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# Metabolic Networking through Enzymatic Sensing, Signaling and Response to Homeostatic Fluctuations

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## 1. Introduction

Metabolic networks include multiple pathways, where certain substrates are transformed to the pathway products through a chain of intermediates generated by sequential action of biological catalysts, i.e. enzymes. Stability of metabolic network and its performing biological functions are achieved through coordination of the network components. For this, critical enzymes of the network not only catalyze the substrate transformation, but also support the network function as a whole by sensing metabolic state, generating signals about the state changes and eventually adjusting the catalytic parameters of the network to abolish the change and signal. Stable function of the metabolic networks under variety of conditions is an important contributor to homeostasis of the living systems, which is defined as their ability to regulate internal environment so that it is maintained stably, also when external environment changes. Independent of the exact mechanism, a basic principle of the metabolic network regulation implies the catalyst ability to change its catalytic properties dependent on other network members. In this paper, pre-requisites and basic mechanisms of the coordination are formulated and exemplified in the data obtained on enzymes and multienzyme complexes. It will be shown that already primary, i.e. purely metabolic, networks without specific regulatory systems are able to coordinate their members. In this case, the same binding sites and members are used for both catalysis and regulation. Increasing complexity of networks may employ additional regulatory binding sites and/or enzymes to coordinate the network function. That is, the more complex networks may include special regulatory enzymes (in particular, those phosphorylating/ dephosphorylating other enzymes), or regulatory binding to the allosteric sites which are structurally separated from the site where the enzymatic catalysis takes place. However, the primary mechanisms of the coordination, i.e. those based on the chemistry of interactions between the enzyme active site with the network metabolites, are realized already in the basic metabolic networks formed by catalysts only. Moreover, they preserve their essentiality also in more sophisticated networks where additional regulatory elements, such as allosteric binding and specialized signal transduction systems, are added to "pure" self-regulated catalysts. The main focus of this paper is to consider the basic mechanisms of the metabolic network coordination and to show their applicability to both the primary and complex networks.

## 2. Pre-requisites for and mechanisms of the metabolic network coordination

Coordination of different enzymes in a metabolic network requires: (1) molecule(s) which concentration would be indicative of the network state/function; (2) binding of such indicator molecule(s) to the network enzyme(s), which results in changes of the enzyme catalytic properties to benefit the network function and stability. It is worth noting that impact of particular metabolic reactions on general metabolic functions is not equal (Sweetlove et al., 2008). For instance, only a limited amount of genes is essential for early embryonic development in zebrafish (Amsterdam et al., 2004). Also within central metabolic network there are enzymatic processes which perturbation greatly affects general performance, and those which changes are easily accommodated/compensated without an obvious effect on basic functions. This is in accord with the study of the global organization of metabolic fluxes, revealing that general metabolic activities are dominated by a limited number of reactions, so called "high-flux backbone" of metabolism (Almaas et al., 2004). Thus, regulation of different enzymes affects the network function to different extent, with the critical, or so called "key", enzymes having the greatest impact. The criticality may be defined by the degree of changes in the whole network upon perturbation of its single member. The regulation of enzymes by indicator molecules may occur through different mechanisms. In the simplest case, the enzymes responding to such indicator molecule(s) would have the same binding site for both catalysis and regulation. In such a case a structural similarity between the enzyme substrate(s) and regulator(s) is expected, if the enzyme complex with the regulator is formed by reversible binding. Alternatively, a regulator may irreversibly modify an enzyme. In this case the reactivities of the modifier and modified group on enzyme may enable the modification even in the absence of the modifier structural similarity to the enzyme substrate, with the enzyme-modifier complex stabilized covalently. In this section, it will be shown that the organization of metabolic networks through intercepting pathways transforming different substrates is well suited to satisfy the coordination requirements already in primary, i.e. consisting of pure catalysts, networks.

### 2.1 Structural similarity between the pathway intermediates.

#### 2.1.1 Substrates and intermediates of their transformation in a pathway have certain similarity in structures.

A pathway within a metabolic network consists of a chain of consecutively working enzymes which transform the pathway substrate by a series of sequential steps. For instance, glucose is a substrate of the glycolytic pathway, where glucose is phosphorylated, isomerised and degraded to smaller molecules. Hence, many of the glycolytic intermediates have at least part of the glucose carbon skeleton and common functional groups, such as alcohol, aldehyde or phosphate ones. Owing to this, the pathway intermediates (e.g., glucose-6-phosphate, or its isomer fructose-6-phosphate) have a certain similarity to glucose and/or each other. Besides, a product of one enzyme in a pathway is the substrate of the enzyme catalyzing the next step. Thus, the consecutive transformation of a substrate molecule in a pathway implies structural similarity between the pathway intermediates. This could underlie partially overlapping binding to enzymes of not only their substrates, but other intermediates of the pathway as well (Fig.1). That is, the substrate of the complex (1) in Fig.1 may later in the pathway generate the breakdown products shown in (2) and (3), but the partial structural similarity of all the bound intermediates shown in Fig.1 still

enables their interaction with the enzyme as shown. For instance, the depicted enzyme may catalyze the phosphorylation of glucose, whereas the breakdown products shown in the complexes (2) and (3) of Fig.1 may correspond to glyceraldehyde-3-phosphate and 3-phosphoglycerate arising on the later stages of the pathway. On the other hand, such breakdown products may interconnect different pathways within a network. For instance, 2-oxoglutarate dehydrogenase, which oxidatively decarboxylates 2-oxoglutarate to  $\text{CO}_2$  and succinyl-CoA, may bind malonate (3C) and glyoxylic acid (2C) (Bunik & Pavlova 2006), which may be regarded as the breakdown derivatives of the 2-oxoglutarate (5C) molecule.

