Archaeal transcriptional regulation – variation on a bacterial theme?

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There is now an increasing body of data available on the DNA-binding properties of several putative transcriptional regulators in the Archaeal domain of life. The evidence points to simple models of promoter occlusion or facilitated recruitment of basal machinery for repressors and activators, respectively. However, little is known about the co-factor requirements, *in vivo* mechanisms and targets of many of these regulators. It is anticipated that the application of post-genomic technologies will begin to shed light on this fascinating area.

Introduction

It is now well established that archaea possess a basal transcription machinery resembling that of eukaryotes (Figure 1). Intriguingly, however, the majority of candidate transcription regulators are homologous to bacterial activators and repressors, with only a few candidate regulators that resemble eukaryotic gene-specific transcription factors [1] (Table 1). To date, only a few archaeal regulatory systems have been characterized at the molecular level. Although the ever-growing number of archaeal genome sequences reveals an increasing list of potential regulators, identifying the downstream targets of the regulators is a considerable technical hurdle, particularly given the extremely rudimentary state of genetic systems available for most archaeal species.

Insight from genetics

A clear exception to this is, of course, found in the halophilic archaea. In particular, the regulatory network regulating gas vesicle biosynthesis has been the subject of many elegant genetic analyses to establish the mechanisms of regulation of the large gas vesicle gene cluster [2]. However, although the identity of the gas vesicle regulators and candidate *cis*-acting motifs have been established, biochemical analysis of this fascinating system has been hampered by the innate intractability of protein biochemistry in these high salt-requiring organisms.

More recently, an elegant series of genetic and biochemical assays has identified the NrpR transcriptional regulator of nitrogen metabolism in the genetically tractable emthanogenic archaeon *Methanococcus maripaludis* [3,4]. The NprR factor was identified following DNA affinity chromatography and the gene encoding the protein was deleted. The resultant mutant strain showed constitutive expression of *nif* and *glnA* supporting a key role for NprR in regulation *in vivo* [4]. NprR acts as a repressor and recent work has revealed that the compound 2-oxoglutarate acts as an inducer by reducing the affinity of NprR for operator sequences [3]. It will clearly be of great interest to determine the molecular mechanism by which NprR impinges on the transcription machinery to effect regulation. However, from the location of the binding sites for NprR it is tempting to speculate that this protein might influence RNA polymerase recruitment by the general transcription factors.

Candidate gene approaches

The majority of the biochemical data available on the mechanisms of archaeal gene regulation have been derived from hyperthermophilic organisms. Thus far, most insights into the molecular mechanisms of archaeal transcription regulation have been derived from studies based on a candidate gene approach [i.e. identifying a possible transcriptional regulator by bioinformatics and

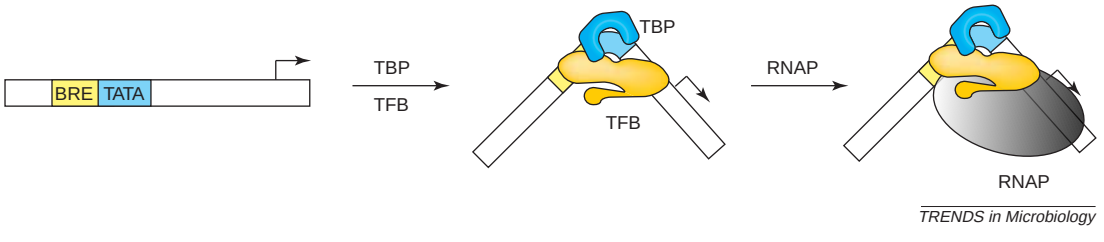

Figure 1. Schematic of the minimal archaeal transcription preinitiation complex derived from *in vitro* studies. The general transcription factors TBP and TFB bind cooperatively to the TATA-box and BRE, respectively. Once bound, they lead to the recruitment of the RNA polymerase (RNAP). There is an additional factor, TFE (not shown), which stimulates the process and functions, at least in part, by facilitating TBP–TATA-box interaction. The positioning of the factors in this cartoon is based on the recent model of the homologous yeast factors TBP, TFIIB and RNAP II (see Ref. [30]).

