Emerging Topics in Life Sciences (2018) **2** 659–669
https://doi.org/10.1042/ETLS20180036

Review Article

Conserved principles of transcriptional networks controlling metabolic flexibility in archaea

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Gene regulation is intimately connected with metabolism, enabling the appropriate timing and tuning of biochemical pathways to substrate availability. In microorganisms, such as archaea and bacteria, transcription factors (TFs) often directly sense external cues such as nutrient substrates, metabolic intermediates, or redox status to regulate gene expression. Intense recent interest has characterized the functions of a large number of such regulatory TFs in archaea, which regulate a diverse array of unique archaeal metabolic capabilities. However, it remains unclear how the co-ordinated activity of the interconnected metabolic and transcription networks produces the dynamic flexibility so frequently observed in archaeal cells as they respond to energy limitation and intermittent substrate availability. In this review, we communicate the current state of the art regarding these archaeal networks and their dynamic properties. We compare the topology of these archaeal networks to those known for bacteria to highlight conserved and unique aspects. We present a new computational model for an exemplar archaeal network, aiming to lay the groundwork toward understanding general principles that unify the dynamic function of integrated metabolic-transcription networks across archaea and bacteria.

Introduction

Metabolism is the sum of all biochemical reactions in the cell. Catabolic pathways oxidize nutrients to provide energy, whereas anabolic pathways synthesize cellular building blocks, structures, and cofactors. The metabolic network is defined as the entirety of metabolic pathways in a given cell, and how those pathways are interconnected [1]. Parts of a metabolic network include metabolites (intermediates), enzymes that catalyze the interconversion of these metabolites, and genes that encode the enzymes [2].

A general definition of transcription-metabolic subnetworks, or TMnets

Flexibility and adaptation of the metabolic network during environmental variability is enabled by the action of the global gene regulatory network (GRN), comprising a web of interacting regulatory molecules called transcription factors (TFs) and the genes they control [3–7]. Often, a given TF or set of TFs regulate a suite of genes that encode enzymes functioning in the same metabolic pathway [5]. These pathways produce small molecules that, in turn, can affect the activity of the TF(s) [8]. These subnetworks, or subsets of the GRN, coordinately activate entire metabolic pathways, but shut off others. This adaptability is required for fitness in response to varying substrate availability. A conserved strategy across archaea and bacteria for regulating a metabolic pathway in response to substrate availability is through transcriptional switches that transition between two or more major metabolic programs (Figure 1) [9]. Here, we define these transcription-metabolic subnetworks, or TMnets, as consisting of a ligand (small molecule or environmental signal), a TF, and the genes encoding the metabolic pathway(s) regulated by the TF. We focus on how the topology, function, and dynamic properties are conserved across archaea and bacteria (Figure 1).

Received: 30 August 2018
Revised: 5 November 2018
Accepted: 8 November 2018

Version of Record published:
14 December 2018

Emerging Topics in Life Sciences (2018) **2** 659–669
https://doi.org/10.1042/ETLS20180036

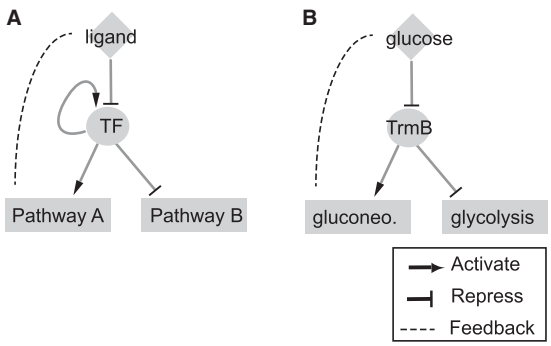

Figure 1. Transcription-metabolic subnetworks — 'TMnets'

Many GRN subnetworks in bacteria and archaea function as transcriptional switches that respond to the availability of a key substrate or product. (**A**) The generalized topology of these TMnets consists of a TF that binds to a ligand to either activate (arrow) or repress (blunt-ended line) the expression level of genes encoding enzymes that function in these metabolic pathways. Often, TFs autoactivate the expression of their own gene (circular arrow). Frequently, metabolic intermediates or end products of these pathways represent the ligand itself, or a substrate for ligand production, hence providing feedback from the metabolic pathway to the transcriptional switch (dotted line) [8]. (**B**) An example TMnet from archaea. When glucose is added to the medium, the TF TrmB is released from DNA binding, de-activating genes encoding enzymes in gluconeogenesis and de-repressing glycolytic genes [10–13]. Given that archaeal subnetworks are less understood, here we explore the conservation of the topological and dynamical properties of such TMnets across bacteria and archaea.

