Review Article

Conserved principles of transcriptional networks controlling metabolic flexibility in archaea

Amy K. Schmid1,2
1Department of Biology, Duke University, Durham, NC 27708, U.S.A.; 2Center for Genomics and Computational Biology, Duke University, Durham, NC 27708, U.S.A.

Correspondence: Amy K. Schmid (amy.schmid@duke.edu)

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 archael 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 archael cells as they respond to energy limitation and intermittent substrate availability. In this review, we communicate the current state of the art regarding these archael networks and their dynamic properties. We compare the topology of these archael networks to those known for bacteria to highlight conserved and unique aspects. We present a new computational model for an exemplar archael network, aiming to lay the groundwork toward understanding general principles that unify the dynamic function of integrated metabolic-transcription networks across archael 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).
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 allosteroy (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 archael 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 archael 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 archael 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 euryarchaeae, TrmB activates gluconeogenic pathways and represses glycolytic pathways [11,12,42]. Recent
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 derepressed [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.
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.
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
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 H2 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 H2 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 H2 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...
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 S0, 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.

Reference
1 Thor, S., Peterson, J.R. and Lubhey-Schultz, Z. (2017) Genome-scale metabolic modeling of archaea lends insight into diversity of metabolic function. *Archaea* 2017, 3 0763848 https://doi.org/10.1155/2017/30763848
2 Schilling, C.H., Letscher, D. and Palsson, B.O. (2000) Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented perspective. *J. Theor. Biol.* 205, 229–248 https://doi.org/10.1006/jtbi.2000.1073
3 Bonnaire, R., Facciotti, M.T., Reiss, D.J., Schmid, A.K., Pan, M., Kaur, A. et al. (2007) A predictive model for transcriptional regulation of physiology in a free living cell. *Cell* 131, 1354–1365 https://doi.org/10.1016/j.cell.2007.10.053
4 Brooks, A.N., Reiss, D.J., Allard, A., Wu, W.J., Salvanha, D.M., Plaisier, C.L. et al. (2014) A system-level model for the microbial regulatory genome. *Mol. Syst. Biol.* 10, 740–749 https://doi.org/10.15252/msb.20145160
5 Martinez-Pastor, M., Tonner, P.D., Darnell, C.L. and Schmid, A.K. (2017) Transcriptional regulation in archaea: from individual genes to global regulatory networks. *Annu. Rev. Genet.* 51, 143–170 https://doi.org/10.1146/annurev-genet-120116-023413
6 Yoon, S.H., Turkarslan, S., Reiss, D.J., Pan, M., Burn, J.A., Costa, K.C. et al. (2013) A systems level predictive model for global gene regulation of methanogenesis in a hydrogeotrophic methanogen. *Genome Res.* 23, 1839–1851 https://doi.org/10.1101/gr.153916.112
7 Gama-Castro, S., Salgado, H., Santos-Zavaleta, A., Ledezma-Tejeda, D., Muniz-Rascado, L., Garcia-Sotelio, J.S. et al. (2016) RegulonDB version 9.0: high-level integration of gene regulation, coexpression, motif clustering and beyond. *Nucleic Acids Res.* 44, D133–D143 https://doi.org/10.1093/nar/gkv1156
8 Ledezma-Tejeda, D., Ichida, and Collado-Vides, J. (2017) Genome-wide mapping of transcriptional regulation and metabolism describes information-processing units in *Escherichia coli*. *Front. Microbiol.* 8, 1486 https://doi.org/10.3389/fmicb.2017.01486
9 Donati, S., Sander, T. and Link, H. (2018) Crosstalk between transcription and metabolism: how much enzyme is enough for a cell? Wiley Interdiscip. *Rev. Syst. Biol. Med.* 10, e1396 https://doi.org/10.1002/wsbm.1396
10 Lee, S.J., Surma, M., Hausner, W., Thomm, M. and Boos, W. (2008) The role of TrmB and TrmB-like transcriptional regulators for sugar transport and metabolism in the hyperthermophilic archaeon *Pyrococcus furiosus*. *Mol. Microbiol.* 69, 247–256 https://doi.org/10.1111/j.1365-2958.2008.06378.x
