

Chemical Organizations in the Central Sugar Metabolism of *Escherichia Coli*

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Summary. The theory of chemical organizations is employed as a novel method to analyze and understand biological network models. The method allows to decompose a chemical reaction network into sub-networks that are (algebraically) closed and self-maintaining. Such sub-networks are termed *organizations*. Although only stoichiometry is considered to compute organizations, the analysis allows to narrow down the potential dynamic behavior of the network: organizations represent potential steady state compositions of the system. When applied to a model of sugar metabolism in *E. coli* including gene expression, signal transduction, and enzymatic activities, some organizations are found to coincide with inducible biochemical pathways.

Key words: chemical organization theory, network analysis, stoichiometry, systems biology, sugar metabolism, *Escherichia coli*

1.1 Introduction

A living cell consists of a tremendous number of components that interact in complicated ways sustaining the processes of life [7]. In order to understand cells, these interactions are commonly portrayed as networks on different levels [2]. Gene regulatory networks describe how genes are regulated, metabolic networks detail how substrates are transformed into products by proteins acting as enzymes, and signal transduction networks focus on how external stimuli

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are sensed and transduced leading to a change in gene expression. With more and more detailed knowledge on the various molecular interactions, the constructed networks modelling cellular processes grow steadily in size and complexity. Novel methods have to be developed to analyze and study them. For example, methods originating from graph theory have been successfully applied to study cellular networks [1]. Other methods concentrate on feasible steady state flux distributions in metabolic networks [10]. In this paper, we employ the theory of chemical organizations [3] as a novel tool to analyze intracellular reaction networks. The network is decomposed into sub-networks that are (algebraically) closed and self-maintaining, revealing the internal structure of the network. Applying the method to a well-established model of *E. coli* sugar metabolism reveals an organizational structure in accordance with biological knowledge. Although the analysis does not lead to novel biological insights in this case, it highlights the potential and the limits of this approach. The paper exemplifies, how organization theory can contribute towards a systems-level understanding of large-scale models of biological systems, contributing to the emerging field of systems biology.

The outline of the paper is as follows. The theory of chemical organizations is introduced in Sect. 1.2. The method is then applied to the sugar metabolism network of *E. coli*, and the results are presented in Sect. 1.3. The discussion follows in Sect. 1.4, and we finally conclude in Sect. 1.5.

1.2 Theory of Chemical Organizations

The theory of chemical organizations [3] extends ideas by Fontana and Buss [4]. It provides a new method to analyze complex general reaction networks. Since the static part of the theory, which is used here, is based solely on network structure and stoichiometric information, no kinetic data is required. The main objective is to determine combinations of network species that are more likely to be present over a long period of (simulation-) time than others. More precisely, the given reaction network is decomposed into sets of molecular species that form algebraically closed and self-maintaining sub-networks. Such species sets are called organizations. The first property – closure – ensures that given the molecular species of an organization, there is no reaction within the reaction network that could create a species not yet present in the organization from the organization species set. The second property – self-maintenance – guarantees that every molecular species that is used-up within an organization can be reproduced by reactions among species of that organization: considering only the reaction network made up by the species contained in the organization, a flux vector² can be found, such that all species of the organization are produced at nonnegative rates from within the organization facilitating maintenance of the organization. Formal definitions

²For a reaction system of n reactions, the flux vector $\mathbf{v} \in \mathbb{R}_+^n$ assigns to each reaction a nonnegative value that represents the reaction’s turnover rate.

of these concepts are given in Sect. 1.2.1. By this approach, the network is analyzed on a more abstract level than by investigating its state space. While in the classic systems approach, the concentrations of all system variables determine the state of the system, here, the system state is characterized by a set of species being present. The theory of chemical organizations delivers a set of organizations, representing all self-maintaining and closed sub-networks of the system. It is shown by Dittrich and Speroni di Fenizio [3], that assuming that the dynamics is modelled using ordinary differential equations, all steady states of the system are instances of organizations, i.e., the species with concentrations greater than zero in a particular steady state are exactly the species contained in a corresponding organization. But not all organizations harbor steady states. For example, an internal cycle not depending on input can fulfill the properties of closure and self-maintenance, yet is thermodynamically infeasible. Furthermore, organizations can contain species with positive production rates. Since organizations may share the same species, the set of organizations together with the set inclusion \subseteq form a partially ordered set that can be visualized in a Hasse diagram providing a hierarchical view on the network under consideration (see Fig. 1.1 for examples). The organizations are vertically arranged according to their size, with organizations containing few molecular species at the bottom. Two organizations are connected by a line, if the upper organization contains all species of the lower organization and there exists no other organization between them. The label of an organization in the Hasse diagram contains a list of species contained in the organization. To keep the labels short, only those species are listed that are not already contained in organizations to which a downlink exists. Hence to get the complete list of molecular species of an organization, it is required to collect the molecular species contained in organizations to which a downlink exists plus the species denoted in the organization label.

