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SHORT COMMUNICATION

Interaction between E-protein and Oct transcription factors in the function of the catfish *IGH* enhancer

Jun-ichi Hikima[a,b], Mara L. Lennard Richard[a,b], Melanie R. Wilson[c], Norman W. Miller[c], Gregory W. Warr[a,b,*]

[a]*Marine Biomedicine and Environmental Sciences Center, Medical University of South Carolina, Charleston, SC 29412, USA*
[b]*Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425, USA*
[c]*Department of Microbiology, University of Mississippi Medical Center, Jackson, MS 39216, USA*

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Summary
Transcriptional control of the immunoglobulin heavy chain (*IGH*) locus in the channel catfish, *Ictalurus punctatus*, is incompletely understood. It is, however, known that 2 variant octamer motifs and a μE5 motif in the core region of the enhancer (Eμ3′) are important in driving transcription, and it has been suggested that interaction between transcription factors (Oct factors and E-proteins) bound to these sites contributes to enhancer function. In this study, the functional relationships between the μE5 motif, the proximal octamer motif, and the factors that bind them have been examined. The results of mutational analysis of these motifs showed that their interaction is important to driving transcription from the enhancer. Furthermore, the catfish Oct transcription factors were capable of a physical interaction with the catfish E-proteins. These results support a role for interaction between transcription factors bound to the octamer and μE5 motifs in the function of the Eμ3′ enhancer.

Introduction

The picture that has emerged from studies of transcriptional control of the *IGH* locus in teleost fish [1–6] is that there is a single enhancer (Eμ3′) that resides between the μ and δ genes. Whilst E-protein binding sites (e.g. μE5 motifs) are clearly of major importance in the function of the Eμ3′ enhancer, octamer motifs can also play a role (as shown in the channel catfish, *Ictalurus punctatus*) in driving transcription [2,4]. The core region of the catfish Eμ3′

Abbreviations: *IGH*, immunoglobulin heavy chain; bHLH, basic helix–loop-helix; CFEB, catfish E-box binding protein; O/E, the octamer#11 and μE5#2 motifs; R#2, Region#2 (core region of Eμ3′ enhancer); WT, wild type; EMSA, electrophoretic mobility shift assay; SE, standard error; EDTA, ethlenediamine tetraacetic acid; TBE, Tris–borate–EDTA; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis.

*Corresponding author at: Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, USA. Tel.: +1 843 762 8869; fax: +1 843 762 8737.
E-mail address: warrgw@musc.edu (G.W. Warr).

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enhancer differs in position, structure and function from the mammalian intronic *IGH* enhancer, Eμ [1,2,7,8]. It contains 2 variant octamer motifs and a consensus μE5 site [4] that are capable of binding to Oct transcription factors and E-box-binding proteins (E-proteins), respectively [9–13]. In contrast the transcription factors that bind to the minimal Eμ enhancer in the mouse *IGH* locus are Ets family members and the TFE3 (bHLH-zip) transcription factor [7,8].

The role of the 2 octamer motifs and the μE5 site in the function of the Eμ3′ enhancer has previously been investigated [4,5]. In summary, (1) the mutational inactivation of the 2 octamer motifs results in the loss of approximately 30% of the activity in the enhancer, (2) mutation of the μE5 site results in a loss of 50% activity, indicating that the μE5 site in this enhancer has an important function, (3) an increased distance between the 2 octamer motifs resulted in increased activity, (4) the binding of Oct transcription factors to the octamer motif induced bending of the DNA (as is also the case with mammalian Oct factors, [14]) and (5) the octamer factors have been suggested to interact with other factors bound to sites within Eμ3′. These interactions may include: Oct factors with E-protein; Oct factors with a coactivator; and Oct factors with an unknown protein binding to a recently described motif present between the 2 octamer sites [4,5,15]. In this study, the contribution to enhancer function of interactions between factors bound to the μE5 site and to the proximal octamer site have been investigated.