Archaea are important but understudied models for understanding the transcriptional regulation of metabolic flexibility

Archaea are ubiquitous, but dominate in energy-limited, extreme environments and thus possess uniquely constrained metabolic capabilities [1,14]. Understanding these capabilities is important because archaea are major players in global geochemical cycles [15] and of interest for biotechnology, for example, enzymes resilient to the harsh conditions of industrial production [16]. However, to realize the full biotechnological potential of archaea, knowledge advances are required to understand archaeal metabolic pathways, their regulation, and dynamic function. Computational modeling of system responses to genomic or environmental perturbations can explain and predict dynamical behavior of interconnected GRNs and metabolic networks [17], ultimately enabling biological systems to be re-engineered for desired outputs such as microbial production of biofuels [18].

Key questions and overview

Despite the importance of archaea, relative to the deep knowledge of bacterial TF function, those of archaea remain unclear. Approximately 50 TFs have been experimentally investigated across all archaeal model organisms (e.g. halophiles, methanogens, thermophiles, reviewed in [5,19]); in contrast, the function of 184 TFs (~60%) is known in *Escherichia coli* alone [7]. What are the conserved underlying biochemical mechanisms of TMnets across archaea and bacteria? What are the unifying dynamic properties of these subnetworks? What are the key differences?

In this review, we compare known TMnets of bacteria and archaea, discussing what topological and dynamical features of TMnets are conserved. How can we leverage the extensive knowledge regarding TMnets in well-studied bacteria? To provide an overview, first we describe the importance of studying transcriptional control of metabolism in enabling global changes in metabolic flux. Second, we compare and contrast the topological features and dynamic functions of known bacterial and archaeal TMnets. Third, we review how the qualitative dynamic properties that emerge from TMnet topology can be revealed through coarse-grained modeling. Such modeling is useful regardless of the species of interest: how and whether dynamic properties are conserved across TMnets remains to be revealed even within *E. coli* [8], much less in understudied species of bacteria and archaea. Finally, we highlight two recently discovered archaeal TMnet examples. We describe the mechanisms underlying metabolic control and, for the first time, model the dynamical properties of the TMnet that regulates sulfur reduction in a thermophilic archaeal model species [20,21]. Using these examples, we argue that characterization and dynamical modeling of TMnets pinpoint knowledge gaps, laying the groundwork toward unifying properties of how metabolic flexibility is regulated.

Why study transcriptional control of metabolism?

Studies that used metabolomics, quantitative proteomics, and/or theory in bacteria have observed pervasive enzyme over-abundance, especially for those that function in central carbon metabolism [22]. Many of these enzymes are regulated at the level of activity by allostery (e.g. in *E. coli*), in which a metabolic intermediate separate from the specific substrate or product of the enzyme inhibits or activates enzyme activity by binding outside of the active site [23]. In archaea, multiple mechanisms important for the regulation of enzyme activity have been discovered recently, including substrate inhibition, product feedback inhibition, and phosphorylation, among others [24–26]. Phosphoproteomics emphasized the importance of phosphorylation in substrate channeling in central carbon metabolism in *Sulfolobus solfataricus* [26]. In bacteria, such regulation changes metabolic flux within seconds, allowing quicker response time to metabolic status and nutrient availability than transcription, which functions on the order of minutes [9]. Changing enzyme levels either by overexpression or by TF knockout also failed to change metabolic flux in several studies, calling into question the importance of transcriptional regulation of metabolic flux [23]. Why, then, are so many metabolic pathways controlled at the transcriptional level? Transcriptional regulation can co-ordinate the level of multiple enzymes in a given metabolic pathway or across multiple pathways. In the case of global TFs, a single TF can co-ordinate a switch-like response of multiple metabolic pathways simultaneously in response to a common stimulus. We therefore focus here on transcriptional control of metabolism.