1.2.1 Formal Definition of Central Concepts

Algebraic chemistry [3] Let \mathcal{M} be a set of elements (called species, molecular species, or just molecules). $\mathcal{P}_M(\mathcal{M})$ denotes the set of all multisets with elements from \mathcal{M} . A multiset differs from a set in the fact that it can contain the same element more than once. Reactions occurring among the species \mathcal{M} can then be defined by a relation $\mathcal{R}: \mathcal{P}_M(\mathcal{M}) \times \mathcal{P}_M(\mathcal{M})$. We call the pair $\langle \mathcal{M}, \mathcal{R} \rangle$ an *algebraic chemistry*.

Closed set [4] A set of species $S \subseteq \mathcal{M}$ is *closed*, if for all reactions $(A \rightarrow B) \in \mathcal{R}$ with $A \in \mathcal{P}_M(S)$, also $B \in \mathcal{P}_M(S)$. In other words: if the educts of a reaction are contained in S , then also its products must be in S . There is no reaction that could create any new species not yet in S from species contained in S .

Self-maintaining set³ [3] Given an algebraic chemistry $\langle \mathcal{M}, \mathcal{R} \rangle$ with $m = |\mathcal{M}|$ species and $n = |\mathcal{R}|$ reactions, its dynamics can be described by $\dot{\mathbf{c}} = \mathbf{M}\mathbf{v}$ with concentration vector $\mathbf{c} \in \mathbb{R}_+^m$, stoichiometric matrix \mathbf{M} , and

flux vector $\mathbf{v} \in \mathbb{R}_+^n$. A set of species $S \subseteq \mathcal{M}$ is called *self-maintaining* if a flux vector \mathbf{v} exists, so that the following three conditions are fulfilled:

(1) For every reaction $(A \rightarrow B) \in \mathcal{R}$ with $A \in \mathcal{P}_M(S)$, its corresponding flux is $v_{A \rightarrow B} > 0$.

(2) For every reaction $(A \rightarrow B) \in \mathcal{R}$ with $A \notin \mathcal{P}_M(S)$, its corresponding flux is $v_{A \rightarrow B} = 0$.

(3) For every species $i \in S$, its concentration change is nonnegative: $\dot{c}_i \geq 0$.

In other words: if we consider only the sub-network made up by the species of S and additionally the species that can be created from S (but are not in S) (conditions (1) and (2)), we can find a positive flux vector, such that no species of S decays (condition (3)). Note that the steady state condition with $\dot{c}_i = 0$ for all species $i \in \mathcal{M}$ is a special case of condition (3).

Organization [3, 4] A set of species $S \subseteq \mathcal{M}$ that is closed and self-maintaining is called an *organization*.

1.3 Application to a Model of Regulated Sugar Metabolism in *E. coli*

In order to demonstrate the feasibility of organization theory as a tool to analyze intracellular reaction networks, we apply it to a relatively small network model encompassing the well-studied sugar metabolism of *E. coli*. If several sugars are available in the growth medium, *E. coli* first exclusively metabolizes its preferred carbon source glucose. Only after depletion of glucose, the bacterium will begin to utilize other available sugars. This diauxic growth phenomenon has been extensively studied in experiments and by mathematical modelling [6, 11, 12], leading to a good understanding of the molecular mechanisms at work. The two main mechanisms facilitating the switch-like behavior are inducer exclusion and catabolite repression. See referenced literature for details of these mechanisms. Extending models by Kremling et al. [6] and Wang et al. [12], Puchalka and Kierzek constructed a reaction network modeling the sugar metabolism of *E. coli* including gene expression, signal transduction, and transport and enzymatic activities [9]. We take this network as an example to demonstrate how the theory of organizations can be applied to intracellular networks. First, the network is adapted as described in the next section. Then, organizations are analyzed for several scenarios representing bacterial growth on different sugar sources.