11 Rechelt, R., Gindner, A., Thomm, M. and Hausner, W. (2016) Genome-wide analysis of the transcriptional regulator TrmB1 in *Pyrococcus furiosus*. *BMC Genomics* 17, 40 https://doi.org/10.1186/s12864-015-2360-0
12 Schmid, A.K., Reiss, D.J., Pan, M., Koide, T. and Baliga, N.S. (2009) A single transcription factor regulates evolutionarily diverse but functionally linked metabolic pathways in response to nutrient availability. *Mol. Syst. Biol.* 5, 282 https://doi.org/10.1038/msb.2009.89
13 Todor, H., Sharma, K., Pittman, A.M.C. and Schmid, A.K. (2013) Protein-DNA binding dynamics predict transcriptional response to nutrients in archaea. *Nucleic Acids Res.* 41, 8541–8558 https://doi.org/10.1093/nar/gkt659
14 Valentinii, D.L. (2007) Adaptations to energy stress dictate the ecology and evolution of the archaea. *Nat. Rev. Microbiol.* 5, 316–323 https://doi.org/10.1038/nrmicro1619
15 Ofte, P., Spiang, A. and Schlieper, C. (2013) Archaea in biogeochemical cycles. *Annu. Rev. Microbiol.* 67, 437–457 https://doi.org/10.1146/annurev-micro-092412-155614
16 Singh, A. and Singh, A.K. (2017) Haloarchaea: worth exploring for their biotechnological potential. *Biotechnol. Lett.* 39, 1793–1800 https://doi.org/10.1007/s10529-017-2434-y
17 Price, N.D., Reed, J.L. and Palsson, B.O. (2004) Genome-scale models of microbial cells: evaluating the consequences of constraints. *Nat. Rev. Microbiol.* 2, 886–897 https://doi.org/10.1038/nrmicro1023
18 Zargar, A., Bailey, C.B., Haushalter, R.W., Eiben, C.B., Katz, L. and Keasling, J.D. (2017) Leveraging microbial biosynthetic pathways for the generation of ‘drop-in’ biofuels. *Curr. Opin. Biotechnol.* 45, 156–163 https://doi.org/10.1016/j.copbio.2017.03.004
19 Karr, E.A. (2014) Transcription regulation in the third domain. *Adv. Appl. Microbiol.* 89, 101–133 https://doi.org/10.1016/B978-0-12-802595-9.00003-2
20 Yang, H., Lipscomb, G.L., Keese, A.M., Schut, G.J., Thomm, M., Adams, M.W. et al. (2010) SurR regulates hydrogen production in *Pyrococcus furiosus* by a sulfur-dependent redox switch. *Mol. Microbiol.* 77, 1111–1122 https://doi.org/10.1111/j.1365-2958.2010.077.issue-5
21 Lipscomb, G.L., Schut, G.J., Scott, R.A. and Adams, M.W.W. (2017) SurR is a master regulator of the primary electron flow pathways in the order *Thermococcales*. *Mol. Microbiol.* 104, 869–881 https://doi.org/10.1111/mmi.13668
22 O’Brien, E.J., Utrilla, J. and Palsson, B.O. (2016) Quantification and classification of *E. coli* proteome utilization and unused protein costs across environments. *PLoS Comput. Biol.* 12, e1004998 https://doi.org/10.1371/journal.pcbi.1004998
23 Kocjanowski, K., Sauer, U. and Chubukov, V. (2013) Somewhat in control — the role of transcription in regulating microbial metabolic fluxes. *Curr. Opin. Biotechnol.* 24, 987–993 https://doi.org/10.1016/j.copbio.2013.03.014
24 Brunsen, C., Esser, D., Rauch, B. and Siebers, B. (2014) Carbohydrate metabolism in archaea: current insights into unusual enzymes and pathways and their regulation. *Microbiol. Mol. Biol. Rev.* 78, 89–175 https://doi.org/10.1128/MMBR.00041-13
25 Esser, D., Hoffmann, L., Pham, T.K., Bracon, C., Qiu, W., Wright, P.C. et al. (2016) Protein phosphorylation and its role in archael signal transduction. *FEMS Microbiol. Rev.* 40, 625–647 https://doi.org/10.1093/femsre/fux020
26 Esser, D., Pham, T.K., Reimann, J., Albers, S.V., Siebers, B. and Wright, P.C. (2012) Change of carbon source causes dramatic effects in the phospho-proteome of the archaean *Sulfolobus solfataricus*. *J. Proteome Res.* 11, 4823–4833 https://doi.org/10.1021/pr300190k
27 Rivera-Gomez, N., Martinez-Nunez, M.A., Pastor, N., Rodriguez-Vazquez, K. and Perez-Rueda, E. (2017) Dissecting the protein architecture of DNA-binding transcription factors in bacteria and archaea. *Microbiology* 163, 1167–1178 https://doi.org/10.1099/mic.0.000504
28 Charoenasawang, V., Wilson, D. and Teichmann, S.A. (2010) Genomic repertoires of DNA-binding transcription factors across the tree of life. *Nucleic Acids Res.* 38, 7364–7377 https://doi.org/10.1093/nar/gkq617
29 Facciotti, M.T., Reiss, D.J., Pan, M., Kaur, A., Vuthoori, M., Bonnaire, R. et al. (2007) General transcription factor specified global gene regulation in archaea. *Proc. Natl Acad. Sci. U.S.A.* 104, 4630–4635 https://doi.org/10.1073/pnas.0611663104
Anantharaman, V., Koonin, E.V. and Aravind, L. (2001) Regulatory potential, phyletic distribution and evolution of ancient, intracellular