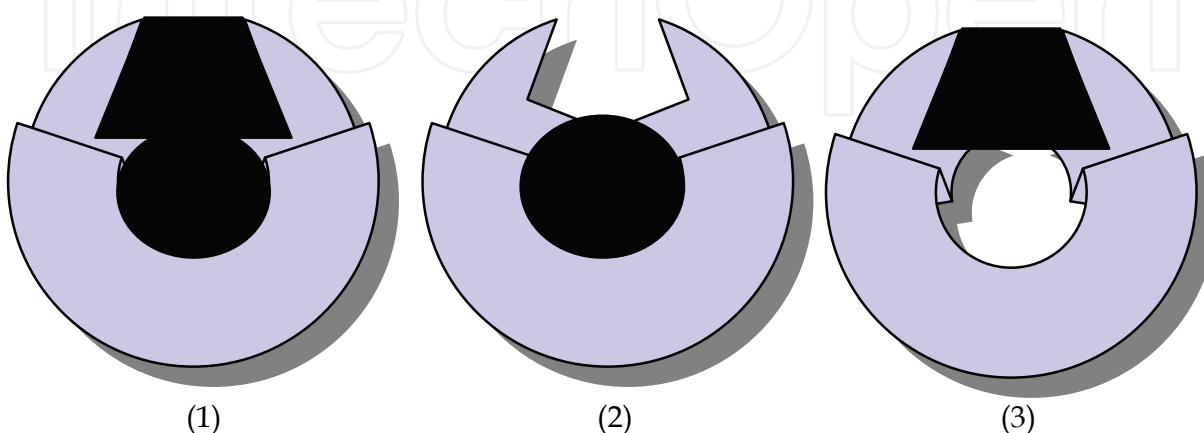


Fig. 1. Binding of a substrate (black in 1) and structurally relevant intermediates (black in 2 and 3), to an enzyme (grey). Certain flexibility of an enzyme structure, realized as a conformational change of its binding site in the presence of a ligand ("hand-glove" model), is schematically presented as a slight structural difference between the enzyme binding site in the free state and when ligand is bound.

Competition for the enzyme binding site between the substrate and intermediate will affect enzymatic transformation of substrate. This change in the enzyme catalytic properties enables the network coordination dependent on the relative concentrations of the intermediates. In the simplest case of the competitive inhibitor, the binding of a non-transformed structural analog of the enzyme substrate blocks the correct binding of the substrate and hence blocks catalysis (Fig.1). However, taking into account multiple binding points, relatively extended structures of both the enzyme ligands and binding sites, and different enzyme-substrate complexes along the reaction pathway, multiple binding modes of ligands are possible (Fig.2), and those may have different consequences for catalysis. A good example of this type of the regulation is provided by the 2-oxoglutarate dehydrogenase, which starts the overall reaction of a key system of the tricarboxylic acid cycle, the 2-oxoglutarate dehydrogenase multi-enzyme complex. The cycle and its auxiliary pathways metabolize different organic acids with up to three carboxylic groups. Extensive kinetic studies of the 2-oxoglutarate dehydrogenase reaction (Bunik et al., 2000; Bunik & Pavlova, 1997a, 1997b; 1996) showed that many of these organic acids and their structural analogs, such as oxalacetate, succinate, glutarate, malonate and 2-oxomalonate, are able to bind to an enzyme-substrate complex. The partial overlap with the binding of the 2-oxoglutarate dehydrogenase substrate, 2-oxoglutarate, occurs by means of either the dicarboxylate (D) group, or both the dicarboxylate and 2-oxo (O) groups. To a certain degree which depends on the structural similarity to 2-oxoglutarate, such binding imitates the pre-catalytic complex of 2-oxoglutarate with the 2-oxoglutarate dehydrogenase (Fig.2, SE). The

catalytically essential interaction with the 2-oxoglutarate dehydrogenase coenzyme, thiamine diphosphate (ThDP) is possible only when the substrate binds to the active site. In this case the formation of a pre-catalytic complex SE is followed by a conformational transition to the catalytic ES complex, where the decarboxylation of 2-oxoglutarate takes place. Following the release of the two reaction products ( $p_1$ ,  $p_2$ ), the free enzyme E is ready for a new catalytic cycle. However, the ES complex is also able to bind another molecule, which may be a dicarboxylate (succinate, malonate, glutarate) or a 2-oxo dicarboxylate (oxalacetate), including the substrate 2-oxoglutarate itself. When bound to ES complex (Fig.2), such binding of a structural analog does not prevent catalysis. Moreover, 2-oxoglutarate may be bound to ES in either an inhibitory ( $S_iES$ ) or an activatory ( $SES$ ) mode. The activatory mode is associated with the slow compared to catalysis conformational