1.3.1 Reaction Network

The original network by Puchalka and Kierzek consists of 92 substances reacting with each other in 120 reactions. The model contains reactions modeling transcription and translation of 21 genes. The uptake and utilization of external glucose, lactose, and glycerol is included in the model as well as

³This concept was termed “*mass-maintaining set*” in [3].

catabolic repression and inducer exclusion, allowing the model to exhibit diauxic growth. Each reaction of the network consists of (up to) three different types of species: educts, products, and modifiers. If a reaction occurs, the educt species are transformed into the product species while the modifiers are not affected. Modifier species only change the reaction rate. Two types of modifiers are used in the model: enzymes, that are required for a reaction to take place, and effectors, that increase the reaction rate acting as an activator, or decrease the reaction rate acting as an inhibitor or repressor. Since algebraic chemistries do not contain modifiers, we have to handle them separately for our analysis as follows. If a reaction does not have modifiers, we take the reaction exactly as it is. In the presence of modifiers, we inspect the reaction rate equation. In case the modifier species concentration has to be greater zero for the reaction rate to become greater zero, we add the modifier species on both educt and product side of the reaction. This is the typical case for enzymes. Only in their presence, the reaction in question can be performed. If the reaction rate can be greater zero even in the absence of the modifier species, we simply ignore them, as they are not necessary for the reaction to take place. They merely increase (or decrease) the reaction rate, acting as nonessential activators (resp. repressors or inhibitors). It is important to note that all inhibitory or negative interactions are ignored by this procedure.

The handling of modifiers as described above cannot be applied to reactions modeling gene expression. Negative interactions can be ignored as before, but activators need special treatment. The model contains five transcription reactions that have activating and/or repressing effectors. With activator concentrations being zero, the transcription reaction rates in the original model are computed to be positive. This corresponds to a basal transcription rate of a gene: even if activators are not present RNA polymerase occasionally binds to the promoter and transcription is initiated, leading to a basal concentration of the respective protein. Applying the procedure as described above to these reactions (i.e., ignoring all activators), would lead to an unconditional transcription of all genes, giving rise to a basal concentration of the corresponding gene products. But as shown below for the transcription of the *lac* genes, basal concentration of proteins might not be sufficient to perform certain metabolic tasks. Consequently, a protein having only basal concentration should be regarded as not being present in our analysis. Only if activators are present increasing the transcription rate so that protein concentrations reach levels that are significantly above basal level – effectively switching the gene on – the corresponding protein should be regarded as being present. Activators and inducers for gene transcription should therefore be modeled as necessary catalysts in gene transcription reactions. The five transcription reactions having effectors are discussed separately:

Transcription of crp: effectors Crp, cAMP. Crp is activated by the binding of cAMP. The activated Crp–cAMP complex negatively regulates the transcription of *crp*. It was also shown that with further increasing concentration of Crp–cAMP this inhibition is overcome and an upregulation occurs [5]. The

inhibition is ignored and since the activation only occurs at high concentrations, it is ignored as well (since the reaction can take place in the absence of the effector species). Hence the effectors Crp and cAMP are ignored for this reaction.

Transcription of *cya*: effectors Crp, cAMP. Crp–cAMP downregulates transcription of *cya*. Being an inhibition, the effector species Crp and cAMP are ignored for this reaction.

Transcription of *lacZY*, *glpFK*, and *glpD*: effectors Crp, cAMP, LacI/GlpR, and Allo/G3P: These genes code for enzymes necessary for lactose and glycerol uptake and utilization. The transcription regulation is similar for both. Two mechanisms are at work for transcription regulation of *lacZY* (*glpFK*, *glpD*). Firstly, repressor LacI (GlpR) represses transcription. If inducer Allo (G3P) is present, it binds to LacI (GlpR) and by this inactivates the repressor. Secondly, Crp–cAMP complex acts as an activator. Both mechanisms are modeled in one reaction equation in the model. We ignore the inhibiting effect of effector species LacI (GlpR). Instead, by adding the inducer Allo (G3P) on both educt and product side of the reaction, we require the inducer to be present for transcription. This is in accordance with biological knowledge: only in the presence of the inducer, the corresponding gene products are synthesized at above basal concentration levels. Mutants not being able to synthesize Crp or cAMP were found unable to grow on several carbon sources [8]. Therefore, we conclude that the presence of Crp and cAMP is also required to synthesize enzymes necessary for carbon uptake and utilization in sufficient concentrations. Accordingly, effectors Crp and cAMP are also added on both educt and product side of the reactions.

The original model contains six reactions that are reversible. We add an explicit back reaction for each of them in our model. Cell growth and cell division is accounted for in the original model by dividing all species concentrations by two on cell division, except for the DNA species. Hence we add decay reactions for all non DNA species that do not already decay in the original model. The remaining species that do not decay are: all 21 promoter species, RNAP, Tscription, Glcex, Lacex, and Glyex. Several species are not produced from within the original network model. Among them are all 21 promoter species, ATP, ADP, and AMP. We assume that they are present in the cell at all times by providing them as external input. We add a reaction of the form $\emptyset \rightarrow \text{inputspecies}$ for each of them. Additionally, RNAP is provided as input. Finally, our network model consists of 92 species and 168 reactions. See Appendix for a complete list of species and reactions. Glucose, lactose, and glycerol in the growth medium are represented by the species Glcex, Lacex, and Glyex. By adding additional input reactions for these species, growth on different sugar sources can be modeled.