Seshasayee, A.S.N., Fraser, G.M., Babu, M.M. and Luscombe, N.M. (2009) Principles of transcriptional regulation and evolution of the metabolic system

Monod, J., Changeux, J.P. and Jacob, F (1963) Allosteric proteins and cellular control systems.

Johnsen, U., Sutter, J.M., Schulz, A.C., Tastensen, J.B. and Schonheit, P. (2015) XacR

Decker, K.B. and Hinton, D.M. (2013) Transcription regulation at the core: similarities among bacterial, archaeal, and eukaryotic RNA polymerases. Annu. Rev. Microbiol. 67, 113–139 https://doi.org/10.1146/annurev-micro-092412-155756

Bell, S.D., Cairns, S.S., Robson, R.L. and Jackson, S.P. (1999) Transcriptional regulation of an archaeal operon in vivo and in vitro. Mol. Cell 4, 971–982 https://doi.org/10.1016/S1097-2765(00)80226-9

Perez-Rueda, E. and Janga, S.C. (2010) Identification and genomic analysis of transcription factors in archaeal genomes exemplifies their functional architecture and evolutionary origin. Mol. Biol. Evol. 27, 1449–1459 https://doi.org/10.1093/molbev/msq033

Anantharaman, V., Koonin, E.V. and Aravind, L. (2001) Regulatory potential, phyletic distribution and evolution of ancient, intracellular small-molecule-binding domains. J. Mol. Biol. 307, 1217–1222 https://doi.org/10.1006/jmbi.2001.4508

Seshasayee, A.S.N., Fraser, G.M., Babu, M.M. and Luscombe, N.M. (2009) Principles of transcriptional regulation and evolution of the metabolic system in E. coli. Genome Res. 19, 79–91 https://doi.org/10.1101/gr.079715.108

Johnsen, U., Sutter, J.M., Schultz, A.C., Tastensen, J.B. and Schonheit, P. (2015) XacR — a novel transcriptional regulator of D-xylose and L-arabinose catabolism in the haloarchaeon Haloferax volcanii. Environ. Microbiol. 17, 1663–1676 https://doi.org/10.1111/emi.2015.17.issue-5

Sharma, K., Gillum, N., Boyd, J.L. and Schmid, A. (2012) The RosR transcription factor is required for gene expression dynamics in response to extreme oxidative stress in a hypersaline-adapted archaeon. BMC Genomics 13, 351 https://doi.org/10.1186/1471-2164-13-351

Schmid, A.K., Pan, M., Sharma, K. and Baliga, N.S. (2011) Two transcription factors are necessary for iron homeostasis in a salt-dwelling archaeon. Nucleic Acids Res. 39, 2519–2533 https://doi.org/10.1093/nar/gkq1211

Plaisier, C.L., Lo, F.Y., Ashworth, J., Brooks, A.N., Beer, K.D., Kaur, A. et al. (2014) Evolution of context dependent regulation by expansion of yeast/famine regulatory proteins. BMC Syst. Biol. 8, 122 https://doi.org/10.1186/s12918-014-0122-2

Moond, J., Changeux, J.P. and Jacob, F (1963) Allosteric proteins and cellular control systems. J. Mol. Biol. 6, 306–329 https://doi.org/10.1016/S0022-2836(00)80001-1

Kim, M., Park, S. and Lee, S.J. (2016) Global transcriptional regulator TrmB family members in prokaryotes. J. Microbiol. 54, 639–645 https://doi.org/10.1007/s12275-016-6362-7