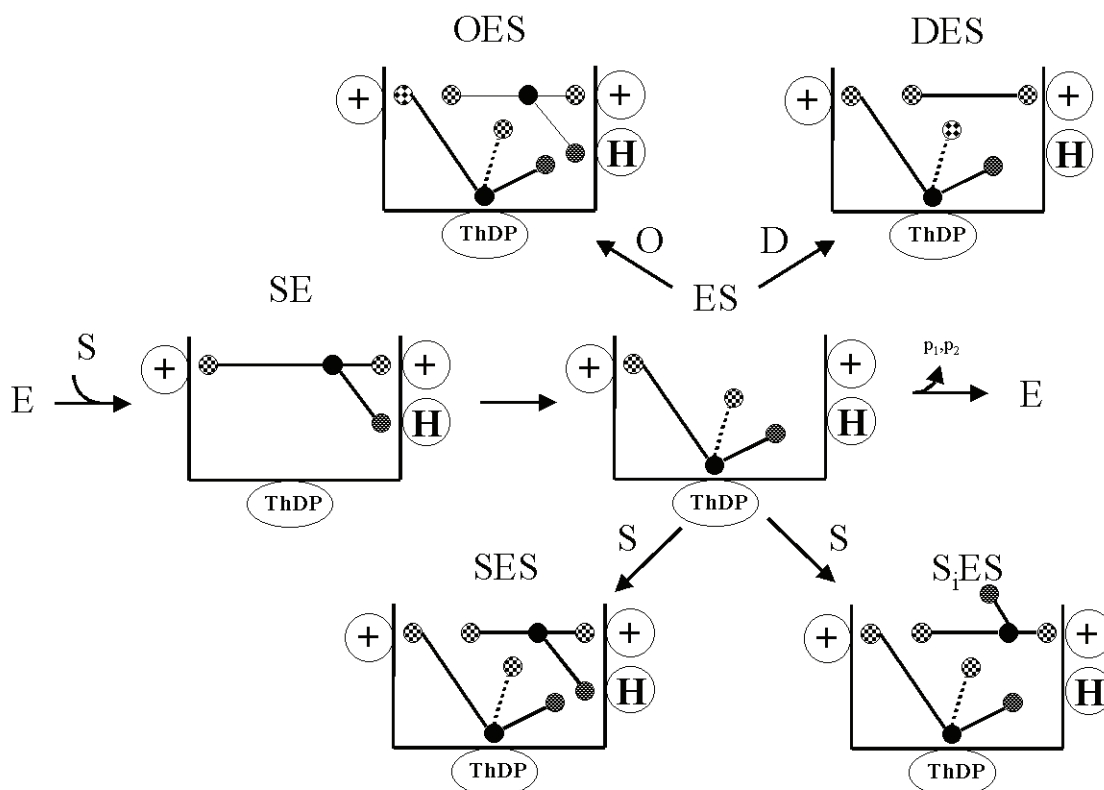


Fig. 2. Enzyme regulation through the multiple binding modes of a ligand, exemplified by the interaction of 2-oxoglutarate dehydrogenase with substrate and its structural analogs. The substrate 2-oxoglutarate (S) and its dicarboxylate structural analogs with (O) or without (D) 2-oxo group may bind to the active site of the 2-oxoglutarate dehydrogenase in different modes, which depend on the enzyme state and interactions realized. The two positively charged (+) and one histidine (H) residues of the enzyme active site interact, respectively, with the two carboxyl and 2-oxo groups of a 2-oxo dicarboxylate. The precatalytic complex SE is transformed into the catalytic complex ES by the formation of the substrate adduct with the coenzyme, thiamine diphosphate (ThDP). The middle part of the figure shows the catalytic cycle. The catalytic bond cleavage occurring in the ES complex is schematically shown by dotted line. The parts below and above the catalytic cycle demonstrate the complexes formed after ES complex binds another molecule of the substrate or its analogs, correspondingly. See the text for other explanations.

transition of the enzyme to a more active state. The inhibitory binding  $S_iES$  is similar to that of dicarboxylates (DES) and, like the bound dicarboxylates, prevents the activation through the conformational transition. Remarkably, only the 2-oxo dicarboxylates are able to block catalysis, obviously because they more closely imitate the precatalytic complex SE, forming the three bonds essential for the SE→ES transformation (Fig.2). Binding of dicarboxylates does not prevent catalysis, but blocks the activatory transition of the enzyme due to binding the second 2-oxoglutarate molecule (complex SES). Thus, dependent on the degree of the structural similarity with the substrate, the substrate structural analogs exhibit different abilities to block catalysis as competitive inhibitors at the stage of the precatalytic complex SE, or to modify catalytic properties as regulators bound to the complex ES (Bunik & Pavlova, 1997a, 1997b). In the latter case, the binding affects the kinetically slow catalysis-associated conformational transition of the protein (Bunik et al., 1991). This phenomenon represents the enzyme hysteretic properties, also called as “enzyme memory”, being important for switching fluxes in the branch points of metabolic networks (Frieden, 1964).

Thus, the network state is encoded by the concentrations of certain intermediates. An enzyme in a network may respond to the network function as a whole through dependence of catalysis on the concentrations of not only the enzyme own substrate(s) and product(s), but also some other intermediates in the network. The response may go beyond the equilibrium binding of different ligands. Indeed, it may involve the reversible, but time-dependent (slow compared to the binding and catalysis) consequences of the binding of regulatory metabolites. Affecting the slow compared to catalysis conformational transition of the enzyme, such regulation does not simply prevent the catalytic transformation, but adjusts it in the time-dependent manner, enabling the enzyme hysteretic properties. This type of sensing mechanism may thus be employed for the time-dependent regulation of distribution of the substrate-dependent fluxes through different pathways. Obviously, it can contribute to the temporal network dynamics (Kholodenko et al., 2010), which is crucial for the network function.

It is remarkable that the mammalian 2-oxoglutarate dehydrogenase multienzyme complex which evolved to the very high catalytic selectivity, hardly allowing for the transformation of any natural 2-oxo acids other than 2-oxoglutarate or 2-oxoadipate, demonstrates a much less pronounced binding specificity at the level of the SE complex formation (Fig.2). That is, the enzyme does bind many of the substrate structural analogs for regulatory purposes. In this regard, it is interesting to note that the participation of the biological catalysts in the network coordination inevitably results in certain constraints to evolution of their catalytic properties to the highest binding specificity or catalytic efficiency. This may contribute to the notion that a higher level of the enzyme regulation, usually inherent in the enzymes functioning within more complex networks, is associated with a lower catalytic power compared to the less regulated orthologues (Hong et al., 1998). The view of enzymes losing catalytic power in order to satisfy regulatory requirements is also in accord with our knowledge from the directed enzyme evolution and protein engineering, which successfully create enzymes with a catalytic power higher compared to that achieved through the natural evolution. The lower catalytic power of the natural enzyme is often considered as a sub-optimal result of the evolution. However, such a view suffers from the lack of understanding that biological catalysts differ from chemical ones by the need to support not only the catalytic process per se, but also the metabolic network coordination/stability. This must impose the selection criteria additional to those increasing the catalytic efficiency and selectivity. For instance, binding of certain substrate structural analogs may interfere with

