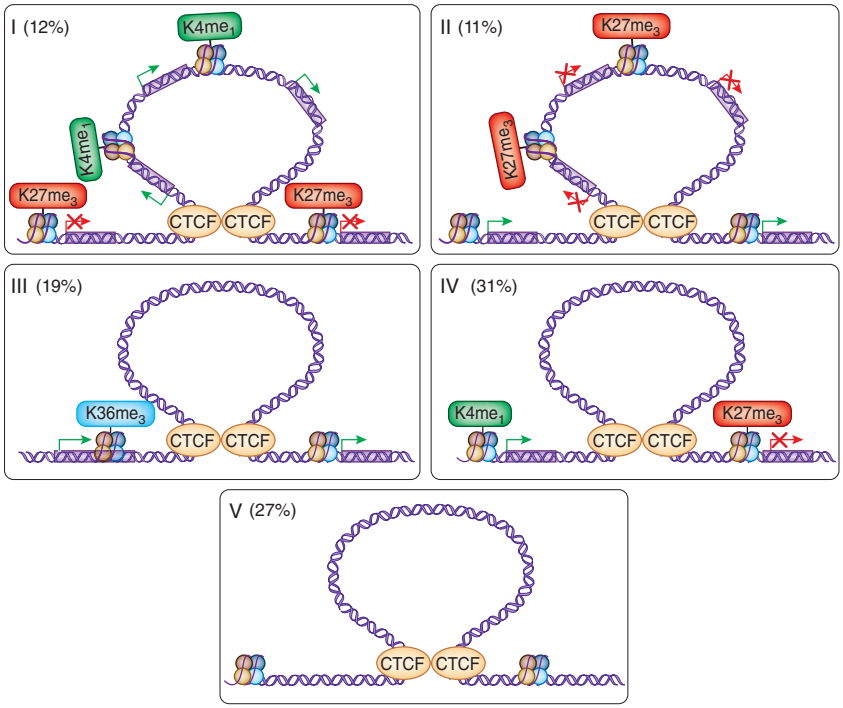

Figure 1 Five distinct chromatin domains are found in association with CTCF in mouse embryonic stem cells. (I) Twelve percent of CTCF-mediated chromatin loops contain active genes (green boxes) and H3K4Me1 in the loop region and inactive genes (red boxes) and repressive marks such as H3K27Me3 outside the loop. (II) Eleven percent of the loops contain inactive genes and repressive marks inside the loop and active genes outside the loop. (III) Nineteen percent of the loops contain H3K36Me3 outside the loop on one side. (IV) Thirty-one percent of the loops contain active genes and H3K4Me1 outside the loop on one side and H3K27Me3 outside the loop on the other side. (V) Twenty-seven percent of the loops do not appear to contain any characteristic gene expression or histone modification patterns.

of genes, as a bridge that connects enhancers to their target genes, thus playing a positive regulatory role. It is unclear whether the apparent discrepancy between the two models actually reflects the ability of CTCF to assume different regulatory roles at different genes.

Another interesting finding is that only a small fraction of the CTCF binding sites (less than 10%) appear to be involved in mediating looping interactions. Although it is possible that a failure to detect other CTCF-mediated looping interactions due to low assay sensitivity accounts for this finding, it is likely that there are actually biological differences between CTCF sites involved in looping and those not involved in looping. To achieve a better understanding of the rules governing chromatin organization, it is necessary to determine what regulates the pairing of the CTCF sites involved in these looping interactions. At present, the authors found no unique properties associated with the CTCF sites involved in looping except that they show a higher degree of enrichment signals for CTCF binding.

Finally, although the focus on CTCF-mediated chromatin loops is an important first step in understanding the higher order structure of chromatin domains, it is highly likely that other types of chromatin looping interactions exist, which may involve additional proteins. To discover such interactions, it will be necessary to have an unbiased view of chromatin structure.

