THE EMBO JOURNAL

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**The EMBO Journal (2013) 32,** 771–772
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# Struggling to let go: a non-coding RNA directs its own extension and destruction

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*The EMBO Journal* (2013) **32,** 771–772. doi:10.1038/emboj.2013.36; Published online 22 February 2013

**In addition to its role in DNA-dependent transcription, RNA polymerase II (Pol II) possesses RNA-dependent RNA polymerase (RdRP) activity (Lehmann *et al*, 2007). In a study published in this issue of *The EMBO Journal*, Wagner *et al* (2013) report the first native cellular function of the RdRP activity of Pol II. The authors find that a mammalian non-coding RNA (ncRNA) can serve as a template for its own extension by Pol II, resulting in its destabilization and a decrease in its potency to repress Pol II.**

Genome-wide RNA profiling has demonstrated that approximately three quarters of the human genome are transcribed (Djebali *et al*, 2012; Pennisi, 2012), leading to a widespread formation of ncRNA since protein-coding genes account for only 3% of the genome. ncRNAs are predominantly localized in the cell nucleus and are generally expressed at lower levels than protein-coding transcripts (Djebali *et al*, 2012). The number of ncRNAs increases with increasing complexity of an organism, and has been proposed to account for much of the additional regulatory functions in higher eukaryotes (Amaral & Mattick, 2008). ncRNAs regulate many stages of gene expression, including transcription (Kaikkonen *et al*, 2011).

One such ncRNA that regulates transcription is mouse B2 RNA. Like its human counterpart Alu RNA, B2 RNA is an abundant ncRNA transcribed by RNA Pol III, the polymerase responsible for transcription of many short untranslated RNAs (Vannini and Cramer, 2012). B2 RNA is synthesized from short interspersed repeat elements (SINEs) that are present in high copy numbers throughout the genome. Upon cellular stresses such as heat shock, B2 RNA is upregulated and represses transcription by binding the active centre cleft of Pol II and preventing contacts with promoter DNA (Yakovchuk *et al*, 2009; Kassube *et al*, 2012). Another mouse ncRNA, B1 RNA, is also transcribed by Pol III from SINEs and upregulated upon heat shock, but while B1 RNA also binds Pol II it fails to repress its activity (Wagner *et al*, 2010).

In their current paper, Wagner *et al* show that B2 RNA in fact serves as a template for its own extension using the RdRP activity of Pol II. In a series of biochemical experiments with purified human Pol II, the authors show that the 3′ end of B2 RNA is extended by 18 nucleotides in a self-templated reaction, whereas B1 RNA is not extended. The extended B2 RNA is a less potent inhibitor of Pol II both *in vitro* and in cells. Treatment of mouse cells with α-amanitin, a potent inhibitor of Pol II, revealed a large increase in overall levels of B2 RNA but not B1 RNA, suggesting that RdRP extension functions to destabilize B2 RNA. Treatment of cells with actinomycin-D, an inhibitor of all DNA-dependent RNA synthesis but not RNA-dependent RNA synthesis, revealed two pools of B2 RNA—one that may be extended and quickly degraded, and a second that cannot be extended and remains stable. It remains unclear whether RNA extension leads to destabilization of the B2 RNA–Pol II complex in cells, but modelling predicts that growth of any RNA template–product duplex in the active centre cleft of Pol II will lead to a partial opening of the polymerase clamp domain and a looser grip on the nucleic acids (Figure 1).

These findings are important not only because they improve our understanding of ncRNA function, but also because they demonstrate for the first time a cellular function of the RdRP activity of Pol II. Although it was known that the RdRP activity of Pol II is used by viruses, such as the hepatitis delta virus, for the replication of their RNA genome (Filipovska and Konarska, 2000; Yamaguchi *et al*, 2001), it was unclear whether it also has cellular functions. Now the authors reveal

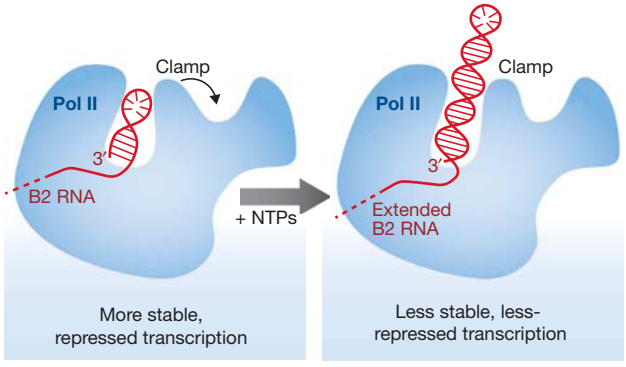


**Figure 1** The RdRP activity of Pol II extends B2 RNA. Extension of B2 RNA in the polymerase active centre cleft is predicted to lead to steric restraints that may destabilize the Pol II–RNA complex. This may explain why extended B2 RNA is a weaker polymerase inhibitor.

that the Pol II RdRP activity can modify a mammalian ncRNA, thereby modulating its function.

In analogy with these new findings, bacterial RNA polymerase also has RdRP activity, which can *de novo* synthesize small ncRNAs using 6S RNA as a template (Wassarman and Saecker, 2006). In contrast to B2 RNA, RdRP-catalyzed transcript synthesis using 6S RNA as a template leads to 6S RNA dissociation from the polymerase. Thus RNA regulation of transcription may be an ancient mechanism, consistent with the idea that today's RNA polymerases arose from an ancient RNA replicase.

Many open questions remain regarding B2 RNA function. How can only one pool of B2 RNAs serve as a template for RdRP-catalyzed extension? What is the role of B2 RNA extension in unstressed cells versus cells exposed to heat shock? What is the factor present in nuclear extracts that the authors observed dissociates B2 RNA from Pol II? With the discovery of a first endogenous target of the RdRP activity of Pol II, it seems likely that other natural targets await discovery and that a systematic search for ncRNAs that interact with Pol II warrants consideration.

## Conflict of interest

The authors declare that they have no conflict of interest.

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