the highest substrate specificity, but be required for regulation. It is also worth noting that the network coordination mechanisms and critical enzymes may differ, dependent on the network. That is, the evolution of different systems does not obligatory follow the same criteria. For instance, the high catalytic selectivity evolved in the mammalian 2-oxo acid dehydrogenase complexes, including the 2-oxoglutarate dehydrogenase one, is not an obligatory feature of these enzyme systems throughout different kingdoms of living organisms. In actinomicete *Corynebacterium glutamicum* a chimeric complex degrading both pyruvate and 2-oxoglutarate is present (Niebisch et al., 2006) instead of the two specific multienzyme complexes transforming either pyruvate or 2-oxoglutarate in many bacteria, plants and animals. Remarkably, the degradation of the two different substrates is performed by the two different gene products of the first component of the 2-oxo acid dehydrogenase multienzyme complex. That is, this chimeric complex uses the substrate-specific first components, like the other known 2-oxoglutarate dehydrogenase complexes. However, the overall control of the processes is changed in such a way that the oxidation of both substrates is regulated not separately, but together within one complex. The chimeric complex also has a very unusual regulation through posttranslational modification of a specific regulatory protein (Schultz et al., 2007, Niebisch et al., 2006). This example illustrates that the species-specific organization of metabolic networks and their coordination mechanisms greatly affect the catalysts evolved in living systems. Other examples illustrating this notion are also given below in Section 4. Thus, the evolution of enzymes and networks is interdependent, which creates an opportunity to change networks through changing their critical enzymes. Obviously, our understanding of the specific network coordination features and how they are met by evolutionary developments will underlie our ability to engineer cells with the designed network coordination. For instance, cells with new signaling circuits may be designed for medical and biotechnological applications (Lim, 2010).

### 2.1.2 Common metabolites interconnect pathways

In biological networks, there are a number of substrates or coenzymes, which are common not only within a pathway, but also between different pathways. For instance, the universal energy store and donor ATP is used in many reactions of a pathway. That is, in glycolysis ATP is the energy donor in the two reactions and is synthesized from ADP in the other two reactions. Other pathways (e.g., fatty acid oxidation) also use this molecule for the same purposes. Universal substrate and coenzyme for many oxidation-reduction steps are  $\text{NAD(P)}^+/\text{NAD(P)H}$  and  $\text{FAD}/\text{FADH}_2$ , respectively. Remarkably, all these ubiquitous components of metabolic networks, as well as some important coenzymes or their derivatives, such as coenzyme A (CoA) or recently discovered thiamine adenine nucleotide (Bettendorff et al., 2007) comprise a common structural block of adenosine (adenine heterocycle bound to ribose) which connects to the catalytic part of the substrate or coenzyme (nicotinamide ring in  $\text{NAD(P)H}$ , isoalloxazine ring in FAD or thiazole ring in adenylated thiamine triphosphate) via the 5'-phosphorylated end of ribose. Moreover, special regulatory systems evolving for the coordination of more complex networks, employ regulators based on the same ubiquitous intermediates. For instance, apart from functioning as the universal redox substrate, interconnecting many pathways, nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) is also the substrate for the regulatory  $\text{NAD}^+$ -consuming enzymes, such as ADP-ribose transferases and poly(ADP-ribose) polymerases (Belenky et al., 2007). The former enable regulatory post-translational modifications of specific enzymes, whereas

poly(ADP-ribosylation) is a pluripotent cellular process important for maintenance of genomic integrity and RNA transcription in cells. Although all molecular mechanisms involved in the function of this regulatory system are not yet well characterized, the process basically depends on the depletion of the network  $\text{NAD}^+$  and ATP (Du et al., 2003) and regulatory action of the intermediates accumulated upon the  $\text{NAD}^+$  degradation, such as ADP-ribose (Perraud et al., 2005) and AMP (Formentini et al., 2009). Thus, the advanced regulation of the highly complex networks is based on the enzymatic sensing of the common key intermediates and their derivatives, occurring already in the primary metabolic networks. Other regulatory derivatives of nicotinamide adenine dinucleotides include the second messengers involved in calcium signaling, such as nicotinic acid-adenine dinucleotide phosphate (NAADP), which differs from  $\text{NADP}^+$  by the presence of a nicotinic acid instead of a nicotinamide moiety (Rutter et al., 2008; Guse & Lee, 2008), and cyclic ADP-ribose (Graeff et al., 2009; Davis et al., 2008; Bai et al., 2005, Yue et al., 2009). As a result, common metabolites interconnecting different reactions of a pathway and different pathways of a network participate in the coordination of primary networks, whereas their derivatives regulate the metabolic networks evolved to a higher complexity. It thus appears that ubiquitous participation of these common intermediates in regulation of primary metabolic networks, associated with the wide representation of their protein binding sites, enabled evolution of the network coordination to further exploitation of these compounds in additional regulatory systems evolved.

Not all of the network indicator molecules are so widely used by the network enzymes as those considered above. Instead, the indicators may also be molecules involved in the processes crucial for the network function and stability. For instance, 2-oxoglutarate is an intermediate synthesized in the tricarboxylic acid cycle. It is irreversibly degraded by either the 2-oxoglutarate dehydrogenase functioning in the cycle or prolyl hydroxylase. The former enzyme exerts an essential control on mitochondrial oxygen consumption under increasing energy demands (Bunik, 2010; Cheshchevik et al., 2010; Cooney et al., 1981), whereas the latter is the hypoxia-inducible factor which controls cellular responses to hypoxia (McDonough et al., 2006; Ginouves et al., 2008). It is therefore obvious that the decrease in the mitochondrial oxidation of 2-oxoglutarate under limited oxygen may influence availability of 2-oxoglutarate for the hypoxia-inducible factor, thus contributing to regulation of the complex network dependent on oxygen sensitivity (Ginouves et al., 2008). In good accord with this assumption, cellular sensing of the 2-oxoglutarate level is involved in retrograde signaling of mitochondria to nucleus, leading to adaptations compensating mitochondrial impairment through the changed expression of key enzymes (Butow and Avadhani, 2004; Bunik & Fernie, 2009).