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Table 1. Archaeal transcriptional regulators and their mode-of-action[a]

Name	Species	Repressor or activator	Ligand	Mode of action	Refs
Mdr1	*A. fulgidus*	Repressor	Metal ions	Blocks RNAP recruitment	[5]
Lrs14	*S. solfataricus*	Repressor	Unknown	Blocks TBP and TFB recruitment	[9,10]
LrpA	*P. furiosus*	Repressor	Unknown	Blocks RNAP recruitment	[6,8]
Ss-LrpB	*S. solfataricus*	Unknown	Unknown	Unknown	[15]
LysM	*S. solfataricus*	Possible activator	Lysine	Unknown	[31]
GvpE	Halophilic archaea	Activator	Unknown	Unknown	[2]
TrmB	*T. litoralis*	Repressor	Sugars	Probably TBP and TFB recruitment	[11]
PhrA	*P. furiosus*	Repressor	Unknown	Blocks RNAP recruitment	[7]
Ptr2	*M. jannaschii*	Activator	Unknown	Facilitates TBP binding	[14]
NrpR	*M. maripaludis*	Repressor	2 oxo glutarate (inducer)	Probably blocks RNAP	[3,4]

[a]Where known, the mechanism whereby the regulator influences transcription is described. In the case of TrmB and NrpR, the position of the operator sequences suggests a likely mode-of-action, but this has not yet been confirmed experimentally.

testing its ability to bind to, and influence transcription from, a candidate promoter (usually its own)].

The first such system to be characterized was the metal-dependent regulator Mdr1 of *Archaeoglobus fulgidus*. This system revealed a bacterial-like mode of negative regulation with RNA polymerase recruitment being blocked by the binding of the repressor molecule [5]. Subsequent work has established that this mechanism is employed by some other negative regulators characterized thus far [6–8] (Table 1), with the exception of the Lrs14 protein [9,10] in *Sulfolobus solfataricus* and probably TrmB [11] from *Thermococcus litoralis*. Lrs14, a member of the archaeal family of bacterial leucine-responsive regulatory protein (Lrp)-like proteins, has been shown to inhibit an earlier step in transcription initiation, namely, the promoter binding of the general transcription factors TBP and TFB [9]. Recent work has identified a possible downstream target for the Lrs14 protein. Lrs14 was found to bind to the promoter region of an alcohol dehydrogenase (*adh*) gene in *S. solfataricus* [12]. However, the effect, if any, of this protein on *adh* transcription has not been determined. Interestingly, virtually all other studies published to date have focused on the growing family of archaeal relatives of the bacterial Lrp family. This is a broadly conserved family of regulators (for review, see Ref. [13]) that includes the only biochemically characterized archaeal transcription activator, Ptr2 [14].

A recent study by Charlier and colleagues has described an extensive dissection of the binding of a *Sulfolobus solfataricus* Lrp-like regulator (Ss-LrpB) to its own promoter [15]. The study reveals that Ss-LrpB binds to three sites, each containing an imperfect inverted repeat structure, in its own promoter region. Interestingly, all three binding sites (boxes 1, 2 and 3) lie upstream of the core promoter elements, the TATA-box and BRE [1]. Box 1 is immediately adjacent to the BRE element with boxes 2 and 3 further upstream. Analysis of binding affinity reveals that boxes 1 and 3 bind Ss-LrpB with reasonably high affinity (10–25 nM), whereas box 2 has significantly lower affinity and indeed is only occupied after boxes 1 and 3 are bound. Therefore, this suggests that the Ss-LrpB might regulate its own promoter activity as a function of Ss-LrpB concentration in the cell. Interestingly, the authors reveal that Ss-LrpB induces extensive DNA curvature at the promoter [15]. Thus, in common with many bacterial systems, a combination of protein–protein and protein–DNA interactions might be inducing the formation of a complex nucleoprotein architecture at the promoter. Furthermore, it is possible that the promoter adopts different conformations depending upon the cellular concentration of Ss-LrpB. Clearly, it is conceivable that by imposing alternate architectures with distinct Ss-LrpB concentrations, Ss-LrpB has the capacity to differentially regulate transcription from its own promoter. What then is the effect of Ss-LrpB on its own promoter activity? The authors do not address this issue directly; however, they do reveal that, when binding to the box 1 BRE-proximal binding site, Ss-LrpB still permits formation of a TBP–TFB–promoter complex, leading to the formation of a quaternary Ss-LrpB–TBP–TFB–DNA complex [15].

Clearly, many intriguing questions remain. The authors speculate that, at low concentrations, Ss-LrpB might stimulate transcription from its own promoter and at higher concentrations auto-repress. If this is the case, it will be of considerable interest to test whether Ss-LrpB influences binding of TBP and/or TFB in a concentration-dependent manner. In this light, it is interesting to note that the only archaeal transcriptional activator characterized biochemically to date, Ptr2, a relative of Ss-LrpB from *Methanococcus jannaschii*, stimulates transcription via recruitment of TBP [14]. If Ss-LrpB is also found to influence TBP and/or TFB binding, will effects be dependent on all three Ss-LrpB binding sites? Similarly, will Ss-LrpB affect recruitment of RNA polymerase (RNAP) to the promoter? Although the RNAP does not directly bind to sites upstream of the BRE [16], it is possible that binding of this enormous enzyme will be influenced by the local geometry of DNA in the vicinity of the promoter. Certainly the binding data of Charlier and colleagues suggests extensive deformation and wrapping of promoter-proximal DNA [15].