1.3.2 Hierarchies of Organizations

We compute the hierarchy of organizations of the network for five different scenarios. The scenarios only differ in which external sugars are supplied as input, resembling bacterial growth on different sugar sources. First, no external sugars are supplied at all. Then, one of the three sugars glucose, lactose, and glycerol is consecutively supplied as the exclusive carbon source. And finally, all three sugars are provided simultaneously. Supplying a sugar source is accomplished simply by adding an input reaction of the form $\emptyset \rightarrow \text{externalsugar}$ to the reaction network. Changing the reaction network also changes the hierarchy of organizations. The resulting hierarchies are depicted in Fig. 1.1. They all consist of four organizations. The labels within organizations refer to sets of species as detailed in Table 1.1. The network model covers the transformation of external sugar into pyruvate, which is then fed into further metabolic processes not considered by the model. These follow-up processes enabling cellular survival are represented by pseudo species Metabolism. Species set *Metabolites* contains all relevant species of this pathway and its presence in an organization hence represents a cell being able to maintain its metabolism and survive.

Starvation: No external sugars are supplied as input. The resulting hierarchy of organizations is depicted in Fig. 1.1(a). The smallest organization Org. 1 contains all input species (21 promoter species, ATP, ADP, AMP, and RNAP). In the presence of the promoters and RNA polymerase, all unregulated genes are transcribed and translated, so that all mRNA and protein species of all 18 unregulated genes are also contained in the smallest organization (cf. *Genes+Enzymes*, Table 1.1). Organizations Org. 2 and Org. 3 contain all species from Org. 1 and additionally Glyex and Lacex, respectively. This seems surprising since these species are not supplied as input in this scenario. But recall that an organization is a set of species that is algebraically closed and self-maintaining. Although the species Glyex and Lacex are not supplied as input, they are still a regular part of the reaction network. Inspecting the networks making up Org. 2 and Org. 3, we find that Glyex and Lacex do not participate in any reaction there. They are isolated nodes in the reaction network. As such, they do not decay, neither are produced, fulfilling the requirements of closure and self-maintenance. The two organizations represent a state in which a fixed amount of Glyex, respectively Lacex entered the system “by accident” and the uptake systems are not induced. In this case, the concentration of the external sugars will not change. Only after the uptake systems have been induced, the external sugars will be used up completely and the system falls back to Org. 1. The largest organization Org. 4 combines Org. 2 and Org. 3. All species of the smallest organization, and Glyex and Lacex are contained. In this scenario, we find no organization containing the metabolites of the network. This indicates that with no external sugar source, the network cannot sustain its metabolism, i.e., the cell is starving.

Growth on glucose: After adding the reaction $\emptyset \rightarrow \text{Glcex}$, the hierarchy of organizations contains again four organizations as shown in Fig. 1.1(b). The smallest organization Org. 1 contains the same species as in the first scenario and additionally Glcex. With Glcex present, all metabolites can be created and maintained. Consequently, all these species are part of the smallest organization, too. With species set **Metabolism** present in the smallest organization, the cell can maintain its metabolism when external glucose is supplied. The remaining part of the organization hierarchy is equivalent to the first scenario without any sugar input.

Growth on lactose: When lactose is supplied as the exclusive external sugar source, the resulting hierarchy of organizations again contains four organizations as depicted in Fig. 1.1(c). The smallest organization contains all unregulated genes and enzymes and additionally Lacex. In Org. 2, only Glyex is added as in the previous cases. Organization Org. 3 contains the species of the smallest organization, all species necessary for taking up and metabolizing external lactose, and the species belonging to the metabolism. Being an organization, the network made up by all these species is algebraically closed and self-maintaining, representing a cell that has switched its *lac* genes on and utilizes external lactose. Figure 1.2(a) details schematically, how Org. 1 is expanded to form Org. 3. Once inducer allolactose is present, the *lac* genes are switched on and LacY and LacZ are synthesized. LacY facilitates the uptake of external lactose while LacZ transforms intracellular lactose and allolactose to glucose and glucose-6-phosphate. Additionally, LacZ transforms lactose to allolactose, closing the positive feedback loop. Glucose then enters the metabolic pathway leading to pyruvate and further metabolic processes. Adding Glyex to Org. 3 results in the largest organization Org. 4. This scenario shows that bacterial growth is possible on lactose as the only carbon source after induction of the lactose uptake system (in Org. 3 and 4).

