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 soma-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,

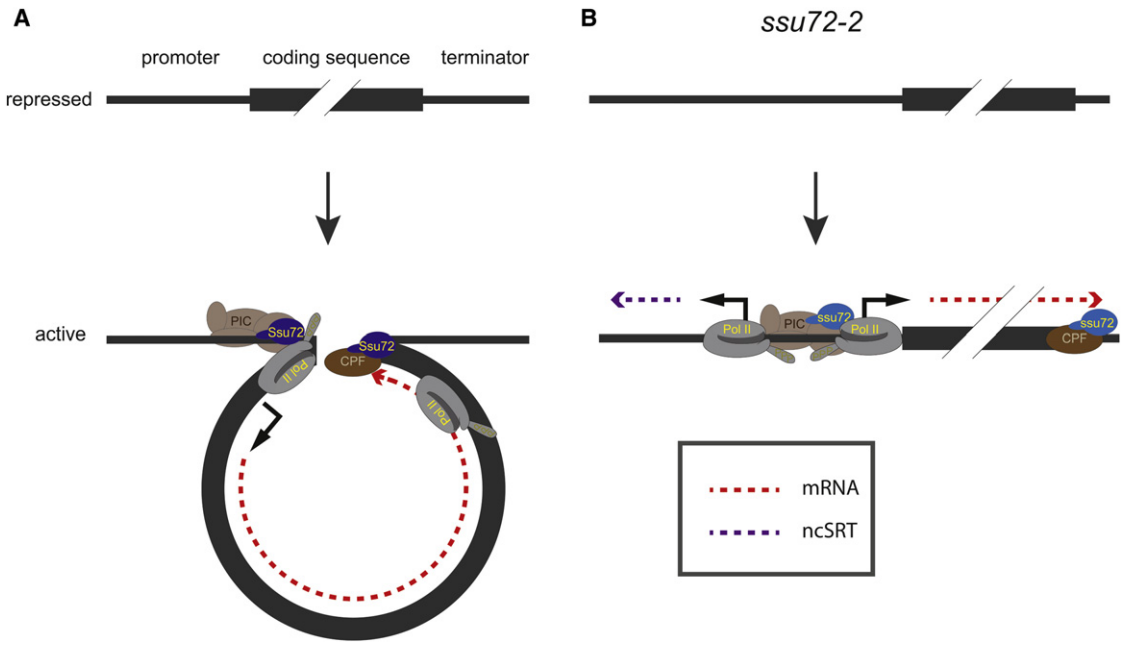

Figure 1. Gene Loops Enhance Transcriptional Directionality
(A) Top: inactive gene, with different portions indicated; bottom: active gene. Actively transcribed genes form an intragenic loop between their promoters and terminators.
(B) A mutation in Ssu72 (*ssu72-2*) results in loss of intragenic looping and divergent transcription of promoter-associated ncRNA.
The following abbreviations are used: PIC, preinitiation complex; Pol II, RNA polymerase II; CPF, cleavage and polyadenylation factor; mRNA, messenger RNA; ncSRT, noncoding Ssu72 restricted transcript. Wild-type Ssu72 appears in dark blue, whereas mutant ssu72-2 appears in light blue.

because they are rapidly degraded by the nuclear exosome (Arigo et al., 2006). They can arise from the nucleosome-free regions associated with promoters or the 3′ end of genes (Xu et al., 2009). It is unclear whether the production of CUTs has any adaptive value or whether it is merely a cost associated with a permissive nucleosome arrangement. However, it is intriguing that CUTs can regulate expression of certain mRNAs by recruiting repressive histone-modifying factors to the promoter (Camblong et al., 2007).

The authors tested the hypothesis that intragenic looping enhances the directionality of transcription by examining the expression of a divergently transcribed ncRNA at the *FMP27* locus in *S. cerevisiae* (Tan-Wong et al., 2012). A mutation in Ssu72 (*ssu72-2*), a component of the cleavage/polyadenylation factor that also interacts with the preinitiation factor TFIIB (Hampsey et al., 2011), blocks intragenic looping and leads to increased accumulation of a divergently transcribed ncRNA and increased RNAPII density over FMP27 promoter (Figure 1). Genome-wide profiling of total RNA in wild-type and *ssu72-2* mutant strains, using strand-specific microarrays, identified many ncRNAs that were induced. In addition to assessing the effect of Ssu72 loss, the authors also examined the effect of loss of Rrp6, a component of the nuclear exosome (Arigo et al., 2006). When RNA from *ssu72-2* cells, *rrp6Δ* cells, and *rrp6Δ ssu72-2* cells was compared with RNA from wild-type cells, the authors observed both CUTs (ncRNAs that accumulate in *rrp6Δ* mutants) and additional ncRNAs that accumulated in the *ssu72-2* mutants. These additional ncRNAs were named Ssu72-restricted transcripts (SRTs). Like CUTs, SRTs frequently arise from promoter regions in a divergent orientation from the gene (Figure 1).