Definition of TMnet topology: mapping how environmental signals, TFs, and their target genes interact

TFs interact directly with environmental signals to control gene expression in bacteria and archaea

Homology has been observed between bacterial and archaeal TFs that activate or repress genes in response to environmental stimuli [27,28]. In archaea, these regulatory TFs differ from the general transcription factors (GTFs [29]) required for the initiation of basal transcription, such as TATA-binding protein (TBP) and RNA polymerase, which resemble those of eukaryotes. GTFs have been recently reviewed in [5,30–32] and are not the subject of focus here. Archaeal regulatory TFs are enriched for DNA-binding domains that strongly resemble those of bacteria, such as helix-turn-helix domains [28,33]. As in bacteria, the majority of archaeal TFs consist of a DNA-binding domain and a partner domain [27,28]. Nearly 50% of known bacterial partner domains bind to a small molecule, also called an inducer or ligand, that affect DNA-binding affinity [34,35]. Recent predictions based on phylogenetic analysis of genome sequences estimate that archaeal TFs share these attributes [27]. To date, ∼40% of TFs studied experimentally in archaea bind a ligand to govern DNA binding [5]; however, this is likely an underestimate given that the specific ligands for some TFs have been predicted but not yet been experimentally characterized [5,36–39].

TMnets with metabolic feedback are pervasive in bacteria

Across bacteria, metabolic switches of varying complexity can be regulated by feedback that integrates metabolic and transcriptional networks, where a given metabolic pathway produces an intermediate, that metabolite changes the conformation of the TF, thereby altering the DNA-binding capability of the TF (Figure 1). For example, LacI derepresses the *lac* operon in the presence of lactose in the growth medium [40]. A recent paper formalized the definition of these metabolic-transcription feedback networks, calling them 'genetic sensory response units' (GENSORs), which consist of an individual TF, the signal that changes its DNA-binding activity, and the cellular response (e.g. gene expression) [8]. Of the GENSOR units studied in the RegulonDB database, a global GRN of *E. coli*, 83% included metabolic feedback [8], suggesting that interaction between metabolism and transcriptional regulation is a general feature enabling environmental adaptation in bacteria. Here we differentiate such GENSOR units from 'transcription-metabolic subnetworks', or TMnets, which include all networks that function as metabolic switches with or without feedback. "Subnetwork" is a general term we have used previously to describe smaller subsets of the global GRN [5], and here we consider TMnets as a special case of such subnetworks (Figure 1A).

TMnets that regulate metabolic flexibility are understudied in archaea

In archaea, the TrmB TF is a widely conserved regulator of central metabolic pathways [41]. In many species of euryarchaea, TrmB activates gluconeogenic pathways and represses glycolytic pathways [11,12,42]. Recent

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https://doi.org/10.1042/ETLS20180036

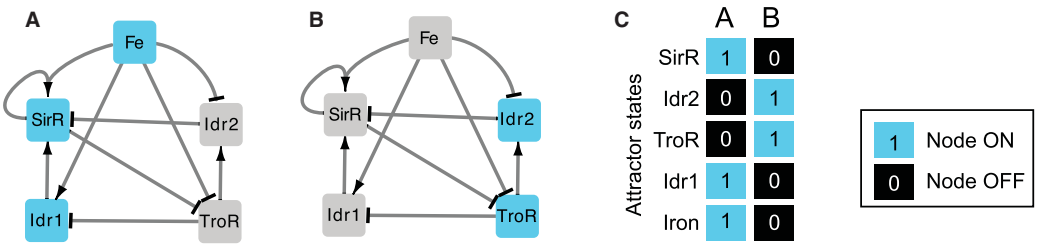
metabolic modeling suggests that the TrmB TMnet functions as a metabolic switch between gluconeogenesis and glycolysis in *Halobacterium salinarum* [13] (Figure 1B). When glucose is spiked into cultures growing on amino acids as a primary source of carbon and energy, transcription of enzyme-coding genes in gluconeogenic pathways is rapidly shut off, whereas glycolytic pathways are de-repressed [13,43]. This topology and switch-like dynamics closely resemble the general TMnet or GENSOR topology of bacteria. Also like the CRP/*lac* system, global control by the TrmB network is hierarchical: although TrmB functions alone as the sole TF required for the switch between central carbon metabolic pathways, TrmB also regulates downstream regulators to generate pulses of expression in peripheral pathways such as cofactor biosynthesis [5,13,43].