Growth on glycerol: Now glycerol is provided as the exclusive carbon source. The resulting hierarchy of organizations is visualized in Fig. 1.1(d). The result is equivalent to the lactose scenario. The smallest organization Org. 1 contains the unconditionally transcribed genes and resulting enzymes, and external glycerol. Organization Org. 3 additionally contains the molecular species necessary for utilizing external glycerol and the metabolism species. Figure 1.2(b) shows, how this organization is formed by expanding Org. 1. Once inducer G3P is present, the genes corresponding to glycerol utilization are switched on and GlpF, GlpK, and GlpD are synthesized. GlpF then enables uptake of external glycerol, GlpK transforms internal glycerol to G3P closing the positive feedback loop, and GlpD transforms G3P to DHAP which in turn fuels the pathway ending in pyruvate and further metabolic processes. Adding Lacex to this organization leads to the largest organization Org. 4. Again we find that once the uptake system for the external sugar is induced, the cell can maintain its metabolism in Org. 3 and 4.

Growth on all sugars: In the last scenario, all three external sugars are supplied as input simultaneously. Figure 1.1(e) depicts the resulting hierarchy of organizations. With external glucose being input, the smallest organization resembles the smallest organization of the glucose scenario, with external lactose and glycerol added. Glucose alone is sufficient for growth, hence the smallest organization already represents a state in which the cell grows (on glucose). The two organizations above the smallest one contain the species necessary for utilizing lactose (Org. 2) and glycerol (Org. 3). They represent states in which the cell metabolizes lactose, respectively glycerol in addition to glucose. The largest organization Org. 4 finally merges Org. 2 and 3, containing all species of the model. Here, all three sugars are metabolized simultaneously. From a biological point of view, only organization 1 is meaningful since the uptake of lactose and glycerol is repressed in the presence of glucose. The existence of the remaining organizations will be discussed in the next section.

Table 1.1. Sets of species as used in Fig. 1.1

Genes+Enzymes	:= {PromCrp, PromCya, PromEIIA, PromEIIBC, PromEI, PromFbp, PromFda, PromGap, PromGlcT, PromGlk, PromGlpD, PromGlpFK, PromGlpR, PromGpm, PromHPr, PromLacI, PromLacZY, PromPfk, PromPgi, PromPyk, PromTpi, RNAP, Tscription, CrpmRNA, CyamRNA, EIIAmRNA, EIIBCmRNA, EImRNA, FbpmRNA, FdamRNA, GapmRNA, GlcTmRNA, GlkmRNA, GlpRmRNA, GpmmRNA, HPrmRNA, LacImRNA, PfkRNA, PgimRNA, PykmRNA, TpimRNA, Crp, Cya, EIIA, EIIBC, EI, Fbp, Fda, Gap, GlcT, Glk, GlpR, Gpm, HPr, LacI, Pfk, Pgi, Pyk, Tpi, AMP, ATP, ADP, cAMP}
Metabolites	:= {Glc, Glc6P, Fru6P, FBP, DHAP, T3P, 3PG, PEP, Pyr, Metabolism, EIIAP, HPrP}
Metabolites*	:= Metabolites \ {Glc}
Glcex	:= {Glcex}
Lacex	:= {Lacex}
Glyex	:= {Glyex}
LacSpecies	:= {Lac, Allo, LacZYmRNA, LacZYmRNA1, LacZ, LacY}
GlySpecies	:= {Gly, G3P, GlpDmRNA, GlpFKmRNA, GlpFKmRNA1, GlpD, GlpF, GlpK}

1.4 Discussion

In all five analyzed scenarios the hierarchy of organizations consists of four organizations, representing four potential steady state species compositions of the system. Some organizations just contain a lower organization and a new species that does not interact with the species of the lower organization (e.g., Org. 2 and 3 in the starvation scenario and in the glucose scenario). In other cases, exactly those species performing a specific cellular function make up the