Of the 605 SRTs and 1,982 CUTs identified in the array profile, the authors focused on the 135 SRTs and 678 CUTs that were transcribed divergently between tandem open reading frames. The *ssu72* mutation resulted in additional RNAPII accumulation upstream of TSSs and over SRTs. Mutations in other factors required for intragenic looping, such as TFIIB (Sua7) and Pta1, also increased divergently transcribed SRTs. Additionally, loss of Ssu72 led to increased histone H4 acetylation over SRT-producing promoters. Overall, this suggests that gene looping decreases divergent transcription by a mechanism that involves histone H4 deacetylation. Loss of the histone H4 deacetylase Rco1 also led to expression of many ncRNAs. However, the ncRNAs induced by loss of Rco1 are derived from the 3′ end of genes, as opposed to SRTs, which are derived from divergent transcription from promoters. This suggests that intragenic looping has a direct role in regulating transcriptional directionality.

To test whether *cis* mutations that affect intragenic looping would also lead to changes in RNAPII directionality, the authors examined the effects of replacing the polyadenylation signal (PAS) in the 3′ UTR with an Rnt1 cleavage signal (RCS). This results in normal termination but blocks polyadenylation and intragenic looping. Replacement of the PAS with RCS in two yeast genes and in the β-globin transgene in human embryonic kidney cells increased the divergent transcription of ncRNAs by 3-fold. This suggests that intragenic looping plays a

conserved role in regulating transcriptional directionality.

These results suggest that formation of gene loops influence unidirectional transcription. How might this work? Based on the acetylation of histone H4 in promoters of genes that exhibit divergent SRTs, the authors postulate that looping leads to directional histone deacetylation and repression upstream of the promoter. An alternative view is that looping leads to directional acetylation within the loop. Also, because recruitment of RNAPII to the promoter is often rate limiting, if intragenic looping permits more efficient recycling of RNAPII for reinitiation, it is tempting to speculate that this might also bias transcriptional directionality. Many components of the preinitiation complex remain associated with the promoter, potentially serving as a scaffold to allow for such recycling. Consistent with this notion, RNAPII associated with the active *hsp70* locus in flies is not readily exchanged with the nuclear pool, suggesting that this locus is somehow "compartmentalized" and that RNAPII is recycled (Zobeck et al., 2010). Resolutions of these questions will await a better understanding of how looping affects chromatin structure, histone acetylation, and RNAPII function.

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IRE1, a Double-Edged Sword in Pre-miRNA Slicing and Cell Death

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IRE1α, the most conserved transducer of the unfolded protein response, plays critical roles in many biological processes and cell fate decisions. Reporting in *Science*, Upton et al. (2012) broadened our understanding of IRE1α as a cell-death executioner, showing that upon ER stress, IRE1α degrades microRNAs to promote translation of caspase-2.

In eukaryotic cells, the endoplasmic reticulum (ER) is a highly specialized organelle responsible for the translation, folding, and modification of approximately one-third of the cell's proteome. Upon accumulation of unfolded/misfolded proteins in the ER, cells activate the unfolded protein response (UPR) that is initiated by three ER transmembrane protein sensors: inositol requiring enzyme 1 alpha (IRE1α), PKR-like ER kinase (PERK), and activated transcription factor 6 alpha (ATF6α). The UPR is essential for normal cellular and organismal physiology and contributes to the etiology of many diseases (Wang and Kaufman, 2012). Although initial UPR activation provides an adaptive response, severe or chronic UPR activation redirects the adaptive response into a proapoptotic response, although the mechanisms are unknown. Among the ER stress sensors, IRE1α is conserved from yeast to humans. IRE1α has both protein kinase and endoribonuclease (RNase) activities that, in metazoans, were originally characterized to initiate removal of a 26 base intron from X-box binding protein 1 (*Xbp1*) mRNA, thereby producing an active transcription factor that induces genes encoding adaptive functions to limit protein misfolding in the ER. However, IRE1α has a growing list of additional mRNA cleavage substrates identified through regulated IRE1-dependent degradation (RIDD) of mRNAs (Han et al., 2009; Hollien et al., 2009). In a recent report in *Science*, Upton et al. showed that IRE1α cleaves a new class of RNAs: microRNAs (miRs) that repress translation through binding to sequences in the 3′ end of mRNAs. IRE1α-mediated cleavage of miRs releases a translational