Materials and methods

DNA constructs

Schematics showing the octamer and μE5 motifs present within the core region of Eμ3′, and the mutations made to them are presented in Figures 1A and 2B. Eight reporter constructs for luciferase expression were used in this study, (1) the minimal c-*fos* promoter (Δ56-Empty; pGL3/Δ56), (2) the minimal c-*fos* promoter with the core region of the catfish enhancer (R#2-WT; pGL3/Δ56/R#2), (3) the core region with double-mutated octamer#11 and μE5 motifs (R#2-ΔOct#11&ΔμE5; pGL3/Δ56/R#2-ΔOct#11&ΔμE5), (4) the core region with mutated octamer#11 motif (R#2-ΔOct#11; pGL3/Δ56/R#2-ΔOct#11), (5) the core region with mutated μE5 site (R#2-ΔμE5; pGL3/Δ56/R#2-ΔμE5#2), (6) the core region with 20 bp addition in the position between octamer#11 and μE5 (O/E+20 bp; pGL3/Δ56/R#2-O/E+20 bp), (7) the core region with 14 bp addition in the position between octamer#11 and μE5 (O/E+14 bp; pGL3/Δ56/R#2-O/E+14 bp), (8) the core region with a 5 bp deletion between octamer#11 and μE5 (O/E-5 bp; pGL3/Δ56/R#2-O/E-5 bp). The reporter constructs #1, 2, and 5 were described in a previous study [11].

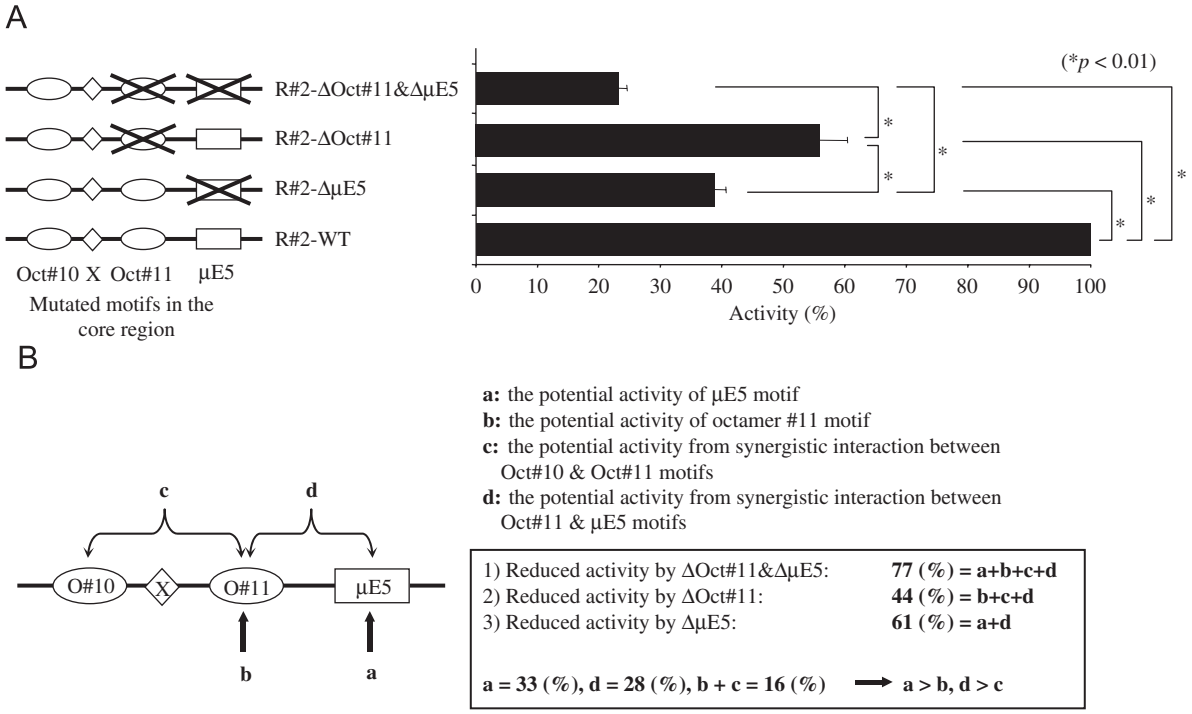

Figure 1 Effect of mutational inactivation of octamer and μE5 motifs in the Eμ3′ enhancer. (A) The Oct#11 and μE5 motifs were inactivated singly or together by site-directed mutation. A schematic illustrating the motifs mutated in the core region (R#2) of the Eμ3′ enhancer is shown on the left. The oval, rectangle, and diamond in the schematic illustrations indicate the motifs to be bound with transcription factors: Oct, E-protein, and an unknown protein (X; [5,6]), respectively. Covered black crosses indicate a mutated motif. Transcriptional activity of the mutated reporter constructs, shown as percent activity relative to the wild type (WT) R#2, upon transient transfection into catfish 1G8 B cells. The standard error (SE) is denoted by the error bars, and the asterisk "*" indicates the significant difference between each of these constructs at $p<0.01$, as determined by two-tailed Student's *t*-test ($n=9$). (B) Schematic showing potential interactions between transcription factors and calculations for the contributions of the Oct and μE5 motifs derived from the results in (A). *Abbreviations*: O#10, octamer#10; O#11, octamer#11.