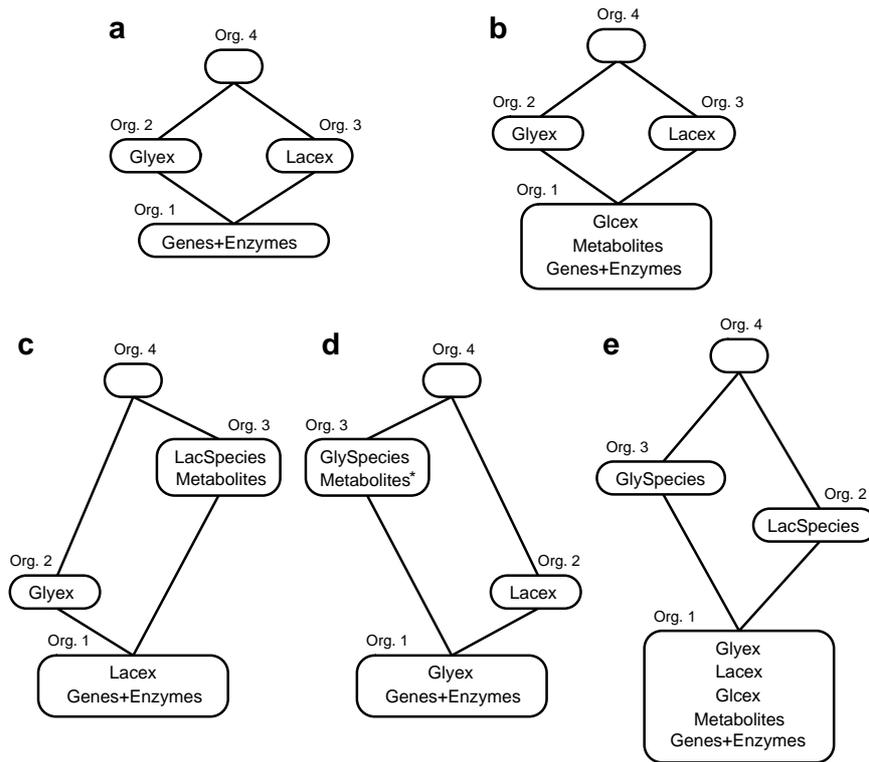


Fig. 1.1. Hierarchies of organizations of the *E. coli* network for five scenarios differing in supplied external sugars, resembling growth on different carbon sources. Organizations consist of the species sets contained in their lower organization(s) plus the species set(s) denoted in their label. Species set labels are detailed in Table 1.1. (a) starvation; (b) growth on glucose only; (c) growth on lactose only; (d) growth on glycerol only; (e) growth on glucose, lactose, and glycerol. See text for details

difference between an organization and its lower neighbor (cf. Org. 2 and 3 in the scenario with all sugars supplied). In these cases a modularity of the analyzed network model is uncovered by organization theory. In this example, the uncovered modules correspond to the inducible uptake systems for lactose and glycerol. Only those organizations that contain the metabolic species correspond to system states facilitating bacterial growth. As expected, such an organization is not found in the scenario without any supplied sugar. For glucose as the exclusive carbon source, all organizations contain the metabolites. For lactose and glycerol, only those organizations contain the metabolites that also contain the species of the respective uptake systems. This result confirms that glucose can be unconditionally utilized, while lactose and glycerol can only be utilized after their respective uptake systems have been induced. The diauxic growth behavior of *E. coli* is not revealed by the hierarchy of organi-

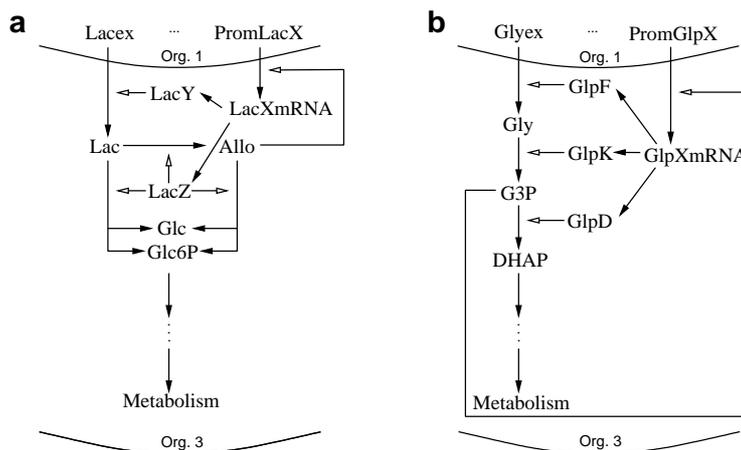


Fig. 1.2. Induction of sugar uptake systems. When lactose or glycerol is the exclusive carbon source, organization Org. 1 corresponds to the state in which the respective uptake systems are not activated and the bacterium is starving (upper part). In organization Org. 3, the systems are induced and the external sugar is utilized. A schematic sketch of the reaction network of organization Org. 3 responsible for utilization of (a) external lactose and (b) external glycerol is shown. Open arrows point from species acting as catalysts to the reactions that are catalyzed. See text for details

zations. In the scenario with three sugars supplied as input, organizations are found that correspond to states where glucose and other sugars are utilized simultaneously. Firstly, this highlights the fact that organizations only represent potential steady states of the system. Further kinetic information is required to determine whether an organization indeed contains steady states or not. And secondly, inhibitory interactions play a crucial role in diauxic growth, but had to be ignored in the conversion of the original network model. Since inhibitory interactions in the original network only decrease reaction rates, they in principle cannot be captured by the theory of organizations in which only the presence or absence of molecular species is considered.