COMPETING FINANCIAL INTERESTS
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The long reach of noncoding RNAs

Elena Sotillo & Andrei Thomas-Tikhonenko

Transcription of genomic loci containing protein-coding genes often yields not only cognate mRNAs but also assorted noncoding RNAs (ncRNAs), which typically map in the vicinity of transcription start sites. A new study shows that far from being random byproducts of gene expression, many long ncRNAs (lncRNAs) are synthesized in a coordinate fashion and control important cellular processes, such as survival in the face of DNA damage.

In 1990, Shirley Tilghman's lab made a puzzling discovery: the very abundant RNA encoded by the H19 gene was transcribed by RNA polymerase II, then spliced and polyadenylated, but unlike canonical messenger RNAs (mRNA), it never associated with the translational machinery[1]. This was later confirmed that the H19 RNA is a fully functional molecule and plays a key role in the imprinting of its own locus[2]. These studies not only ushered in the lncRNA era but also set off a prolonged debate on whether lncRNAs act locally (in *cis*) or globally (in *trans*). There is plenty of evidence in support of various *cis* modes of action. Up to 70% of protein-coding transcripts are thought to be transcribed in both sense and antisense directions[3], and the X-chromosome–encoded Xist RNA 'coats' and silences its own chromosome[4]. However, on p. 621 of this issue, Howard Chang and for lncRNAs[5].

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cis trans

Transcribe locally, act globally

Hung *et al.* used ultrahigh-resolution microarray technology to identify more than 200

lncRNAs that are encoded in close proximity to 56 cell-cycle–controlling genes (cyclins, cyclin-dependent kinases (cdks), cdk inhibitors and so on)[5]. Predictably, during cell-cycle progression, self renewal and neoplastic transformation, levels of the cell-cycle–related mRNAs fluctuated—but so did levels of lncRNAs encoded in their vicinity.

When these fluctuating lncRNAs were grouped based on expression patterns, co-localized lncRNAs usually ended up in the same clusters, suggesting that adjacent ncRNAs are regulated in concert. In principle, they could act locally by regulating the nearby mRNA levels. However, the authors found that the expression of lncRNA clusters did not correlate either positively or negatively with expression of the nearest mRNAs. This finding led the authors to reject the idea that most of lncRNAs function in *cis* and challenged them to identify an alternative mode of action. They focused on a particular lncRNA that is induced by p53, a master regulator of diverse cellular processes ranging from senescence to apoptosis.

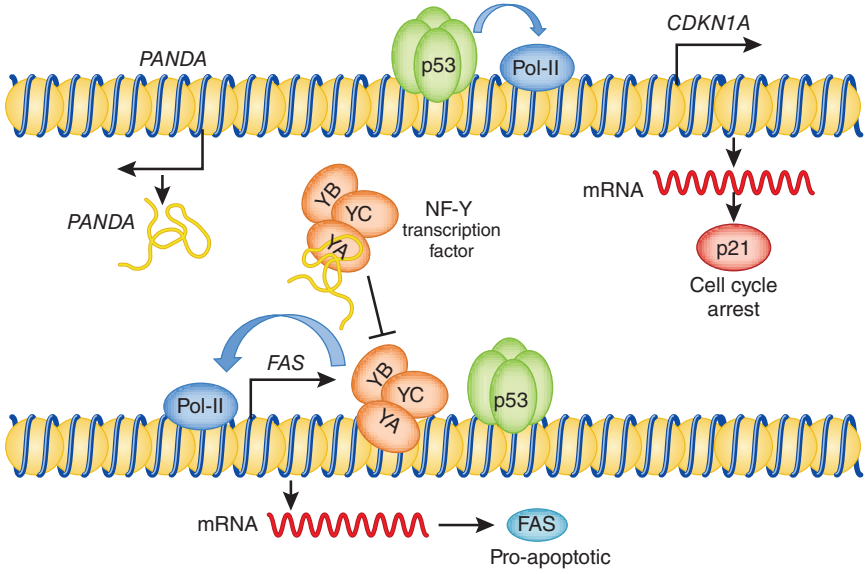

Figure 1 Model of pro-survival effects of *PANDA* lncRNA during the DNA damage response. Upon DNA damage, p53 activates expression of target genes such as *CDKN1A*, which encodes the cell cycle regulator p21. p53 also activates expression of *PANDA* lncRNA encoded upstream of *CDKN1A*. *PANDA* physically interacts with the NF-YA subunit of the NF-Y transcription factor and prevents it from cooperating with p53 on the promoters of p53-dependent pro-apoptotic targets, such as *FAS*.

