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Short Report
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Engineered Promoter Selectivity of an ECF Sigma Factor

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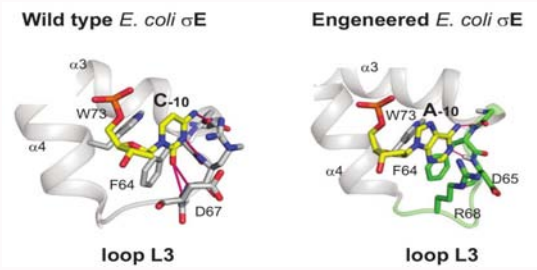
Abstract

In bacteria, promoter melting by alternative sigma factors is achieved by flipping out the base at the position -10. The identity of this base is determined by the sequence of the variable loop L3 of the sigma factor. Here, we solved the solution structure of an engineered *Escherichia coli* sigmaE region 2 that recognizes an adenine instead of a cytosine in position -10. Our results demonstrate at the atomic level how Extra Cytoplasmic Function (ECF) sigma factors can be rationally engineered to modify their promoter selectivity.

Keywords: Bacterial transcription initiation; ECF sigma factor; Promoter melting; Promoter specificity



Graphical Abstract:

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Introduction

In bacteria, regulation of gene expression mainly occurs at the transcriptional level [1]. A major way to redirect gene expression consists in the usage of alternative sigma factors, the promoter specific subunit of the bacterial RNA polymerase [2]. Extra Cytoplasmic Function (ECF) sigma factors serve to mount specific responses upon environmental stimuli, are activated using different mechanisms and interact with other regulation networks [3,4].

In sharp contrast with housekeeping sigma factors, ECF sigma factors have a narrow range of promoter targets and redirect a smaller but critical part of the overall transcription towards specific genes. This stringent selectivity was linked to their mechanisms of promoter recognition and melting [5,6]. While the housekeeping sigma factor opens promoters by flipping out two bases at the -10 promoter element, the ECF sigma factor pushes out a single base which is specifically recognized by atoms of a variable loop [7,8]. Notably, the envelope stress sigma factor σ^E of *Escherichia coli* requires a cytosine in position -10 (with respect to the transcription start site) to initiate transcription. We previously showed that by substituting the loop L3 of σ^E by the one of σ^W from *Bacillus subtilis*, the chimera protein σ^E-L3-σ^W initiates transcription only when an adenine is found in position -10 (Figure 1a). To understand how flipped bases are accommodated by ECF sigma factors, we investigated the structure of the chimera in complex with the -10 promoter element GTAAAA non template strand.

We used solution NMR to determine how an adenine is selected by the engineered sigma factor (Figure 1c and Supplementary table 1). Intermolecular NOEs allow the precise positioning of the adenine in the same cavity than observed for the wild type version of σ^E (Figure 1b). Briefly, the base stacks against F64 and W73 and forms hydrogen bonds with the backbone atoms of the loop L3. The adenine does not bury as far as the cytosine does (Figure 1c and 1d). Indeed, while the binding of wild type σ^E is in slow exchange regime, the chimera protein binds its single strand DNA target in fast exchange regime, in line with a lower affinity. The recognition of A_{-10} shows common features with how the housekeeping sigma factor selects A_{-11} from the -10 promoter element [7] (Figure 1e

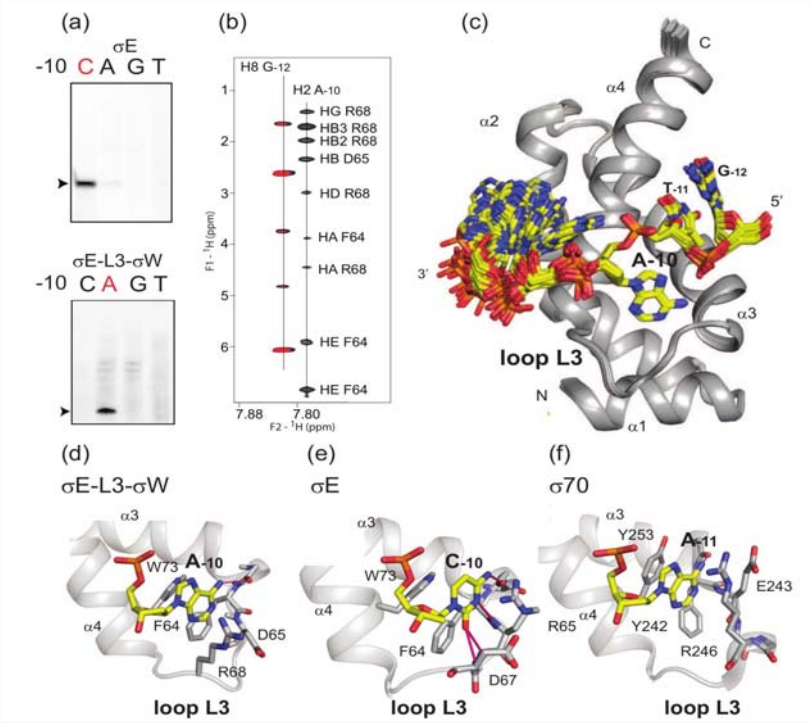

Figure 1: NMR solution structure of the region 2 of the chimera protein bound to GTAAAA.
(a) *In vitro* transcription assays showing the promoter specificity of σ^E and σ^E-L3-σ^W [8]. (b) Overlay of the 2D ^{1}H-^{1}H F_1f-F_2f (red, intramolecular RNA NOEs) and F_2f (black) NOESY experiments showing the intermolecular NOEs with the signal of H2 A_{-10}. (c) NMR solution structure of the complex formed by σ^E-L3-σ^W and GTAAAA (PDB ID 5OR5 and BMRB ID 34172). Molecular basis for the recognition of the flipped base by the engineered sigma factor (d), σ^E (e) and the housekeeping sigma factor (f).

and 1f). In both cases, the base is surrounded by an aromatic residue on one side and an arginine-glutamate couple on the other side. In the three structures, the -10 base is specifically recognized by backbone atoms of loop L3. The analysis of the three structures suggest that the position of the arginine-glutamate couple along the sequence of loop L3 could control the specificity for the flipped-out base and fine-tunes promoter selection.

In bacteria, orthogonal gene expression pathways are generated by different binding specificity for the -35, -10 promoter elements and other *cis* motifs [9-11]. Being able to control the -10 promoter element recognition and promoter selectivity offers the possibility to design novel sigma factors and environmentally-induced gene expression systems.

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