1.5 Conclusion

We have demonstrated how the theory of chemical organizations can be employed to uncover modularity in intracellular reaction network models. The theory operates on a high level of abstraction as only the presence or absence of species is considered compared to the continuous state space considered in classic approaches. Consequently, concentration dependent interactions (e.g., non-essential activation of enzymes or inhibitory interactions) cannot be taken into account. Nevertheless, profound results can be obtained. Organizations represent potential steady state species compositions of the model. The hierar-

chy of organizations, reflecting the structure of the network model, provides a new perspective on the system and its potential dynamic behavior. The movement of the system through state space can be mapped to a movement in the space of its organizations [3], leading to a reduction in dimensionality. Organizations, being closed and self-maintaining sub-networks, can be separately analyzed using classic methods. Especially for large networks, analyzing small sub-networks is more feasible than studying the whole network at once. With species in organizations typically having more interactions among each other than with outside species, organizations can also be used for network visualization. By grouping species belonging to one organization closely together, a clearer graphical representation of the whole network can be achieved. Since only stoichiometry is required for the analysis, the method can be applied to a broad range of network models ranging from chemical and biochemical networks to social networks. The results presented in this paper suggest that the theory of organizations will be a helpful tool for studying and understanding large-scale intracellular network models.

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Appendix

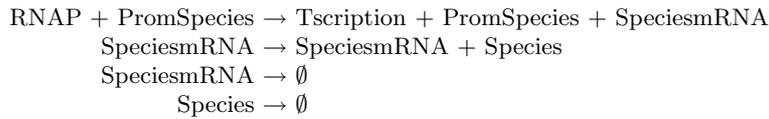
A List of Species

Species Names	Substances
ATP, ADP, AMP, cAMP	ATP, ADP, AMP, and cyclic AMP
RNAP, Tscription	RNA polymerase and RNAP bound to DNA
Crp, PromCrp, CrpmRNA	catabolite repressor protein, gene, and mRNA
Cya, PromCya, CyamRNA	adenylate cyclase, gene, and mRNA
EIIA, PromEIIA, EIIAmRNA	PTS system enzyme IIA ^{Glc} , gene, and mRNA
EIIAP	phosphorylated PTS system enzyme IIA ^{Glc}
EIIBC, PromEIIBC, EIIBCmRNA	PTS system enzyme IIBC ^{Glc} , gene, and mRNA
EI, PromEI, EIImRNA	PTS system enzyme I, gene, and mRNA
Fbp, PromFbp, FbpmRNA	fructose bis-phosphatase, gene, and mRNA
Fda, PromFda, FdamRNA	fructose bisphosphate aldolase, gene, and mRNA
Gap, PromGap, GapmRNA	glyceraldehyde-3-phosphate dehydrogenase, gene, and mRNA
GlcT, PromGlcT, GlcTmRNA	glucose transporter, gene, and mRNA
Glk, PromGlk, GlkmRNA	glucokinase, gene, and mRNA
GlpD, PromGlpD, GlpDmRNA	glycerol-3-phosphate dehydrogenase, gene, and mRNA
GlpFKmRNA, GlpFKmRNA1	glpFK operon mRNA
GlpR, PromGlpR, GlpRmRNA	glp regulon repressor, gene, and mRNA
Gpm, PromGpm, GpmmRNA	phosphoglycerate mutase, gene, and mRNA
HPr, PromHPr, HPrmRNA	PTS system HPr protein, gene, and mRNA
HPrP	phosphorylated PTS system HPr protein
LacI, PromLacI, LacImRNA	lac operon repressor, gene, and mRNA
LacZYmRNA, LacZYmRNA1	lac operon mRNA
Pfk, PromPfk, PfkRNA	phosphofructokinase, gene, and mRNA
Pgi, PromPgi, PgimRNA	phosphoglucose isomerase, gene, and mRNA
Pyk, PromPyk, PykmRNA	pyruvate kinase, gene, and mRNA
Tpi, PromTpi, TpimRNA	triose phosphate isomerase, gene, and mRNA
PromGlpFK, GlpF, GlpK	glpFK operon, glycerol faciliator and kinase
PromLacZY, LacZ, LacY	lac operon, β -galactosidase, and lactose permease
Glcex, Glyex, Lacex	extracellular glucose, glycerol and lactose
Glc, Gly, Lac	intracellular glucose, glycerol and lactose
Allo	Allolactose
Glc6P	glucose-6-phosphpate
G3P	glycerol-3-phosphate
Fru6P	fructose-6-phosphate
FBP	fructose-1,6-bisphosphate
DHAP	dihydroxy-acetone-phosphate