Trans-fixed by p53

An emerging concept in the RNA field is that lncRNAs can bind and alter the activity of transcription factors[6], and the broader the function of the transcription factor, the longer the reach of the interacting lncRNA. For example, *MEG3*, the maternally expressed gene-3, was found to increase p53 levels and activity through direct interaction with p53 or indirectly by inhibiting the dedicated ubiquitin ligase MDM2 (refs. 7,8). Also, a locus encoding the long intergenic noncoding (linc) RNA lincRNA-p21 was discovered 15 kb upstream of *CDKN1A*. Notably, it clearly impinges on the p53 pathway[9]. Its transcription (along with that of *CDKN1A*) is induced after exposure to DNA damaging agents such as doxorubicin[9], the effects of which are mostly mediated by p53. Once activated, lincRNA-p21 binds to the heterogeneous nuclear ribonucleoprotein K (hnRPN-K), which interacts with repressive transcriptional complexes and assists p53 in inhibiting gene expression[9]. Thus, p53 both regulates and is regulated by the *CDKN1A* locus. Now it turns out that this feedback loop has another kink.

Hung *et al.*[5] identified the lncRNA *PANDA* (P21 associated ncRNA DNA damage activated), which is located between *CDKN1A* and lincRNA-p21. *PANDA* is one of 12 lncRNAs that showed expression changes in response to p53 activation via DNA damage. Its close proximity to *CDKN1A* (only 5 kb upstream on the antisense strand) suggested that it might regulate *CDKN1A* expression. However, upon DNA damage, *PANDA* was induced appreciably earlier than *CDKN1A* mRNA. Also, knockdown of *PANDA* had no effect on p21 expression. Rather, knockdown of *PANDA* selectively enhanced induction of p53-regulated pro-apoptotic genes such as *FAS* and *APAF1*. These p53 targets are distinguished from cell-cycle–related p53 targets by the presence of binding sites for the transcription factor NF-Y in their promoters[10].

NF-Y connection

NF-Y is a heterotrimeric complex composed of NF-YA, NF-YB and NF-YC, of which NF-YA is an important regulatory subunit[11]. There is a close and complex relationship between NF-Y and p53. On the one hand, p53 seems to rely on NF-Y to repress transcription of many of its target genes[12]. On the other hand, NF-Y has been shown to function as a *trans* activator of a subset of p53 targets, such as *FAS*, that lack a TATA box in their promoter region and require binding of both NF-Y and p53 (ref. 10).

Hung *et al.*[5] hypothesized that *PANDA* might function through sequestration of NF-YA away from NF-Y–p53 co-regulated promoters. Using RNA chromatography and chromatin immunoprecipitation, they showed that *PANDA* indeed binds to NF-YA and its knockdown increases the presence of NF-YA at promoter regions of p53-dependent pro-apoptotic target genes[5] (**Fig. 1**). This could lead to increased cell death in response to DNA damage. Consistent with this idea, reducing *PANDA* levels with short interfering RNA resulted in increased rates of apoptosis[5]. Whether pro-survival effects of *PANDA* during DNA damage response are mediated through 'eviction' of NF-YA and ensuing repression of FAS remains to be determined. At the very least, it is consistent with the prevailing view that NF-Y transcription factors help orchestrate p53-dependent responses to DNA damage[13]. Thus, although *PANDA* might be little more than 'junk' RNA for the purpose of p53-dependent cell cycle arrest, it plays a key role in protecting the stressed cell from apoptotic death.

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