## 2.2 Biological network buffers

Some metabolites may be present in metabolic networks at very high concentrations. For instance, cells may contain 1-10 mM tripeptide glutathione (L- $\gamma$ -glutamyl-L-cysteineylglycine) (Mieyal et al., 1995; Lopez-Mirabal & Winter, 2008) or 10-200 mM of dipeptides carnosine (beta-alanyl-L-histidine) and anserin (N2-methyl-carnosine) (Boldyrev, 2007). This creates an opportunity for the network coordination, which employs such highly abundant molecules as general intracellular sensors. For instance, the buffering or redox properties of these compounds may be used to integrate functional outcome of different enzymes which influence the pH or redox potential of intracellular milieu. The dipeptides are mostly known as general buffers and metal chelators. The latter property may be responsible for their



antioxidant action, which was also suggested to be due to the direct chemical reactions between the dipeptides and reactive oxygen species (ROS) and/or the secondary products arising due to ROS, such as the products of lipid peroxidation. That is, the conjugation of carnosine with  $\alpha,\beta$ -unsaturated aldehydes formed from lipid peroxidation was shown as an important mechanism for the aldehydes detoxification (Aldini et al., 2002; Guiotto et al., 2005). It should be noted, however, that in case of bifunctional lipoxidation products such reactions may also damage the protein function. For instance, the bifunctional lipoxidation-derived aldehyde 4-oxo-2-nonenal can cross-link carnosine to proteins, causing an irreversible protein modification *in vitro* (Zhu et al., 2009), although occurrence of such processes *in vivo* is not known.

Significance of the very specific pattern of the dipeptide levels during development and of a number of species- and tissue-specific modifications of the basic carnosine structure is quite intriguing, but remains an enigma, as no specific protein/enzyme targets of the dipeptides have been revealed up to date (Boldyrev, 2007). Nevertheless, the exact structure of the dipeptides may be used as a chemical signature of different species, which may point to yet undefined function of such specificity for the network regulation. In contrast, the specific structure of glutathione is known to be required for its reduction by the NADPH-dependent glutathione reductase and also for the glutaredoxin-dependent deglutathionylation of proteins (Mieyal et al., 1995). Although the antioxidant function of glutathione employs its direct chemical reaction with ROS as well as that of the dipeptides, it goes beyond that, involving the bi-directional communication between the network enzymes. As will be shown in Section 2.3, the redox equilibrium between glutathione and its oxidized form, glutathione disulfide, may affect the enzymatic function of many enzymes in the network through the reversible chemical modification of the protein thiol groups. On the other hand, other enzymes affect the state of the glutathione redox buffer by generating ROS oxidizing glutathione to its disulfide and through the NADPH-dependent reduction of the glutathione disulfide (Gitler and Danon, 2003). Thus, the glutathione-dependent communication between the network enzymes is under control of the network function indicators NAD(P)H/NAD(P)<sup>+</sup> and ROS. Besides, the glutathione/glutathione disulfide redox buffer is also in redox equilibrium with other cellular thiols and disulfides of low molecular weight, which may control availability of some important SH-comprising substrates and coenzymes. Reactions between different cellular thiols and disulfides are represented by Equations 1-4, with the relative concentrations of different products dependent on the redox potentials of the participating redox couples (\*RS<sup>-</sup>/<sup>\*</sup>RS-SR<sup>\*</sup> and #RS<sup>-</sup>/<sup>#</sup>RS-SR<sup>#</sup>) in the medium:



For instance, an important substrate of many enzymes, CoA, contains thiol group which enters the thiol-disulfide exchange reactions with the glutathione/glutathione disulfide (Gilbert, 1982). Furthermore, cystine or glutathione disulfide may oxidize dithiol group of reduced lipoic acid to its disulfide (Konishi et al., 1996). In living systems, lipoic acid is used as a covalently attached cofactor of the multienzyme complexes which oxidatively

decarboxylate 2-oxo acids (Bunik & Strumilo, 2009). In the course of catalysis, the redox active disulfide of lipoic acid undergoes redox cycling between the dithiol and disulfide forms. Addition of low molecular weight disulfides of cysteine or glutathione inhibits the catalysis in accordance with the redox potentials of the thiols and disulfides participating in the reaction, pointing to the formation of their mixed disulfides with the complex-bound lipoate (Bunik, 2000). The mixed disulfide of the complex-bound lipoic acid with glutathione was also observed in situ (Applegate et al., 2008).

### 2.3 Common reactivities of the enzyme functional groups and structural elements

Complementary to the commonality of metabolites in the network, the enzyme structures and reactivities have much in common too. For instance, the common adenine nucleotide moiety discussed in Section 2.1.2 binds to the nucleotide binding domain of proteins, comprising an open twisted  $\beta$  sheet surrounded by  $\alpha$  helices on both sides - so called Rossmann fold (Branden and Tooze, 1999). The thiol/disulfide groups of the protein cysteine/cystine residues may undergo different thiol-disulfide exchange reactions (Equations 1-4) with the glutathione/glutathione disulfide or other SH/disulfide-comprising members of the network, including both the protein (thioredoxin, protein disulfide isomerase) and low molecular weight (cysteine/cystine; CoA/CoA-disulfide) components. Increasing network concentration of glutathione disulfide usually signals the prevalence of oxidative conditions and loss of the network reducing power. As a result, the thiol groups of proteins (\*RS-) enter the reaction with glutathione disulfide (#RS-SR#), forming the mixed disulfide according to Equation 1 (Gilbert, 1984; Mieyal et al., 1995; Shelton et al., 2005). Similar to enzymes, the DNA-binding proteins regulating transcription may also change their function upon glutathionylation. For instance, the NF $\kappa$ B transcription factor is shown to be glutathionylated under hypoxic conditions in situ, with the loss of its DNA-binding activity causing the hypoxic cancer cell death (Qanungo et al., 2007). Currently, no enzyme has been shown to serve as a catalyst of this reaction (S-glutathionylation) in situ, although potential prototypes are reported, including human glutaredoxin 1 and the pi isoform of glutathione-S-transferase (Gallogly & Mieyal, 2007; Qanungo et al., 2007). The backward reaction of the protein deglutathionylation is catalyzed by glutaredoxins. The reversible post-translational modification of proteins through glutathionylation is important not only to protect the protein cysteine residues from irreversible oxidation under oxidative conditions. This modification also serves to transduce redox signals in order to either normalize homeostasis or, if this turns to be impossible, to start the death program. For instance, accumulation of hydrogen peroxide leads to depression of mitochondrial metabolism due to glutathionylation of 2-oxoglutarate dehydrogenase, with the peroxide consumption restoring both the metabolism and free thiols in the enzyme (Applegate et al., 2008). However, when the transcription factor p65-NF $\kappa$ B is S-glutathionylated, this potentiates the cell death through apoptosis (Qanungo et al., 2007). Such network switches between different states, which are dependent on the degree of homeostatic deviations, are widely spread in living systems. In particular, initial increase in the network hydrogen peroxide elicits antioxidant gene expression to reduce the peroxide, but if the peroxide level continues to increase, the prooxidant genes are expressed, inducing the cell death (Veal et al., 2007).

