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NON-CODING RNA

Decoy pumilio for genomic stability

Pumilio homologue 1 (PUM1) and PUM2 are RNA-binding proteins that bind to motifs known as pumilio response elements (PREs) in many mRNAs and stimulate their degradation. The levels of PUM proteins must be strictly controlled to avoid pathologies such as neurodegeneration, but how this is achieved is unknown. Lee *et al.* identified a long non-coding RNA (lncRNA), which they termed *NORAD* (non-coding RNA activated by DNA damage), and showed that it sequesters PUM proteins and suppresses PUM-mediated RNA degradation and genomic instability.

Looking to identify lncRNAs involved in the DNA damage response, the authors characterized *NORAD* as a highly conserved and ubiquitously expressed lncRNA in mammalian cells and tissues. Following DNA damage, its expression is induced up to ~1,400 copies per cell, similar to highly abundant mRNAs. Inactivation or depletion of *NORAD* in diploid human cells caused chromosomal instability (CIN), as evidenced by aneuploidy, mitotic errors such as anaphase bridges and mitotic slippage, and chromosomal rearrangements. Restoring *NORAD* expression significantly reduced aneuploidy, establishing that *NORAD* is essential for genomic stability.

NORAD contains five copies of a ~400-nucleotide-long domain ('*NORAD* domain'), and the authors found that PUM2 binds to all five *NORAD* domains. PAR–CLIP (photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation) experiments, as well as the analysis of published PAR–CLIP data, revealed that *NORAD* is the most highly represented target of PUM2. There are 15 conserved PREs in *NORAD*, mostly clustering in or around the *NORAD* domains (by contrast, 90% of other PUM-bound transcripts have two or fewer PREs), and endogenous PUM2 binds to at least seven of them.

HCT116 cells were found to express an average of ~15,000 PUM1 and ~2,000 PUM2 proteins per cell. Considering the expression levels of *NORAD* in these cells (~500–1,000 copies per cell), the authors hypothesized that it could sequester most, if not all, PUM proteins and thus prevent the degradation of their mRNA targets. Consistent with this, PUM2 targets were significantly downregulated in *NORAD*$^{-/-}$ cells, and PUM1 or PUM2 overexpression produced a similar gene expression signature. Importantly, overexpression of PUM2 (and, to a lesser extent, of PUM1) was sufficient to induce aneuploidy, whereas inactivation of PUM2 or PUM1 in HCT116 *NORAD*$^{-/-}$ cells partially suppressed CIN. Conversely, knockout of PUM1 and PUM2 together induced aneuploidy, further demonstrating the need to keep PUM expression levels within a narrow range.

Finally, the authors found that the PUM2 target genes downregulated in *NORAD*$^{-/-}$ cells were significantly enriched for regulators of mitosis, DNA repair and DNA replication, of which the inactivation of many has been shown to induce genomic instability. Together, the results demonstrate that both the PUM proteins and their negative regulator *NORAD* are important for maintaining genomic stability, and that the precise regulation of PUM activity is achieved by the decoy capacity of *NORAD*.

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> "the precise regulation of PUM activity is achieved by the decoy capacity of *NORAD*"

ORIGINAL ARTICLE Lee, S. *et al.* Noncoding RNA *NORAD* regulates genomic stability by sequestering PUMILIO proteins. *Cell* http://dx.doi.org/10.1016/j.cell.2015.12.017 (2016)