

Differently Regulated Gene-Specific Activity of Enhancers Located at the Boundary of Subtopologically Associated Domains: TCR α Enhancer.

Abstract

Enhancers activate transcription through long-distance interactions with their cognate promoters within a particular subtopologically associated domain (sub-TAD). The TCR α enhancer (E α) is located at the sub-TAD boundary between the TCR α and DAD1 genes and regulates transcription toward both sides in an ~1-Mb region. Analysis of E α activity in transcribing the unrearranged TCR α gene at the 5'-sub-TAD has defined E α as inactive in CD4⁻CD8⁻ thymocytes, active in CD4⁺CD8⁺ thymocytes, and strongly downregulated in CD4⁺ and CD8⁺ thymocytes and $\alpha\beta$ T lymphocytes. Despite its strongly reduced activity, E α is still required for high TCR α transcription and expression of TCR $\alpha\beta$ in mouse and human T lymphocytes, requiring collaboration with distant sequences for such functions. Because V α J α rearrangements in T lymphocytes do not induce novel long-range interactions between E α and other genomic regions that remain in cis after recombination, strong E α connectivity with the 3'-sub-TAD might prevent reduced transcription of the rearranged TCR α gene. Our analyses of transcriptional enhancer dependence during T cell development and non-T lineage tissues at the 3'-sub-TAD revealed that E α can activate the transcription of specific genes, even when it is inactive to transcribe the TCR α gene at the 5'-sub-TAD. Hence distinct requirements for E α function are necessary

at specific genes at both sub-TADs, implying that enhancers do not merely function as chromatin loop anchors that nucleate the formation of factor condensates to increase gene transcription initiated at their cognate promoters. The observed different regulated E α activity for activating specific genes at its flanking sub-TADs may be a general feature for enhancers located at sub-TAD boundaries.

Antisense oligonucleotides (ASOs) are short synthetic single-stranded DNA/RNA-like oligonucleotides, which are designed to selectively bind RNA (Watson-Crick pairing) to regulate protein expression. Here, protein expression is regulated by affecting processing or translation of target RNA or by activation of RNase H and subsequent degradation of target RNA, due to their potential to modify protein expression, ASOs are currently used to treat diseases such as hereditary transthyretin amyloidosis. In order to use ASOs as therapeutic agents, they are modified to achieve better bioavailability and higher target RNA specificity. For this purpose, DNA nucleotides of ASOs are replaced with locked nucleic acid (LNA) nucleotides. Here, GapmeRs, made of LNA bases flanking a central DNA sequence, provide a potent target mRNA inhibition. Mechanistically, GapmeRs form DNA-RNA hybrids after binding their target RNA. These DNA-RNA hybrids are

recognized by RNase H catalyzing RNA cleavage.

BIOGRAPHY

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