Concluding remarks: where next?

In contrast to the *in vitro* experiments, *in vivo* Ss-LrpB will be binding to a DNA template that is compacted by association with small basic chromatin proteins [17]. It is tempting to speculate that the conformational alterations induced by Ss-LrpB might impact upon local chromatin architecture *in vivo* and thereby influence the transcriptional status of the core promoter. Furthermore, the Sul7d family of chromatin proteins, and Alba in *Sulfolobus*, has been shown to be subject to the post-translational modifications of methylation and acetylation, respectively [18,19]. One of the key questions remaining in the study of archaeal transcription is whether *in vivo* transcription

regulators might target chromatin modifying activities to promoters as part of their function.

Beyond the immediate direct and indirect interactions between Ss-LrpB and core machinery, is it possible that a small molecule ligand could influence Ss-LrpB binding and, therefore, its effect on transcription? The identification of additional genes regulated by Ss-LrpB could facilitate identification of such a co-regulatory molecule.

The phenomenon of auto-regulation of regulator transcription has proven to be a valuable tool for understanding the biochemical basis of regulator function. However, it is clear that most regulators will have downstream targets and it will only be with the identification of these targets that we will begin to gain insight into the true physiological roles of the regulators. It is to be hoped that the combination of improving systems for genetic manipulation of archaea, married with post-genomic technologies such as microarray platforms, will enable us to begin to dissect the complex regulatory hierarchies controlling archaeal gene expression. However, even in organisms lacking genetic tools, it should be possible to begin to gain insight into the *in vivo* function of regulators. In particular, the combination of chromatin immunoprecipitation technologies with array interrogation will reveal the genomic binding-sites of regulatory molecules and hopefully give insight into the identity of co-regulated genes. A second technique that will likely be of great value is the ROMA (run off transcription microarray analyses) technology [20]. In this remarkable system, total genomic DNA can be transcribed by a defined *in vitro* basal transcription system. Comparisons can then be made between the profiles of transcription products obtained before and after the addition of a candidate regulator to the reaction. In this light, the Ptr2 activator of *M. jannaschii* has been demonstrated to activate transcription from promoters embedded in the context of genomic DNA [14]. It is eagerly anticipated that this promising initial result can be extended to interrogation of arrays with *in vitro* transcription products. Comparison of the transcript profile generated in the presence and absence of regulator will enable the identification of targets of that molecule.

Finally, as alluded to previously, it has been demonstrated that archaeal chromatin proteins have the ability to impact upon transcriptional processes *in vitro* [19,21,22]. There is a curious dichotomy in the distribution of chromatin protein in the two most highly studied branches of the archaea. The Euryarchaea possess bona fide histone homologues [23], but no evidence has been found for post-translational modification of these species *in vivo* [24]. By contrast, crenarchaea do not have histones, but in the crenarchaeote *Sulfolobus solfataricus* two of the major chromatin proteins, Sso7d and Alba are post-translationally modified. The situation becomes even more intriguing with the observation that Alba is found in many euryarchaeotes [17,19,25]; however, it is not yet known if Alba is acetylated in those species.

Recent work has provided evidence for a role of archaeal chromatin proteins in modulating gene expression *in vivo*. *Methanococcus voltae* possesses two genes encoding archaeal histone homologues and, in addition, a single Alba homologue. Deletion mutants for these genes have been generated in *M. voltae*. Interestingly, none of the genes are essential for viability. However, 2D protein gel analysis revealed that a diverse range of proteins showed significantly altered levels in the mutant strain compared with wild-type, indicating that these chromatin proteins could indeed have a role in regulating transcription from some promoters [26].

It will be of great interest to determine whether, as with bacterial nucleoid proteins [27–29], the archaeal chromatin proteins function at a local level to serve as co-activators or co-repressors. Alternatively, it is possible that archaeal chromatin (and its covalent modification in *Sulfolobus* species) could influence comparatively large-scale regions of chromatin in a paradigm more akin to that observed in eukaryotes. Clearly, it will be important to begin to dissect the chromatin occupancy of promoters and transcribed sequences *in vivo* and to identify novel factors that impinge upon the large-scale architecture of archaeal chromatin.

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