Another recently described example of a TMnet in archaea is the iron homeostasis regulatory network in *H. salinarum* [38,44]. Unlike the TrmB TMnet, the iron regulatory network is comprised of four DtxR family TFs that regulate each other with complex double interlocked feedback loop architecture (Figure 2). The extensive transcriptional feedback between the TFs is hypothesized to increase the homeostatic range and enable the resilience of *H. salinarum* to extremely low levels of iron typical in saturated salt lake habitats [44]. The network enables cells to switch between the iron starvation response and iron uptake during replete conditions to maintain iron homeostasis. Interestingly, such interlocking feedback loops between TFs have so far only been observed in eukaryotes, enabling stable phenotypes such as circadian oscillations or cell fate determination [45–48]. Although the topologies are known for many archaeal TMnets (recently reviewed in [5,19]), the dynamic properties remain unclear for the majority of these networks.

TMnet topology explains and predicts dynamical properties of metabolic flexibility

Theoretical work has shown that the topology, or structural layout, of subnetworks in which two or more TFs regulate each other can lead to characteristic and predictable gene expression dynamics [5,49,50]. Such dynamical principles appear to generalize across TF types, environmental response, and organism [49]. In contrast, only a few well-understood TMnets have been used as the system of choice for detailed, quantitative mathematical models. For example, the *E. coli lac* operon is frequently used for modeling bacterial TMnets [40], usually using systems of ordinary differential equations (ODE) [51,52]. The TrmB TMnet is a model TMnet for archaea [13,43,53]. Although fine-grained ODE models of these systems are highly accurate in regard to quantitative explanation of how integrated GRNs and metabolic networks function over time [13,52], extensive knowledge of kinetic parameters for the network of interest is required [51]. Such parameters include rate constants for transcript production and degradation, TF-inducer-binding affinities, high-resolution time course transcriptomic data, and other kinetic and/or thermodynamic parameters that only come from detailed experiments ([52]; summarized in [51,54]). More recently, thermodynamic models of the *lac* operon have shown using fewer parameters that the free energy of repressor–DNA interaction, or Bohr parameter, is predictive of the fold change in gene expression over a wide range of inducer concentrations and number of repressor molecules per cell [55].

However, it remains unclear whether the principles learned through kinetic modeling of the *lac* operon in *E. coli* and ODE modeling of the TrmB regulon in *H. salinarum* are conserved across TMnets in other bacterial and archaeal species. As an alternative, Boolean models of TMnets have qualitatively recapitulated bistability, a known dynamic feature of well-studied bacterial networks such as the *lac* [54] and arabinose operons [56] in *E. coli*. Using Boolean modeling, the iron homeostasis network in *H. salinarum* was also predicted to exhibit bistability during constant iron exposure and oscillatory dynamics under fluctuating iron availability (Figure 2) [44]. This suggests that higher complexity archaeal TMnets can enable multiple dynamic regimes depending on model parameters. These studies posited that network topology, or structure, was sufficient for qualitative prediction of network dynamic properties [54,56]. It is important to note, however, that the computational difficulty of such prediction scales with the size of the network [51], and therefore smaller networks (~3–5 nodes), such as those of interest here, are an excellent case study for Boolean modeling. This suggests that such coarse-grained modeling is an excellent candidate for predicting dynamic properties when network topology is known but not detailed kinetic parameters. Such models can suggest targeted experimental tests, obviating time-consuming trial and error [44]. This is an especially useful property for less studied systems such as TMnets in archaea. In the ensuing section, we use Boolean models to demonstrate that archaeal TMnets can function as bistable transcriptional switches that enable adaptation to variation in environmental availability of a critical growth substrate.



