SulfoSYS (Sulfolobus Systems Biology): towards a silicon cell model for the central carbohydrate metabolism of the archaeon Sulfolobus solfataricus under temperature variation

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Abstract

SulfoSYS (Sulfolobus Systems Biology) focuses on the study of the CCM (central carbohydrate metabolism) of Sulfolobus solfataricus and its regulation under temperature variation at the systems level. In Archaea, carbohydrates are metabolized by modifications of the classical pathways known from Bacteria or Eukarya, e.g. the unusual branched ED (Entner–Doudoroff) pathway, which is utilized for glucose degradation in S. solfataricus. This archaeal model organism of choice is a thermoacidophilic crenarchaeon that optimally grows at 80°C (60–92°C) and pH 2–4. In general, life at high temperature requires very efficient adaptation to temperature changes, which is most difficult to deal with for organisms, and it is unclear how biological networks can withstand and respond to such changes. This integrative project combines genomic, transcriptomic, proteomic and metabolomic, as well as kinetic and biochemical information. The final goal of SulfoSYS is the construction of a silicon cell model for this part of the living cell that will enable computation of the CCM network. In the present paper, we report on one of the first archaean systems biology projects.

Systems biology

After an initial period of a substantial diversity of activities that were called and accepted to be systems biology, the discipline has now narrowed down to a defined area of science. The various definitions agree that it is the science that attempts to discover principles governing the emergence of biological function from the interactions of components of living systems [1,2]. The non-linearity and complexity of those interactions invokes mathematics, quantitative experimentation and system-wide approaches. However, these three aspects are derived properties, not defining properties, of systems biology.

For reasons of higher homogeneity and genetic accessibility, systems biology has been particularly strong in micro-organisms. These have included both prokaryotes, such as Escherichia coli, and eukaryotes, with Saccharomyces cerevisiae as a main object of study. These organisms will have some systems biology principles, such as the summation law for control coefficients [3,4], in common. Others, such as those related to signal transduction, are notably different. For yet others, such as distribution of control [5] and heterogeneity of regulation [6], the situation is still unclear.

Archaea

The third domain of life on earth, i.e. the Archaea, have been much less subject to systems biology studies. Since their discovery, Archaea represent an important comparative lineage to study the evolution and characteristics of central
cellular functions in living cells. Archaea represent a mosaic of bacterial and eukaryotic as well as archaeal-specific features. They have gained special interest because mechanisms involved in information processing (e.g. transcription, translation, and replication) represent a simpler version of the eukaryotic equivalents and are different from the bacterial equivalents, and because Archaea harbour several unique metabolic features. The archaeal CCM (central carbohydrate metabolism) is characterized by unusual pathways and enzymes, many of which differ from their bacterial or eukaryotic counterparts (for a review, see [7]), and their regulation as well as energetics is not understood.

Many archaeal species that have been obtained as laboratory cultures to date are adapted to extremely high temperatures. They also experience large and frequent temperature changes in their natural environments, probably more pronounced than mesophilic microbes, and therefore require effective adaptation mechanisms. Temperature changes are the most difficult to deal with for organisms, because of the extreme heat permeability of all known external membranes of living organisms, and the rather substantial temperature dependence of both activity and stability of most proteins. Recently, it was pointed out that biochemical networks should be expected to be exquisitely sensitive to temperature changes unless a temperature-compensation mechanism is in place: even slight differences between the rates of individual reactions in metabolic pathways should cause rapid accumulation or depletion of intermediates with various deleterious effects [8].

To prevent substantial and rapid changes in the concentration of important metabolites with change in temperature, the rates of individual reactions in metabolic pathways must therefore change by precisely the same extent. Organisms could adapt by (i) having identical temperature coefficients of the enzymes, (ii) exhibiting metabolic regulation, (iii) adjusting $V_{\text{max}}$ Values (e.g. through enzyme phosphorylation), (iv) adjusting translation or protein stability, (v) adjusting transcription or mRNA stability, (vi) rewiring the metabolic flow, (vii) synthesizing compatible solutes, (viii) exporting ‘overflow’ metabolites, or (ix) going into dormancy. The hypothesis is that several of these mechanisms contribute to different extents, and the aim of this study is to quantify each of these adaptations in a systems biology approach. As the issue should be most acute for (hyper)thermophiles, the study is performed with the crenarchaeon Sulfolobus solfataricus. In this integrated systems biology approach, the CCM of S. solfataricus and its regulation under temperature variation is analysed by the integration of genomic, transcriptomic, proteomic, metabolomic, kinetic and biochemical information.

