Final Report Summary - DPE CORE PROMOTERS (Molecular Studies of Transcription and Core Promoter Regulation of Critical Developmental Genes)

The regulation of gene transcription is critical for the proper development and growth of an organism. The core promoter is the ultimate target sequence of a multitude of factors that control the initiation of transcription. Core promoters encompass the RNA start site and consist of functional sub-regions, termed core promoter elements or motifs, which confer specific properties to the core promoter. Our focus is on the unique contribution of the core promoter to transcriptional regulation of gene networks. The downstream core promoter element (DPE) was originally discovered as a TFIID recognition site that is located downstream of the Inr element. Importantly, the DPE is conserved from Drosophila to humans. The study of the molecular mechanisms that regulate DPE transcription as well as the identification of DPE promoters and DPE-specific transcription factors will provide new insights into the biological role of the DPE.

We addressed the following specific aims:

1. To study DPE-specific transcriptional activation by Caudal

Caudal is a DPE-specific transcriptional activator that is a master regulator of genes involved in development. To elucidate the mechanism by which Caudal preferentially activates DPE transcription, we performed a structure-function analysis of the Caudal protein and mapped a region within Caudal that contributes to the preferential DPE activation. We defined a region that is sufficient to confer core promoter preferential activation to a heterologous protein. Furthermore, by analyzing transcriptional activation by the mouse Caudal-related Cdx proteins, we discovered that the preferential activation of core promoters is evolutionarily conserved. Importantly, we identified and characterized a co-activator that mediates between Caudal binding at the enhancer and the core promoter region.

Furthermore, we analyzed the contribution of specific core promoter elements to the innate immune response and discovered that the combination of multiple core promoter elements contributes to the transcriptional outcome. Interestingly, the caudal gene contains two promoters encoding a maternal TATA-dependent transcript and a zygotic DPE-dependent transcript. Both transcripts encode the same protein. Our data suggests the potential existence of an auto-regulatory feedback loop where a zygotically DPE-dependent caudal transcript is induced by the maternal Caudal protein.

2. To test how widespread is the function of DPE in diverse developmental pathways and gene regulatory networks

We have previously shown that nearly all Drosophila Hox genes, which are involved in early development of the embryonic body plan, contain DPE motifs. To discover additional gene regulatory networks (GRNs), which are regulated via the core promoter, we examined the core promoter composition of genes that are involved in early embryonic development. This analysis led us to explore the importance of core promoter functions in the dorsal-ventral developmental gene regulatory network and in particular, in Dorsal target genes. This network includes multiple genes that are activated by different nuclear concentrations of Dorsal, an NFkB homolog transcription factor, along the dorsal-ventral axis. We have shown that multiple Dorsal target genes are evolutionarily conserved and functionally dependent on the DPE. Moreover, we demonstrated that over two-thirds of Dorsal target genes contain DPE sequence motifs, which is significantly higher than the proportion of DPE-containing promoters in Drosophila genes. Importantly, using hybrid enhancer-promoter constructs in Drosophila cells and embryo extracts, we demonstrated that the core promoter composition is an pivotal determinant of transcriptional activity of
Dorsal target genes.

3. To discover DPE-specific transcription factors

In search of a transcription factor that supports DPE-dependent transcription, we employed a biochemical complementation approach and identified the Drosophila TBP (TATA binding protein)-related factor 2 (TRF2) as an enriched factor in the fractions that support DPE-dependent transcription. Furthermore, TRF2 was also enriched in fractions that bound DPE-containing DNA-affinity columns. There are two protein isoforms of TRF2 – short and long TRF2. We have shown that overexpression of short TRF2 preferentially activates transcription of DPE-dependent promoters and knock down of TRF2 preferentially reduces DPE-dependent transcription. We performed DNA microarray analysis and discovered the enrichment of DPE-promoters among short TRF2 up-regulated genes. It was previously shown that TRF2, unlike TBP, fails to bind DNA containing TATA-boxes. Using microfluidic affinity analysis we discovered that short TRF2-bound DNA oligos are enriched for Inr and DPE motifs. Taken together, our findings highlight the role of short TRF2 as a preferential core promoter regulator.

Our research supports a broad role for DPE in key biological processes. Our findings are important for understanding transcription and development, and are likely to shed light on the regulation of gene expression under normal and pathological conditions, such as leukemias and lymphomas, in which the regulation of Hox gene expression becomes abnormal.

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