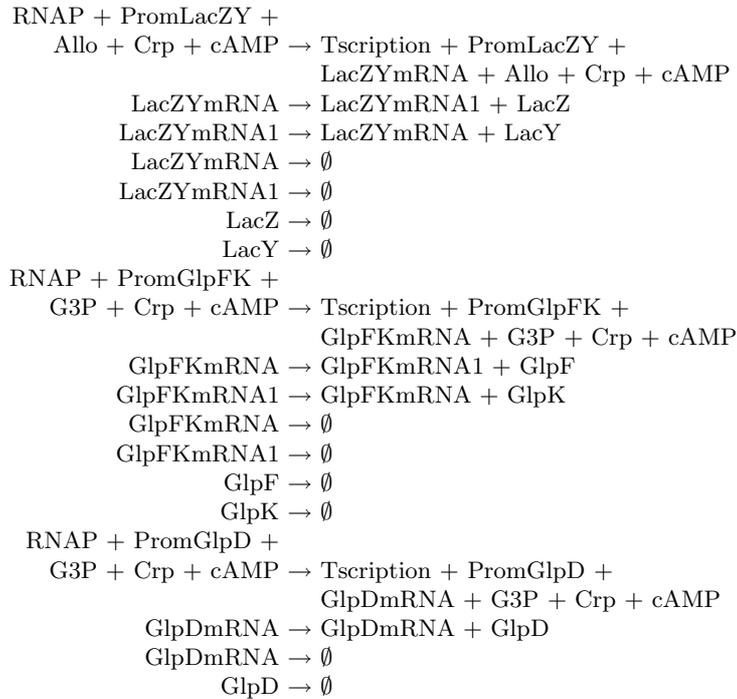
Species Names	Substances
T3P	glyceraldehyde-3-phosphate
3PG	3-phospho-glycerate
PEP	phosphoenolopyruvate
Pyr	pyruvate
Metabolism	further metabolic processes

B Reaction Network

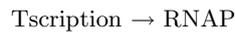
1. Synthesis and decay is identical for species Crp, Cya, EIHA, EIIBC, EI, Fbp, Fda, Gap, GlcT, Glk, GlpR, Gpm, HPr, LacI, Pfk, Pgi, Pyk, and Tpi:



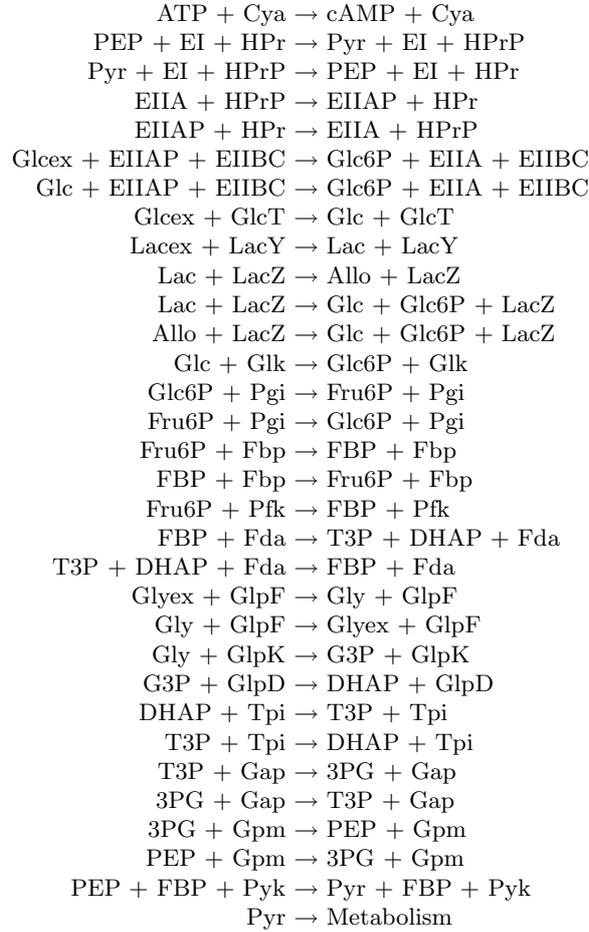
2. Synthesis and decay of inducible species LacZY, GlpFK, and GlpD:



3. Unbinding of RNAP:



4. Signal transduction, transport and metabolic reactions:



5. Decay reactions for species ATP, ADP, AMP, cAMP, EIIAP, HPrP, Glc, Gly, Lac, Allo, Glc6P, G3P, Fru6P, FBP, DHAP, T3P, 3PG, PEP, Pyr, and Metabolism have the form:



6. Input reactions for ATP, ADP, AMP, RNAP, PromCrp, PromCya, PromEIIA, PromEIIBC, PromEI, PromFbp, PromFda, PromGap, PromGlcT, PromGlk, PromGlpD, PromGlpR, PromGpm, PromHPr, PromLacI, PromPfk, PromPgi, PromPyk, PromTpi, PromGlpFK, and PromLacZY have the form:

