RNAs non-specifically inhibit RNA polymerase II by binding to an unexpected surface

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Many RNAs are known to act as regulators of transcription in eukaryotes, including certain small RNAs that directly inhibit RNA polymerases both in prokaryotes and eukaryotes. We have examined the potential for a variety of RNAs to directly inhibit transcription by yeast RNA polymerase II (Pol II) and find that unstructured RNAs are potent inhibitors of purified yeast Pol II. Inhibition by RNA is achieved by blocking binding of the DNA template, and requires binding of the RNA to Pol II prior to open complex formation. RNA is not able to displace a DNA template that is already stably bound to Pol II, nor can RNA inhibit elongating Pol II. Unstructured RNAs are more potent inhibitors than highly structured ones and can also block specific transcription initiation in the presence of basal transcription factors. Crosslinking studies with ultraviolet light show that unstructured RNA is most closely associated with the two large subunits of Pol II that comprise the template binding cleft, but that the RNA has contacts in a basic residue channel behind the back wall of the active site. These results are distinct from previous observations of specific inhibition by small, structured RNAs in that they demonstrate a sensitivity of the holoenzyme to inhibition by unstructured RNA products that bind to a surface outside the DNA cleft. These results are discussed in terms of the need to prevent inhibition by RNAs, either though sequestration of nascent RNA or pre-emptive interaction of Pol II with the DNA template.

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