

## [P-S-057] IDENTIFICATION OF ETS1 AS ASE BINDING PROTEIN: UNIQUE ROLE IN AGE-RELATED GENE REGULATION

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**Introduction:** We previously discovered the ASE/AIE-mediated genetic mechanism for age-related regulation of gene expression, the first molecular mechanism for age-related homeostasis, through transgenic mouse studies of human factor IX (hFIX) as well as protein C genes. A key genetic element, ASE, has specific Ets consensus sequences (G/CAAGGAAG) of particular importance for its roles in physiology and age-related human diseases.

**Methods:** Systematic electrophoretic mobility-shift assays (EMSAs) and competitive EMSAs with murine liver nuclear extracts (NEs) were carried out with double stranded 22-mer oligonucleotide probes containing all possible combinations of ASE-like sequences. Supershift assays were then carried out with a set of antibodies to known Ets family proteins to identify a nuclear protein specifically binding to ASE. Overexpression experiments were finally carried out using 293T cells to confirm the protein binding.

**Results:** EMSAs and competitive EMSAs showed functional ASEs (G/CAAGGAAG) bind only a unique nuclear protein, but not other proteins which bind to other nonfunctional ASE-like elements. In supershift EMSA, only anti-Ets1 antibody produced a strong and unique supershift pattern. NEs prepared from 293T cells transfected with Ets1 expression vector gave a clearly enhanced intensity of the shifted band over that generated by NEs prepared from non-transfected control cells, and supershift analysis further dramatically enhanced the shifted band intensity.

**Conclusions:** Our results unambiguously support that the nuclear protein, which specifically binds to functional ASEs (G/CAAGGAAG) is Ets1. Although Ets1 has been known for its role as a proto-oncogene, this study now assigns another novel and critical function to Ets1 in physiologic regulation of age-related gene expression. As we previously reported that ASE also plays a unique tissue-specific regulator, Ets1 appears to be a truly multi-functional transcription factor.

Kurachi S, Zhang K, Kasama E, Kurachi K. IDENTIFICATION OF ETS1 AS ASE BINDING PROTEIN: UNIQUE ROLE IN AGE-RELATED GENE REGULATION. *J Thromb Haemost* 2007; 5 Supplement 2: P-S-057

**Date:** Sunday, July 8, 2007

**Session Info:** Poster: Vitamin K dependent factors, incl. Factor IX

**Room:** Exhibition Area