Another important network coordination system is based on the complete oxidation-reduction of protein vicinal thiols according to Equations 1-2, where #RS-SR# depicts the

protein disulfide. Usually the reductant  $^*RS^-$  is a small protein with the redox-active thiol/disulfide group, thioredoxin. In many enzymes this results in essential changes of the enzymatic activities, leading to metabolic switches (Gitler & Danon 2003). A classic example is the light-dark regulation of metabolic activity of chloroplasts, which is based on the light-induced reduction of the disulfide bonds in several key proteins, switching on the photosynthetic reactions of carbon fixation in plants through multiple mechanisms shown in Fig.3 (Buchanan, 1991; Danon & Mayfield, 1994; Jacquot et al., 1997; Gitler & Danon 2003).

Not only metabolic enzymes, but also ion channels (Aon et al., 2007), transcription and translation factors (Chen PR, 2006; Hayashi, 1993; Jacquiersarlin & Polla, 1996; Ueno et al., 1999; Levings & Siedow, 1995; Danon & Mayfield, 1994), cytokines (Schenk et al., 1996), growth factors (Blum et al., 1996; Gasdaska et al., 1995), hormonal action (Boniface & Reichert, 1990; Makino et al., 1999) and even intercellular communication (Meng et al., 2010) may be regulated by the thioredoxin-dependent mechanism.

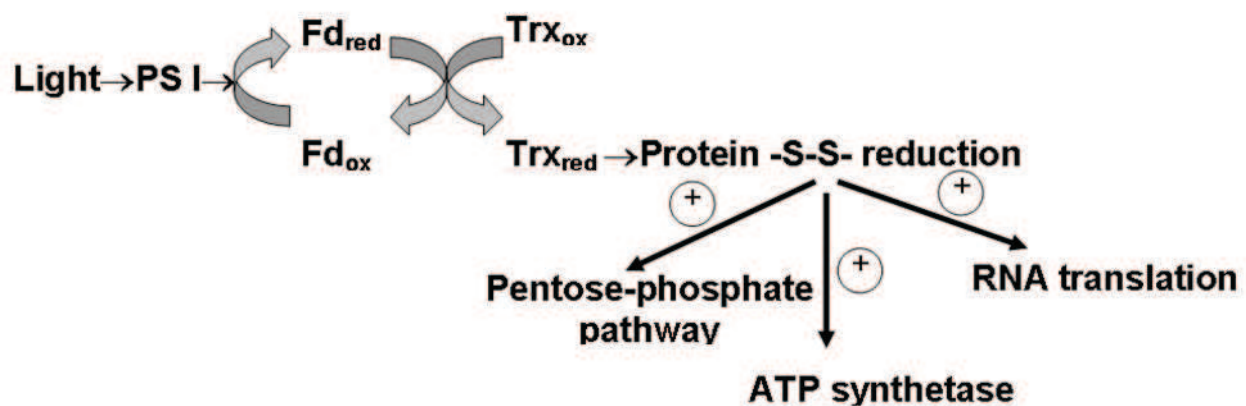


Fig. 3. Thioredoxin-mediated regulation of chloroplasmic metabolism by light. Reduction of the chloroplasmic photosystem I (PS I) by light enables electron flow to the redox-protein ferredoxin (Fd) which reduced form serves as the thioredoxin (Trx) reductant in the reaction catalyzed by the ferredoxin-dependent thioredoxin reductase. Thioredoxin reduces the regulatory disulfides in several chloroplasmic proteins, which leads to their activation. The latter occurs in the key enzymes of the photosynthetic pentose-phosphate pathway, the energy producing ATP synthetase and the translation-regulating protein.

Many aspects of the redox regulation by reversible oxidation/reduction of protein thiols/disulfides are similar to another important posttranslational modification of proteins, involved in signal transduction, the phosphorylation/dephosphorylation (Fig.4). This modification usually occurs at the protein amino acid residues tyrosine, serine, threonine or histidine. Remarkably, the redox state of the nicotinamide adenine dinucleotides may be among the signals which are transduced by both the phosphorylation/dephosphorylation and thiol-disulfide-dependent systems. For instance, in response to increased NADH/NAD<sup>+</sup> ratio the pyruvate dehydrogenase complex is inactivated by phosphorylation (Roche et al., 2003), whereas the 2-oxoglutarate dehydrogenase complex is inactivated according to another mechanism controlled by thioredoxin (Bunik 2003). Thus, different coordination mechanisms may be involved in the transduction of the same signal through different enzymes. In general, the tight interplay between the redox states of the thiol/disulfides and nicotinamide adenine dinucleotides makes the pathways controlled by the coordination mechanism (1) in Fig.4 directly dependent on the network indicator

molecules (i.e. SH/-S-S-; NAD(P)H/NAD(P)<sup>+</sup> and ROS). In contrast, the phosphorylation/dephosphorylation-dependent coordination mechanism (2) does not directly depend on the cellular ADP/ATP ratio, but rather switches off/on certain pathways in response to specific signals of different nature. That is, although such signals may still be generated by depletion in cellular ATP, this would not prevent the ATP-dependent protein phosphorylation under such conditions. However, depletion of NADPH directly decreases the NADPH-dependent reduction of the glutathione disulfide.