Lee, S.J., Surma, S., Seitz, S., Hausner, W., Thomm, M. and Boos, W. (2007) Differential signal transduction via TrmB, a sugar sensing transcriptional repressor of Pyrococcus furiosus. Mol. Microbiol. 64, 1499–1505 https://doi.org/10.1111/j.1365-2958.2007.05737.x

Todor, H., Gooding, J., Ikayeva, O.R. and Schmid, A.K. (2015) Dynamic metabolite profiling in an archaeon connects transcriptional regulation to metabolic consequences. PLoS One 10, e0135693 https://doi.org/10.1371/journal.pone.0135693

Martínez-Pastor, M., Lancaster, W.A., Tonner, P.D., Adams, M.W.W. and Schmid, A.K. (2017) A transcription network of interlocking positive feedback loops maintains intracellular iron balance in archaea. Nucleic Acids Res. 45, 9990–10001 https://doi.org/10.1093/nar/gkk662

Zordan, R.E., Miller, M.G., Gálvez, D.J., Tuch, P.B. and Johnson, A.D. (2007) Interlocking transcriptional feedback loops control white-opaque switching in Candida albicans. PLoS Biol. 5, e256 https://doi.org/10.1371/journal.pbio.0050256

Shen-Orr, S.S., Milo, R., Mangan, S. and Alon, U. (2002) Network motifs in the transcriptional regulation network of Escherichia coli. Nat. Genet. 31, 64–68 https://doi.org/10.1038/ng881

Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D. and Alon, U. (2002) Network motifs: simple building blocks of complex networks. Science 298, 824–827 https://doi.org/10.1126/science.298.5594.824

Cheng, P., Yang, Y. and Liu, Y. (2001) Interlocked feedback loops contribute to the robustness of the Neurospora circadian clock. Proc. Natl. Acad. Sci. U.S.A. 98, 7408–7413 https://doi.org/10.1073/pnas.117120298

Alon, U. (2007) An Introduction to Systems Biology: Design Principles of Biological Circuits, Chapman & Hall/CRC Mathematical and Computational Biology Series, Chapman & Hall/CRC, Boca Raton, FL

Shoval, O. and Alon, U. (2010) SnapShot: network motifs. Cell 143, 326-e1 https://doi.org/10.1016/j.cell.2010.09.050

Wang, R.S., Saadatpour, A. and Albert, R. (2012) Boolean modeling in systems biology: an overview of methodology and applications. Phys. Biol. 9, 055001 https://doi.org/10.1088/1478-3975/9/5/055001

Wong, P., Gladney, S. and Keating, J. (1997) Mathematical model of the lac operon: inducer exclusion, catabolite repression, and diauxic growth on glucose and lactose. Biotechnol. Prog. 13, 132–143 https://doi.org/10.1021/bp970003a

Todor, H., Dulmage, K., Gillum, N., Bain, J.R., Muehlbauer, M.J. and Schmid, A.K. (2014) A transcription factor links growth rate and metabolism in the hypersaline adapted archaeon Halobacterium salinarum. Mol. Microbiol. 93, 1172–1182

Veliz-Cuba, A. and Stigler, B. (2011) Boolean models can explain bistability in the lac operon. J. Comput. Biol. 18, 783–794 https://doi.org/10.1089/cmb.2011.0031

Pazo-Melja, M., Barnes, S.L., Belliveau, N.M., Chur, G., Einax, T., Lewis, M. et al. (2018) Tuning transcriptional regulation through signaling: a predictive theory of allosteric induction. Cell Syst. 6, 456–469 https://doi.org/10.1016/j.cels.2018.02.004

Jenkins, A. and Macauley, M. (2017) Bistability and asymmetry in a Boolean model of the l-arabinose operon in Escherichia coli. Bull. Math. Biol. 79, 1778–1795 https://doi.org/10.1007/s11538-017-0306-0

Domagalski, E.H.P.L. and Jones, B.F. (1990) Trace metal geochemistry of Walker, Mono, and Great Salt Lakes. In Fluid-Mineral Interactions: A Tribute to H.P. Eugster (Spencer, R. ed.), vol. 2, pp. 315–353, The Geochemical Society, Washington, DC, U.S.A.