**Project structure**

The SulfoSYS (Sulfolobus systems biology) project is one of 11 projects funded within the European trans-national research initiative ‘Systems Biology of Microorganisms’ (SysMO; http://www.sysmo.net/), supported by six European partner countries (Austria, Germany, Norway, Spain, The Netherlands and the U.K.). In the SulfoSYS project, 11 academic partners from five European countries participate (Table 1). The overall SulfoSYS structure comprises three work packages (Fermentation and Perturbation, Biochemistry, Genomics and Post-Genomics) embedded in the modelling platform (Figure 1). The Fermentation and Perturbation work package offers a central fermentation unit, which provides biological samples for all partners. In addition, S. solfataricus knockout as well as expression strains with increased levels of key enzymes are constructed. The Biochemistry work package contributes biochemical and kinetic information of enzymes and analyses their regulatory properties, all in response to temperature change. Finally, the Genomics and Post-Genomics work package performs whole-cell high-throughput analyses (Transcriptomics, Proteomics, Metabolomics and Comparative Genomics), which allow for the analysis of changes at different
The model system: *Sulfolobus solfataricus* (P2, DSM 1617)

*S. solfataricus* P2 is a thermoacidophilic crenarchaeon that grows optimally at 80°C (growth range 60–92°C) and pH 2–4 [10]. The organism is a strict aerobe and grows heterotrophically on various carbon sources, amino acids and peptides [11]. No autotrophic or anaerobic growth is observed.

As mentioned above, (hyper)thermophilic archaea are still lacking from the common systems biology model organisms. *S. solfataricus* qualifies for such a role, as (i) its genome has been sequenced and annotated [12], (ii) it can be cultured easily under defined growth conditions [11], (iii) comprehensive biochemical and functional genomics data have been assembled over 25 years (e.g. [13]; reviewed, in [7,14,15], but see [15a]), and (iv) the organism is meanwhile amenable to genetic manipulations. Methods for the construction of directed gene-deletion mutants [16–18] and overexpression strains [19,20] have been established. In addition, proteins of this thermophile surpass proteins from mesophiles in terms of (i) rigidity (favouring crystallization, purification and protein–protein interaction studies), and (ii) the exploitation of thermostable bioproducts in biotechnological applications (extremozymes, white biotechnology). Therefore, and also
because of its use as a production strain for proteins and its use for the development of drugs and diagnostics, *S. solfataricus* is an emerging biotechnology organism of the domain Archaea.

**The molecular network**

The network under study is the CCM of *S. solfataricus*, i.e. the catabolic branched ED (Entner–Doudoroff) pathway, the gluconeogenic EMP (Embden–Meyerhof–Parnas) pathway, the reverse ribulose monophosphate pathway for pentose formation and the tricarboxylic acid cycle, as well as glycogen and trehalose metabolism (Figure 2). The initial focus of the project is on the archaeal-specific branched ED pathway of *S. solfataricus* [21–26]. This modified pathway has been reported first for *S. solfataricus* and *Thermoproteus*.