D **Evidence for node and edge activities in the iron regulatory subnetwork of *H. salinarum***

source node	edge	target node	evidence	references
SirR	activates	SirR	ChIP-qPCR	Martinez-Pastor et al., 2017
SirR	represses	TroR	ChIP-qPCR, RT-qPCR in ΔsirR	Martinez-Pastor et al., 2017
Idr2	represses	SirR	ChIP-chip, transcriptomics in Δidr2	Schmid et al., 2011
TroR	represses	Idr1	ChIP-qPCR, RT-qPCR in ΔtroR	Martinez-Pastor et al., 2017
Idr1	activates	SirR	ChIP-chip, transcriptomics in Δidr1	Schmid et al., 2011
Idr2	represses	SirR	ChIP-chip, transcriptomics in Δidr2	Schmid et al., 2011
Fe	represses	TroR	ChIP-qPCR, ΔtroR phenotype, ICP-MS	Martinez-Pastor et al., 2017
Fe	represses	Idr2	ChIP-chip, Δidr2 phenotype, ICP-MS	Schmid et al., 2011; Martinez-Pastor et al., 2017
Fe	activates	SirR	ChIP-qPCR, Δidr2 phenotype, ICP-MS	Schmid et al., 2011; Martinez-Pastor et al., 2017
Fe	activates	Idr1	ChIP-chip, Δidr1 phenotype, ICP-MS	Schmid et al., 2011; Martinez-Pastor et al., 2017

Figure 2. Double interlocked feedback loop topology of the iron TMnet switches iron acquisition on during starvation and off during sufficiency

(**A**) Topology and activity of the DtxR TMnet switch in the presence of iron. Blue nodes are active (ON), gray nodes are inactive (OFF). Edge designations are as in Figure 1. (**B**) Topology and activity of the DtxR TMnet in the absence of iron. (**C**) Attractor states reached in model simulations. Columns A and B resulted from the topologies shown in subpanels (**A**) and (**B**), respectively. (**D**) Summary of experimental evidence supporting TMnet topology. Subpanels (**A**) and (**B**) were adapted from [44].

New Boolean models demonstrate the conservation of TMnets and their dynamic properties across archaeal species

Example 1: Double interlocked feedback governs iron acquisition and predicts two steady states in *H. salinarum*

Iron is required for key metabolic processes but is toxic in excess, so tight regulation of iron homeostasis is widely conserved. In hypersaline environments, iron levels are extremely low [57], and halophiles are resistant to iron starvation [38,44,58]. Recently, we described the topology and dynamics of the TMnet governing iron acquisition in *H. salinarum* [44]. This TMnet consists of four TFs (Idr1, Idr2, SirR, and TroR) whose amino acid sequence strongly resembles those of the DtxR family of metal repressors in bacteria [59,60] (Figure 2A,B). Each of these four TFs directly regulates expression of at least one of the other three (Figure 2D, [44]), comprising two interconnected feedback loops (Figure 2A,B). Despite the conservation of these TFs with those of the DtxR family, such complex feedback topology between iron-responsive TFs has not yet been observed in bacteria [7,59].

Boolean modeling of this network suggested that, like the SurR TMnet, two steady states are achieved based on the experimentally validated topology, one in which Idr1 and SirR are active in the presence of iron, the other in which TroR and Idr2 are active under iron starvation (Figure 2C). However, these steady states are achieved only after three time steps, suggesting a slower transition between states ([44], Supplementary File 1). Simulations suggested that this TMnet enables maintenance of iron homeostasis despite drastic variations in extracellular iron levels, consistent with dynamic properties of interlocking feedback architecture in eukaryotes, such as those regulating yeast colony phenotypes [45]. These results suggest that archaea can use bacterial-type TFs in a eukaryotic regulatory network topology to adapt to harsh environments. Computational predictions of gene networks in other archaeal genomes also revealed that other species harbor multiple DtxR homologs whose predicted regulons overlap [60], narrowing the scope of organisms to investigate the extent of conservation of complex TMnets allowing iron homeostasis across archaea.

