EVALUATION OF REGULATORY DNA REGIONS FOR MATRIX METALLOPROTEINASE EXPRESSION

Yunlong Liu (1,2), Hui B. Sun (1,3), Hiroki Yokota (1,2,3,4)

(1) Biomedical Engineering Program, and (3) Departments of Anatomy and Cell Biology, and (4) Mechanical Engineering, Indiana University - Purdue University Indianapolis, Indianapolis, IN 46202
(2) Department of Biomedical Engineering, Purdue University, West Lafayette, IN 47907

ABSTRACT

We described a novel method to identify critical regulatory DNA regions for gene regulation using the responses of a family of matrix metalloproteinase (MMP) genes and tissue inhibitor of metalloproteinases (TIMP) genes to mechanical stimuli as a model biological system. First, the role of seven known transcription factor (TF) binding motifs such as AP1, AP2, NFY, NFκB, PEA3, Sp1, and STAT was estimated in response to mechanical shear stress using a DNA-transfection-based biochemical assay (PROCO assay). Secondly, the predicted role of the selected TF binding motifs was modeled using the observed mRNA expression level of MMP genes (PROBE model). Lastly, the critical regulatory DNA regions in the flanking DNA sequences were evaluated. The biochemical and computational results support that both 5'-end and 3'-end flanking DNA sequences contribute to regulation of a family of MMP and TIMP genes.

METHODS

Messenger RNA Expression Data

The mRNA expression level under mechanical shear at 2 dyn/cm^2 for 0, 1, 3, 6, 12, and 24 hours in MH7A human synovial cells were determined by reverse transcription and polymerase chain reactions. The 17 genes in this study included 13 MMP genes (MMP1, 2, 3, 7, 8, 9, 10, 11, 13, 14, 15, 16, 20), and 4 TIMP genes (TIMP1, 2, 3, and 4).

TF Binding Motifs

Seven TF binding motifs such as AP1, AP2, NFY, NFκB, PEA3, Sp1, and STAT were considered in this study for MMP and TIMP genes. The 5'-end and 3'-end flanking genomic DNA sequences were retrieved from the database in GenBank and the site of transcription initiation was identified using the procedure developed on UCSC Genome Center [1]. MatInspector and SignalScan were used to identify the selected TF binding sites on the regulatory DNA sequences [2,3].

PROCO Assay

In order to estimate the role of TF binding motifs, we conducted PROCE assay [4]. In brief, exogenous double-stranded DNA fragments consisting of a specific TF binding motif were transferred into MH7A synovial cells. The transferred DNA fragments would act as competitors against endogenous TF binding sites, and downregulation of mRNA expression levels by the transfer would indicate that the DNA fragments are a potential stimulator of gene expression. The mRNA level of MMPs and TIMPs was determined with and without DNA transfer. Alteration in the mRNA level was used to estimate the role of TF motifs.

PROBE Model

The quantitative relationship between the observed mRNA expression profile and numbers of the selected TF binding motifs on putative regulatory DNA sequences was established using PROBE formulation [5,6]. Using model error as a measure for evaluation of regulatory DNA regions, we determined a functional contribution of the 5'-end and 3'-end regulatory sequences to regulation of MMP and TIMP genes. In this study, we used the linear PROBE model without considering interactions among TF binding motifs.

RESULTS AND DISCUSSION

Estimated Role of TF Binding Motifs

A transfer of ~15-bp DNA fragments consisting of the biologically known TF binding motifs such as AP1, NFY, NFκB, PEA3, Sp1, and STAT repressed mRNA expression of many MMPs, indicating that these TF binding motifs on endogenous chromosomes would stimulate MMP expression (Fig. 1). Particularly, AP1 (fos/jun binding sites) and PEA3 (ets binding sites) exhibited their strong stimulatory role. A transfer of AP2 binding motif, on the other hand, elevated MMP2 mRNA expression suggesting the inhibitory role of AP2 (Fig. 1).

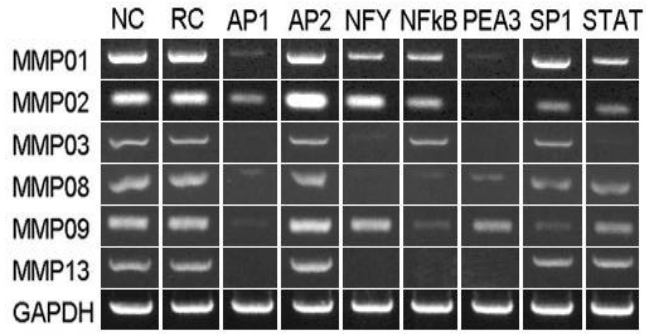

Fig. 1. Messenger RNA level of MMP1, 2, 3, 8, 9, and 13 in PROCO assay. The labels are: "NC" – normal control without any DNA transfer, and "RC" – random control with a transfer of random DNA oligonucleotides. GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as control.