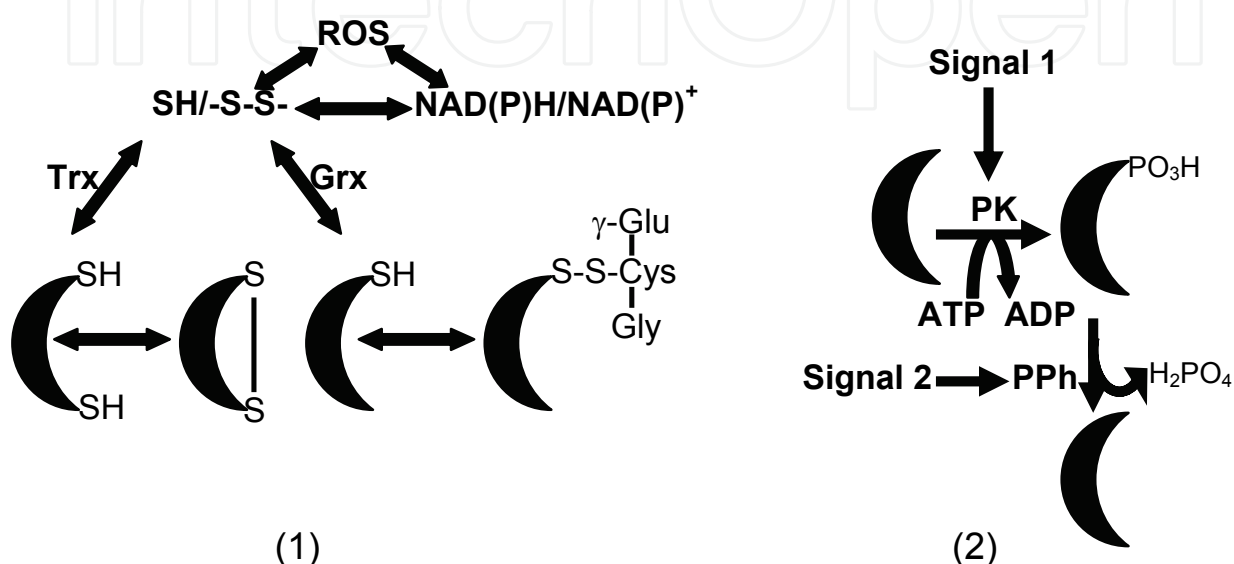


Fig. 4. Comparison of the two types of the protein regulation by post-translational modification. (1) - Modification of the protein cysteine residues in non-photosynthetic organisms; (2) - phosphorylation of the protein tyrosine, threonine, serine or histidine residues. The regulated protein is depicted as a crescent. Full oxidation/reduction of the neighbouring thiols in a protein is under the control of thioredoxin system (Trx), comprising thioredoxin and the thioredoxin reductase which in non-photosynthetic organisms is NADPH-dependent. S-glutathionylation of a protein thiol by glutathione (L- $\gamma$ -glutamyl-L-cysteinyl-glycine) is controlled by glutaredoxin system (Grx), comprising glutaredoxin, glutathione and the NADPH-dependent glutathione reductase. Through catalysis by the thiol-disulfide oxidoreductases, the redox state of the nicotinamide adenine dinucleotides is related to that of cellular thiols. Both the redox states, i.e. those of the dinucleotides and thiols, are also directly related to the ROS production and scavenging. Thus, the coordination mechanism (1) may integrate signals from cellular thiols, ROS and nicotinamide adenine dinucleotides. In contrast, the coordination mechanism (2), involving the signal- and protein-specific protein kinases (PK) and protein phosphatases (PPh), does not directly depend on the overall ATP/ADP ratio.

It should be noted, however, that the network evolution to increased complexity and high differentiation precludes any of the regulation types to operate at the level of whole network. Indeed, in simpler bacterial organisms certain examples exist where the protein thiol oxidation into disulfide bonds or reduction of the disulfide bonds to thiols is determined by the redox status of the environment. That is, the oxidizing milieu of the bacterial periplasm stimulates the former, whereas reducing conditions of cytoplasm

promotes the latter (Eser et al., 2009). Also in mammals under some experimental settings it is possible to observe the correlation between the global intracellular change in the cellular glutathione/glutathione disulfide ratio and function of the redox-dependent transcription factors (Haddad et al., 2000). However, in view of the known reducing nature of intracellular milieu, significance of the thiol-disulfide exchange-based regulation of proteins in such a milieu had been questioned (Ziegler, 1985). For instance, the redox potential of the transcription factor OxyR is -185 mV, while thiol/disulfide redox potential of cytoplasm was estimated between -260 and -280 mV, which raises the question of how OxyR may be activated by the disulfide formation at all (Aslund et al., 1999). Further studies showed that such simplified thermodynamic consideration is not plausible for predicting the coordination mechanisms in biological networks, because the local reaction kinetics cannot be neglected, even when the overall thermodynamics is unfavorable (Danon 2002; Toledano et al., 2004). In particular, transient activation of OxyR upon local accumulation of hydrogen peroxide was supposed to occur (Aslund et al., 1999). Indeed, it was shown that activation of OxyR due to specific disulfide bond formation occurs very fast and results in a metastable protein conformation that is locally strained. The rapid kinetic reaction path and conformational strain, respectively, are supposed to drive the oxidation and reduction of OxyR (Lee et al., 2004). Also Hsp33, a member of a newly discovered heat shock protein family, is a cytoplasmically localized protein, with its reactive cysteines responding quickly to oxidizing conditions by forming the disulfide bonds. The latter activates the chaperone function of Hsp33 (Jacob et al., 1999). Thus, it was shown that the disulfide bonds can indeed be formed within the reducing intracellular environment. Moreover, the disulfide bonds can also be reduced in the oxidizing extracellular space (Hogg 2003; Yang & Loscalzo, 2005; Yang et al., 2007).

Worth noting, the local accumulation of hydrogen peroxide necessary for cellular signaling through the thiol/disulfide-dependent processes is in some cases allowed by the phosphorylation-induced inactivation of peroxiredoxin I, which in its dephosphorylated state efficiently degrades hydrogen peroxide (Woo et al., 2010). This provides a good example of the cross-talk between the regulatory systems coordinating the network through the thiol-disulfide exchange and phosphorylation reactions. Thus, significance of the local effects due to the thiol-disulfide exchange was established, owing to which most types of the network coordination in real systems should take into account local concentrations and kinetic effects. That is, most of the effects involving the network coordination through the posttranslational modification of the network enzymes occur transiently and in response to a local signal.