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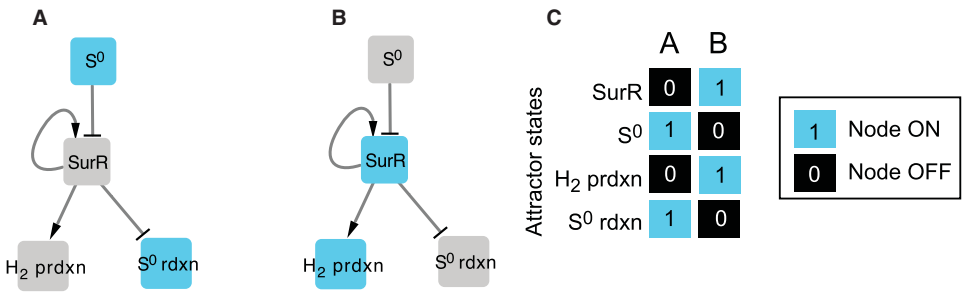
https://doi.org/10.1042/ETLS20180036



D **Evidence for node and edge activities in the SurR subnetwork of *P. furiosus***

source node	edge	target node	evidence	references
S^0	represses	SurR	X-ray crystal structure, EMSA, footprinting with SurR cysteine mutant	Yang et al., 2010
SurR	activates	SurR	In vitro transcription, footprinting, EMSA	Lipscombe et al.,2009
SurR	represses	S^0_redxn	Tanscriptomics, EMSA, footprinting, ΔsurR and cysteine mutant qRT-PCR, ΔsurR growth phenotype	Schut et al., 2007; Lipscombe et al., 2009; Lipscombe et al., 2017.
SurR	activates	H_2_prdxn	Tanscriptomics, EMSA, footprinting, ΔsurR and cysteine mutant qRT-PCR, ΔsurR growth phenotype	Schut et al., 2007; Lipscombe et al., 2009; Lipscombe et al., 2017.

Figure 3. SurR redox status responds to the availability of elemental sulfur, which functions as a regulatory switch between sulfur reduction and hydrogen production

(**A**) Topology and activity of the SurR TMnet switch in the presence of elemental sulfur (S^0). (**B**) Topology and activity of the SurR TMnet in the absence of S^0. (**C**) Attractor states reached in model simulations. Columns A and B resulted from the topologies shown in subpanels (**A**) and (**B**), respectively. (**D**) Summary of experimental evidence supporting TMnet topology. Edge designations are as in Figure 1. Node colors are as in Figure 2.

Example 2. SurR is a reversible redox switch that balances S^0 reduction and H_2 production based on substrate availability

Pyrococcus furiosus is a desirable target for biotechnology because it thrives at high temperature and possesses unique metabolic capabilities [61,62]. *P. furiosus* produces hydrogen gas as a byproduct of fermentation of organic carbon to organic acids [63], so understanding the pathways and regulation of H_2 production is of interest for downstream bioengineering for boosting biological production of H_2. H_2 production is inhibited in the presence of elemental sulfur, which is reduced in the presence of organic carbon to hydrogen sulfide and carbon dioxide [64,65]. The transcriptomic response of *P. furiosus* to S^0 is rapid: within 10 min of S^0 addition, nearly 20 S^0 reduction genes are induced. Simultaneously, over 30 H_2 production genes are down-regulated [64]. Lipscomb et al. identified SurR, a TF that regulates this transcriptomic response program [66]. SurR was co-purified in an affinity capture experiment with the promoter of *mbh1*, the gene encoding one of the hydrogenase enzymes [66].

