoding genes are eliminated from somatic cells in the process, in addition to a large amount of repetitive noncoding DNA. As in *A. suum*, many of the eliminated genes are predicted to function in

basic cellular processes (e.g., transcription). Also like in *A. suum*, breakpoints in lampreys appear to share no conserved sequences, but the authors noticed short palindromic sequences at multiple junctions of germline-specific and somat-retained sequences.

Together, these two studies demonstrate that chromatin diminution in giant roundworms and in lampreys serves to spare somatic cells the costs of replicating and maintaining large quantities of unneeded DNA and also represents a highly efficient “throw-away approach” to gene regulation for an unexpectedly high number of genes whose products are only desired or even only tolerated in the germline.

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A New Direction for Gene Looping

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Upon binding to a promoter, RNA polymerase II can synthesize either a coding mRNA or a divergently transcribed noncoding RNA. In a recent issue of *Science*, Tan-Wong et al. (2012) find that intragenic looping increases the proper orientation of RNA polymerase II, reducing the production of divergent noncoding transcripts.

Chromatin frequently assumes higher-order arrangements that facilitate transcriptional regulation. For example, chromatin loops can bring distal regulatory elements into close proximity to promoters (Krivega and Dean, 2012). Such loops can promote gene expression by allowing distal enhancers to contact a promoter; they can also function to insulate neighboring chromatin domains. Genes themselves can also loop through interaction of the promoter with the terminator (O'Sullivan et al., 2004). Intragenic looping is transcription dependent and requires components of the transcription preinitiation complex (TFIIB) and pre-mRNA 3'-end processing complex (Hampsey

et al., 2011) (Figure 1). Chromosome conformation capture (3C) has revealed intragenic looping of many genes, including the yeast genes *GAL10* (2.1 kb), *HEM3* (1.0 kb), and *FMP27* (7.9 kb), as well as the mammalian genes *BRCA1* and *CD68* and the HIV-1 provirus (Hampsey et al., 2011). Although intragenic looping requires transcription, loss of looping does not strongly affect transcription (Singh and Hampsey, 2007). For a few genes, it has been suggested that intragenic looping might affect their reactivation rate after repression, a phenomenon called transcriptional memory. However, the general functional significance of intragenic looping still remains unclear.

In a recent issue of *Science*, Proudfoot, Steinmetz, and colleagues described work suggesting that intragenic looping plays an important role in regulating divergent transcription, reducing the production of divergently transcribed noncoding RNAs (ncRNAs) (Tan-Wong et al., 2012). The phenomenon of divergent transcription is common to most active promoters in diverse organisms (Seila et al., 2009). Upon assembly of the preinitiation complex, RNA polymerase II (RNAPII) can initiate and transcribe in either direction, one producing an mRNA and the other producing a short, rapidly degraded ncRNA. These cryptic unstable transcripts (CUTs) are widespread but scarce,