COMPILING SIGMA-70-DEPENDENT PROMOTERS

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1. INTRODUCTION

Promoters are sequences that precede transcriptional start sites and determine recognition by RNA polymerases to initiate transcription. Most bacterial genes are regulated at the level of transcription, and expression from different classes of promoters is under the control of RNA polymerases with different sigma factors. The eubacterial core RNA polymerase is comprised of one β, one β’ and two α subunits. Promoters are recognized by the holoenzyme (Eσ) formed by the reversible association of the core with a σ factor. This σ subunit confers specificity in promoter sequence recognition and is essential for transcription initiation. Global switches in transcription patterns are known to be related to promoter selectivity of multiple RNA polymerases with different sigma factors, where the competition of sigma factors for core occupancy in each situation plays a key role. Typically, bacteria have a major sigma factor that recognizes a large number of promoters, especially those controlling expression of the housekeeping genes. In Gram-negative bacteria, this factor is σ70. Other specialized sigma factors such as σ28, σ38, σ32 and σ54 share high sequence homology with σ70 and are included in the so-called σ70 family. The sigma factor σ54 constitutes a different group of its own, with characteristics that differ greatly from the σ70 family. The RNA polymerase with σ54 recognizes a specific subset of promoters with highly conserved sequences and architecture, completely different from other
promoters, and where transcription initiation is always dependent on additional transcriptional factors\(^9\) (de Lorenzo, Chapter 10 in this volume).

In both *Pseudomonas putida* and *Pseudomonas aeruginosa*, the genes for \(\sigma^{70}, \sigma^{38}, \sigma^{32}, \sigma^{28}\) and \(\sigma^{54}\) factors have been found and characterized\(^{11, 30, 43, 72, 77, 104, 140, 161, 165, 166}\). Moreover, multiple ORFs coding for putative extracytoplasmic function (ECF) sigma factors that constitute a phylogenetically and functionally distinct subgroup within the \(\sigma^{70}\) family have been found in their genome\(^{130, 139}\). Most members of this subgroup respond to signals from the extracytoplasmic environment. However, for many of them their specific function and target promoters remain unknown\(^{106}\). In both strains, at least five of these ECF sigma factors are organized in five analogous clusters that seem to be involved in iron uptake, a function that appears to be crucial for these strains. Some of the remaining *P. putida* ECF sigma factors seem specific and are related to the ability of this strain to colonize the rhizosphere, while *P. aeruginosa* has specific ECF sigma factors related to heme uptake and virulence associated secretion systems\(^{106}\).

In this chapter we present a compilation of 149 \(\sigma^{70}\)-dependent *Pseudomonas* promoters in order to characterize the DNA sequence that is recognized by the \(\sigma^{70}\)-RNA polymerase in this genus. The promoters were selected after screening all *Pseudomonas* promoters published or recorded in available databases that had been defined as being \(\sigma^{70}\)-dependent and in which the transcriptional start point has been determined experimentally. We are aware that some of these promoters may require an alternative sigma factor of the \(\sigma^{70}\) family for transcription since strict \(\sigma^{70}\)-dependency is normally difficult to establish *in vitro*. However, the promoters selected for this analysis were originally reported to be \(\sigma^{70}\)-dependent, with the exception of Pm-pWW0 from *P. putida*, which depends on both \(\sigma^{32}\) and \(\sigma^{38}\), but also shows significant basal activity *in vivo* with \(\sigma^{70}\) (Ref. 105). In the *P. aeruginosa* promoter algC, we also found a good \(-12/-24\) consensus sequence for \(\sigma^{54}\)-dependent promoters. However the authors ruled out the possibility of this promoter being \(\sigma^{54}\)-dependent since it is regulated by AlgR1\(^{187}\). Therefore, this promoter has been included in the compilation as \(\sigma^{70}\)-dependent.

### 2. Compilation

#### 2.1. Compilation Procedure

The promoters included in the compilation were obtained in three ways. A first set was selected after downloading from GenBank (release 135.0) all *Pseudomonas* sequences that included the word “promoter” within their annotations. Then the selected sequences were screened for the presence of an