In a series of thorough *in vivo* and *in vitro* studies, the topology of the SurR TMnet was characterized, including SurR mechanism of DNA binding and its effect on metabolism (Figure 3A). When elemental S is absent, SurR binds to a GTT-n3-AAC motif, activating expression of its own gene and genes whose products are involved in hydrogen production (e.g. all three hydrogenases SI, SII, and [NiFe]-hydrogenase MBH) [21,66]. Under these conditions, SurR also represses genes encoding enzymes required for the reduction in elemental sulfur to H_2S (e.g. protein disulfide oxidoreductase, sulfur reductase) [21,66]. When S^0 is present in the environment, a CXXC motif within SurR is oxidized, and SurR undergoes a redox switch that changes its conformation and disrupts SurR-DNA binding [20]. Repression of S^0 reduction genes is relieved, and H_2S production proceeds [21]. This experimental evidence is summarized in Figure 3D. Interestingly, the resultant topology of the SurR TMnet resembles that of bacterial TMnets such as GENSOR units (Figure 1A; [7,8]).

Here, we applied Boolean modeling to explain and predict dynamic behavior of the SurR TMnet (Figure 3A,B). A series of Boolean logic functions describes the topology (Table 1). Given that the SurR TMnet consists of

Table 1. Boolean logic functions: SurR TMnet
SurR is the DNA-binding and gene expression regulation activity of the SurR TF; S^0 is the presence of elemental sulfur; H_2 is the expression of genes encoding hydrogen production enzymes and hydrogen production *per se*; and S^0 rdxn is the expression of genes encoding enzymes in the sulfur reduction pathway and the production of H_2S *per se*. $\wedge$, *AND*; $\neg$, *NOT*.

Node	Logic function
S^0	S^0
SurR	$SurR \wedge \neg S^0$
H_2	$SurR$
S^0 rdxn	$\neg SurR$

four nodes, each with two possible states (i.e. ON or OFF), we simulated the model in each of the 2^4 possible starting states using a synchronous update scheme with six time steps (Supplementary File 1). This means that we examined the state (activity) of each node across time steps of equal duration, with each node state updating simultaneously at each time step. The transition state graph, or output, for 8 of the 16 simulations resulted in a steady state (also known as a fixed point, or 'attractor' [51]) with S^0 and S^0 reduction ON; but SurR and H_2 OFF (Figure 3A,C, attractor state (A)). Four of the 16 simulations resulted in the opposite steady state, with S^0 and S^0 rdxn OFF; but SurR and H_2 ON (Figure 3B,C, attractor state (B)). Four of the simulations resulted in attractor states with S^0 reduction ON in the absence of S^0, a biologically impossible state, and so was discarded. In biological terms, model results explain the existing data, where SurR essentially acts as a switch [20]: in the presence of S^0, SurR-DNA binding is disrupted, relieving repression of the sulfur reduction pathway, and deactivating the H_2 production pathway (Figure 3D). These opposing steady states are reached within the first time step of the simulations, consistent with the empirical observation of the rapid transcriptional response to S^0 (Figure 3D, [64]).

Toward dynamic modeling and unifying properties of TMnets across archaea

Here we have presented two examples of TMnet dynamics in archaea. Using computational modeling of network topology to predict the dynamic properties of these example archaeal networks, we observed here and previously [44] that such switches display conserved dynamic properties with those of bacteria, but govern uniquely archaeal metabolic pathways. The SurR TMnet behaved as a rapid switch between two opposing metabolic pathways, a property highly conserved with bacterial TMnets [8]. Despite the more complex inter-TF feedback, a topology conserved with those of eukaryotes [45], the DtxR iron network exhibited bistable dynamics similar to that of the SurR TMnet, albeit with a slower response time (Figures 2 and 3, Supplementary File 1). Is bistability a unifying property of TMnet dynamics across archaea? Are simple or complex topologies more frequently observed? The field of transcriptional regulation in archaea is currently poised to answer these questions: experimental evidence now exists to support the functional knowledge for ~50 archaeal TFs [5,19]. Notable examples of putative switch-like TMnets include TFs that regulate central carbon metabolism (TrmB [41], GlpR [67], and XacR [36]), which are discussed in the ensuing section.