**Figure 2 | The molecular network: CCM of *S. solfataricus***

The engaged glycolytic reactions of the branched ED pathway and gluconeogenesis via the EMP pathway are shown. Furthermore, the reverse ribulose monophosphate (RuMP) pathway, which is used for pentose generation as well as glycogen and trehalose metabolism, is indicated (broken arrows). The two phosphoproteins of *S. solfataricus* identified are indicated (P). In addition, the results of the transcriptomic/proteomic CCM analysis [14] are given: induction during growth on yeast/tryptone (↑YT) and glucose medium (↑G). The allosteric regulation of the GAPN by glucose 1-phosphate is indicated by a dotted arrow. Enzymes: ALDH, aldehyde dehydrogenase; ENO, enolase; FBPase, fructose-1,6-bisphosphatase; GA, glucan-1,4-α-glucosidase; GAD, glucose dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAPN, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase; GKD, glycerate kinase; GL, glucolactonase; GLGA, glycogen synthase; GLGP, glycogen phosphorylase; KD(P)GA, 2-keto-3-deoxy-(6-phospho)-gluconate aldolase; KDGK, 2-keto-3-deoxygluconate kinase; PEPS, phosphoenolpyruvate synthetase; PGAM, phosphoglycerate mutase; PGK, phosphoglycerate kinase; TIM, triose phosphate isomerase; TreT, trehalose glycyltransferase synthase; TreY, malto-oligosyltrehalose synthase; TreZ, malto-oligosyltrehalose trehalohydrolase. Intermediates: DHAP, dihydroxyacetonephosphate; Fructose 1,6P2, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; GAP, glyceraldehyde 3-phosphate; KD(P)G, 2-keto-3-deoxy-(6-phospho)-gluconate; KD(P)Gal, 2-keto-3-deoxy-(6-phospho)-galactonate.
above 80°C and hyperthermophiles (organisms with optimal growth temperatures that are physiological for this organism, suggesting that metabolic thermoadaptation might play an important role in the utilization of the two ED branches. One example that strongly supports this hypothesis is the almost exclusive presence of GAPN or GAP oxidoreductase in hypertherophilic archaea. Both enzymes avoid the formation of the thermolabile 1,3-bisphosphoglycerate (half-life of less than 2 min at 60°C, [30]) by the direct oxidation of GAP to 3-phosphoglycerate. The pathway then foregoes the production of ATP via the classical GAPDH–PGK enzyme couple. Therefore this part of the CCM is highly suited to analyse possible temperature-dependent shifts and the resulting re-routing of metabolic flow between pathways in response to these temperature changes.

The EMP pathway is supposed to be active only in the gluconeogenic direction, since no classical or archaeal-type sugar kinase activities (glucokinase, hexokinase or phosphofructokinase) have been detected in S. solfataricus and no obvious candidate genes encoding homologues have been identified in the genome. As characteristic enzymes of the anabolic EMP pathway, candidate genes encoding PEPS, PGK, GAPDH and fructose bisphosphatase (archaeal-type, class V) were identified in the genome.

Achievements and outlook

Several enzymes of the branched ED pathway have been characterized previously (e.g. [21–24,26,27]). The ongoing reconstruction of the pathway using the genome sequence information, however, revealed several paralogues for some of the enzymes, raising questions about their function in the network. In addition, most importantly for a joint systems biology approach, besides providing scientific fundamentals [e.g. SOPs (standard operating procedures), recombinant enzymes, appropriate strains], an efficient communication platform has been established (eGroupWare, open source). Furthermore, SOPs have been improved and/or established in the different work packages [e.g. control of genomic strain stability, stock handling, fermentation, metabolite isolation, proteome analysis (iTRAQ)]. The first models for the network under investigation have been developed, and the first experimental pilot projects were performed, in which all partners tested and, if necessary, refined their protocols and generated first-run quantitative data on the same shared biomass samples. The same biomass sample philosophy was of course a vital standardization SOP needed to gain cohesive and meaningful results.
The long-term goal of this highly integrated project is to build a sufficiently precise replica for this part of the living cell, i.e. a ‘silicon cell model’ that should enable the computation of life at the systems level. This model will be the first detailed kinetic model of a pathway in an archaeon, and should highlight differences as well as similarities between members of all three domains of life. The model should definitely enable us to look at the general properties of metabolic control analysis and at robustness. With additional experimentation determining the changes in enzyme levels with the temperature, we shall also be able to determine to what extent the organism manages its defence against temperature fluctuation and variations through regulated gene expression, through direct metabolic regulation or through signal transduction leading to covalent modification. The results will be the first instance of a comprehensive, ‘live’ dataset of an archaeon’s carbon and energy metabolism. It should also serve as a scaffold for storing and managing other data concerning the functioning of the organism. And it will be ‘alive’ indeed: its system behaviour can be calculated by anyone through our web interface (see http://jjj.bio.vu.nl/).

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