Model Error in PROBE Formulation

The predicted role of the selected TF binding motifs was used to model the mRNA expression profile of MMP and TIMP genes in response to mechanical shear. The model used the genomic DNA sequences in the 5'-end flanking region and/or the 3'-end flanking region. The results revealed that the error for the model with the 5'-end DNA sequences was smaller than the model with the 3'-end DNA sequences, but the model with both flanking DNA sequences gave the minimum model error (Fig. 2).

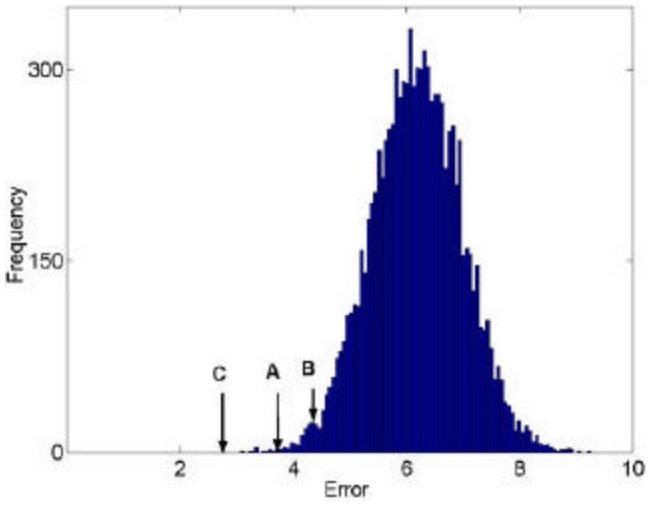

Fig. 2. Monte Carlo simulation and model error using PROCO data including 6 MMP genes. The model error for 1180-bp franking DNA sequences was 3.7 (A: 5-end only), 4.4 (B: 3'-end only), and 2.6 (C: 5'-end and 3'-end) with 6.2 ± 0.8 (randomly scrambled data in Monte Carlo simulation, N = 10,000).

Observation vs. Modeling

The observed and modeled mRNA expression profiles are illustrated (Fig. 3). The normalized model error for PROBE formulation including 17 genes was 4.3 (5'-end only), 6.4 (3'-end only), and 3.5 (5'-end and 3'-end) where the minimum error was given for a linear combination of 5'-end and 3'-end in a ration of 0.66 to 0.33 (Fig. 4).

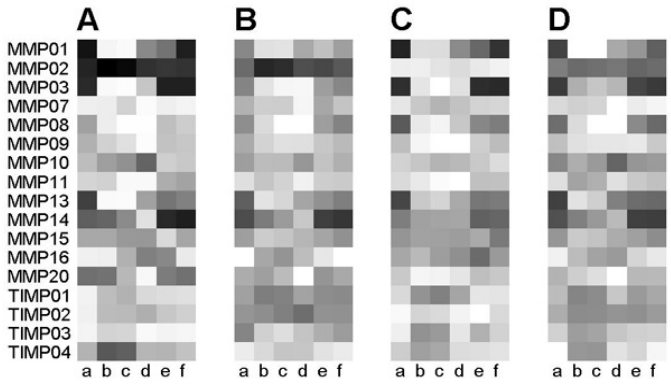

Fig. 3. Observed and modeled mRNA expression profiles of MMP and TIMP genes in response to mechanical shear for 0 (control), 1, 3, 6, 12, and 24 hours. The darker color indicates higher mRNA expression levels. (A) Observed pattern. (B) Modeled pattern using 5'-end sequences. (C) Modeled pattern using 3'-end sequences. (D) Modeled pattern using 5'-end and 3'-end sequences.

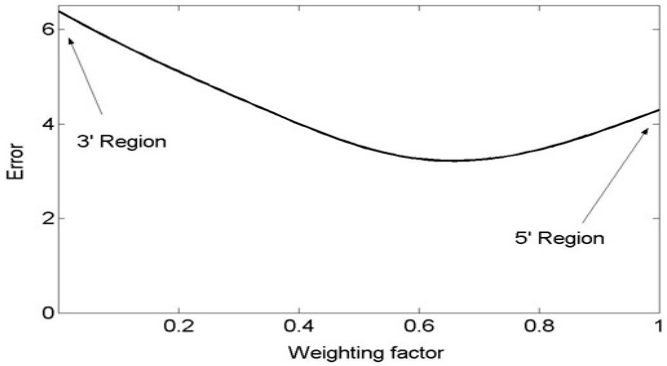

Fig. 4. Model error as a ratio of weight to the 5'-end sequences to weight to the 3'-end sequences.

ACKNOWLEDGEMENT

This study was supported by NIH R03AR47628 (to H.B.S.) and NIH R21RR17012 (to H.Y.).

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