Hubmacher, D., Matzanke, B.F. and Arendt, S. (2007) Iron-uptake in the Euryarchaeon Pyrococcus furiosus. Environ. Microbiol. 9, 1663–1676 https://doi.org/10.1111/j.1462-2920.2007.01051.x

Leyn, S.A. and Rodionov, D.A. (2015) Comparative genomics of DvR family regulons for metal homeostasis in Archaea. J. Bacteriol. 197, 451–458 https://doi.org/10.1128/JB.02386-14

Zeldes, B.M., Keller, M.W., Loder, A.J., Straub, C.T., Adams, M.W. and Kelly, R.M. (2015) Extremely thermophilic microorganisms as metabolic engineering platforms for production of fuels and industrial chemicals. Front. Microbiol. 6, 1209 https://doi.org/10.3389/fmicb.2015.01209
62 Adams, M.W.W. and Kelly, R.M. (2017) The renaissance of life near the boiling point — at last, genetics and metabolic engineering. Microb. Biotechnol. 10, 37–39 https://doi.org/10.1111/1751-7915.12463

63 Fiala, G. and Stetter, K.O. (1986) Pyrococcus furiosus sp. nov. represents a novel genus of marine heterotrophic archaeabacteria growing optimally at 100°C. Arch. Microbiol. 145, 56–61 https://doi.org/10.1007/BF00413027

64 Schut, G.J., Bridger, S.L. and Adams, M.W.W. (2007) Insights into the metabolism of elemental sulfur by the hyperthermophilic archaean Pyrococcus furiosus: characterization of a coenzyme A — dependent NAD(P)H sulfur oxidoreductase. J. Bacteriol. 189, 4431–4441 https://doi.org/10.1128/JB.00031-07

65 Bridger, S.L., Clarkson, S.M., Stirrett, K., DeBarry, M.B., Lipscomb, G.L., Schut, G.J. et al. (2011) Deletion strains reveal metabolic roles for key elemental sulfur-responsive proteins in Pyrococcus furiosus. J. Bacteriol. 193, 6498–6504 https://doi.org/10.1128/JB.05445-11

66 Lipscomb, G.L., Keese, A.M., Cowart, D.M., Schut, G.J., Thomm, M., Adams, M.W. et al. (2009) SurR: a transcriptional activator and repressor controlling hydrogen and elemental sulphur metabolism in Pyrococcus furiosus. Mol. Microbiol. 71, 332–349 https://doi.org/10.1111/mmi.2009.71.issue-2

67 Martin, J.H., Rawls, K.S., Chan, J.C., Hwang, S., Martinez-Pastor, M., McMillan, L.J. et al. (2018) GspR is a direct transcriptional repressor of fructose metabolic genes in Haloferax volcanii. J. Bacteriol. https://doi.org/10.1128/JB.00244-18

68 Kanai, T., Akerboom, J., Takedomi, S., van de Werken, H.J., Blombach, F., van der Oost, J. et al. (2007) A global transcriptional regulator in Thermococcus kodakaraensis controls the expression levels of both glycolytic and gluconeogenic enzyme-encoding genes. J. Biol. Chem. 282, 33659–33670 https://doi.org/10.1074/jbc.M703424200

69 Rawls, K.S., Yacovone, S.K. and Maupin-Furlow, J.A. (2010) GspR represses fructose and glucose metabolic enzymes at the level of transcription in the halooarchaeon Haloferax volcanii. J. Bacteriol. 192, 6251–6260 https://doi.org/10.1128/JB.00827-10

70 Pickl, A., Johansen, U. and Schonheit, P. (2012) Fructose degradation in the halooarchaeon Haloferax volcanii involves a bacterial type phosphoenolpyruvate-dependent phosphotransferase system, fructose-1-phosphate kinase, and class II fructose-1,6-bisphosphate aldolase. J. Bacteriol. 194, 3088–3097 https://doi.org/10.1128/JB.00200-12

71 Sutter, J.M., Johansen, U. and Schonheit, P. (2017) Characterization of a pentono lactonase involved in D-xylose and L-arabinose catabolism in the halooarchaeon Haloferax volcanii. FEBS Microbiol. Lett. 364 https://doi.org/10.1093/femsle/fnx140

72 Johanse, U., Sutter, J.M., Zais, H. and Schonheit, P. (2013) L-Arabinose degradation pathway in the halooarchaeon Haloferax volcanii involves a novel type of L-arabinose dehydrogenase. Extremophiles 17, 897–909 https://doi.org/10.1007/s00792-013-0572-2