Switches regulate carbon source use in archaea

The global regulons governed by the TrmB family of TFs have been characterized across several species [11,12,68]. These TFs typically regulate gene-encoding enzymes in glycolysis and gluconeogenesis [41]. In *H. salinarum*, TrmB activates gluconeogenesis and co-ordinates precursor supply with growth rate, providing a dynamic switch in response to glucose, and enabling rapid response to environmental conditions [13,53]. The GlpR TF of *Haloferax volcanii* functions in catabolite repression [69]. In the presence of glucose and glycerol, GlpR represses genes whose products function in fructose uptake and oxidation, and induces those involved in glycerol degradation [67,69]. When fructose is available, it is transported through the phosphotransferase system and converted to fructose-1-phosphate (F-1-P) [70]. F-1-P binds to GlpR and disrupts its interaction

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with DNA, derepressing gene-encoding enzymes in the modified Embden–Meyerhoff pathway that oxidizes fructose [24,67]. XacR of *H. volcanii* activates an unusual pentose degradation pathway that is promiscuous for both xylose and arabinose [36,71,72]. Despite the knowledge of carbon source regulation, gaps remain that currently preclude dynamic modeling. In some TMnets, the ligand remains unknown (e.g. XacR [36], TrmB in *Thermococcus kodakaraensis* [68]). How gene expression changes over time remains unknown in some cases (e.g. GlpR [67]). Therefore, viewing TMnets from a dynamic modeling perspective aids in pinpointing important knowledge gaps, prioritizing experiments.

How widely conserved are switches exhibiting bistable dynamics?

The mechanism of action for all three of these TMnets regulating central carbon metabolism appears to resemble that of the SurR switch described here: a ligand disrupts the TF-DNA-binding interaction, derepressing genes encoding enzymes of one pathway, but de-activating a second pathway [13,36,66,67]. However, in each case, additional complexities may be at play. TrmB in *H. salinarum* and GlpR in *H. volcanii* each directly regulate at least two other TFs in response to carbon source availability [12,13,67]. In the SurR network, key nodes or edges could still be missing. For example, the expression of other TFs of unknown function was induced by S^0, including PF2051, an ArsR family homolog [64]. Genome-wide transcriptomics and TF-DNA-binding location analysis for Idr1 and Idr2 in *H. salinarum* suggested that these two TFs independently and oppositely regulate iron uptake systems, and also jointly regulate nearly 20 genes (Figure 2D, [38]). However, the direct targets of TroR and SirR (except for the genes encoding the other DtxR TFs) remain unknown. Is interaction between these TFs required for dynamical flexibility of the respective metabolic pathways they govern?

Given these complexities, dynamic modeling can elucidate the set of minimal components that are necessary and sufficient to produce observed dynamic metabolic responses to substrates. We posit here that these components should at least include: (a) knowledge of the specific inducing ligand; (b) how TF-ligand interaction affects DNA binding; (c) time course transcriptomics (e.g. RNA-seq) of target genes in response to the signal in TF knockout vs. wild-type backgrounds. This core of TMnet components and data could then be used to search across diverse archaea for conserved TMnet properties. Such searches will require the development of systematic computational methods for comparing networks. For example, one could quantify the similarities between TMnet components such as cis-regulatory-binding sequences or nodes (TFs and target genes). Alternatively, in the absence of homologous nodes, perhaps the overall structure of the TMnets *per se* should be compared. Dynamic modeling assists in pinpointing key knowledge gaps that, when filled, will enable future re-engineering of archaeal transcriptional switches that control metabolism.

Summary

- Transcription-metabolic subnetworks (TMnets) often function as switches in bacteria and archaea, transitioning between metabolic pathway activity during nutrient fluctuation.
- Coarse-grained modeling methods are useful tools to delineate qualitative dynamic properties of understudied organisms such as archaea.
- TMnet responses to different stimuli across archaeal lineages produce strikingly similar dynamics.
- Many recently discovered archaeal TFs are nearly ready for dynamic modeling.

Abbreviations

GENSORs, genetic sensory response units; GRN, gene regulatory network; GTFs, general transcription factors; ODE, ordinary differential equations; TBP, TATA-binding protein; TFs, transcription factors.

Funding

This work was funded by National Science Foundation [grants 1651117 and 1615685 to A.K.S.]

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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