### **3. Coordination of the network through generation of secondary signal molecules**

Binding of the indicator molecules as discussed in the section 2.1 may coordinate the network through the direct regulation of a critical enzyme with the indicator bound. However, the indicator binding may also coordinate network through generation of a secondary signal molecule, which would bind to other members of the network, not binding the indicator itself. Activation of an alternative catalytic pathway in the indicator-enzyme complex may induce generation of such secondary signal. This is exemplified in, but not limited to the widely known side reaction of partial dioxygen reduction with the formation of ROS. The latter are well suited for the signaling function because their reactivity allows for chemical modification of catalysts, whereas their concentration is tightly controlled by

the cellular ROS scavenging systems, intimately linked to the network indicator molecules NAD(P)H/NADP<sup>+</sup> and glutathione/glutathione disulfide. In multisubstrate enzymatic reactions, the partition between different catalytic routes, such as the main physiological and side reactions, may be controlled by the substrate ratio. Function of the 2-oxo acid dehydrogenase multienzyme complexes, each catalyzing the substrate-specific overall reaction of oxidative decarboxylation of pyruvate, 2-oxoglutarate or branched chain 2-oxo acids according to Equation 5, provides a good example of such regulation.

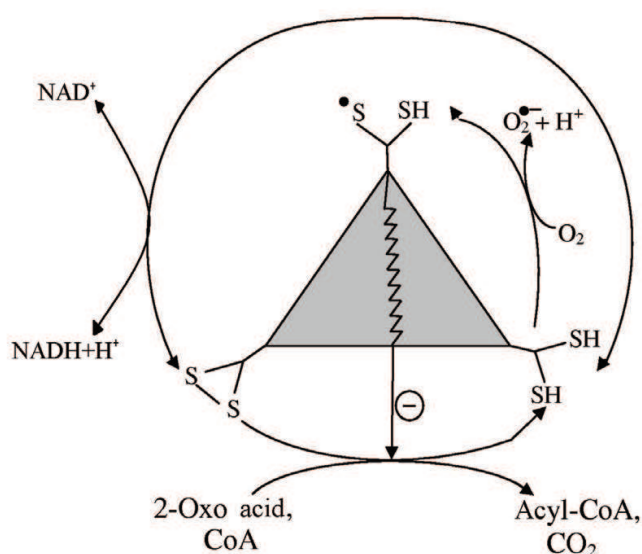
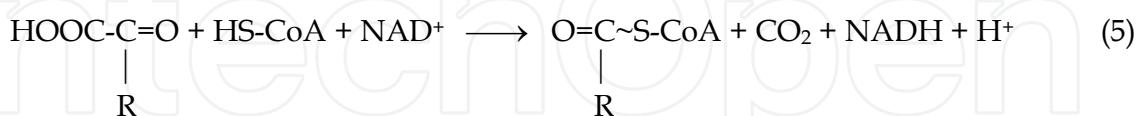


Fig. 5. Oxidative decarboxylation of a 2-oxo acid by the multienzyme 2-oxo acid dehydrogenase complexes. The overall physiological reaction occurring through the dihydrolipoyl intermediate, results in the formation of acyl-CoA, CO<sub>2</sub> and NADH. This comprises partial reactions at the bottom and at the outside left part of the figure. The catalytic disulfide/dithiol groups of the covalently bound lipoyate residues of the complexes are schematically shown at the triangle corners. The side reaction of the one electron reduction of dioxygen is activated in the absence of the terminal substrate NAD<sup>+</sup>, shown inside the circle at the right. The inactivation of the first enzymatic component of the complex by the thiyl radical of the complex-bound lipoyate is indicated by the negative sign beside the arrow connecting the radical species and the component reaction at the bottom. See text for further discussion.

The complexes consist of multiple copies of the three catalytic components: the substrate specific 2-oxo acid dehydrogenase, dihydrolipoyl acyltransferase and dihydrolipoyl dehydrogenase. The physiological process (Equation 5) is defined in Fig.5 by the circled arrows and arrows outside the circle at the bottom and left sides. The process includes the intermediate acylation/reduction of lipoyl residues covalently bound to the second component, dihydrolipoyl acyltransferase. While this route uses the energy of the 2-oxo acid oxidative decarboxylation to reduce NAD<sup>+</sup> into NADH (reaction at the left of Fig. 5),

dioxygen is reduced instead of  $\text{NAD}^+$  in the side reaction (inside the circle at the right of Fig.5). As seen from the figure, the side reaction may be activated when the dihydrolipoyl intermediate arises in the presence of 2-oxo acid and CoA, but the terminal substrate  $\text{NAD}^+$  is absent. Due to reversibility of the terminal dihydrolipoyl dehydrogenase reaction (reaction at the left of Fig. 5), the side reaction of dioxygen reduction may also be activated in the absence of 2-oxo acid and CoA, when the complex-bound lipoic acid is reduced by NADH. In so far, the activation of the side reaction involving dioxygen is controlled by availability of the substrates of physiological reaction, i.e. 2-oxo acid, CoA,  $\text{NAD}^+$ , the reaction product NADH and their ratio, which defines the steady-state level of the complex-bound dihydrolipoyl intermediate. The mechanism of the dioxygen reduction in the side reaction of the 2-oxo acid dehydrogenase complexes is such that only one electron is transferred from the dihydrolipoyl intermediate to dioxygen (Bunik & Sievers, 2002). As a result, the system operating through this route generates the two radical species: the superoxide anion radical and complex-bound thiyl radical (Fig.5). Both radical species may be considered as the secondary signal molecules. The superoxide anion radical belongs to ROS affecting the enzymatic function (i) directly, (ii) through shifting the thiol-disulfide redox status (Fig.4) to a higher oxidation state and (iii) through other signaling systems including those of phosphorylation/dephosphorylation (Woo et al., 2010). In particular, ROS inactivate aconitase and succinate dehydrogenase (Nulton-Persson & Szwedda, 2001; Bulteau et al., 2003), which belong to the same pathway (TCA cycle) as the pyruvate and 2-oxoglutarate dehydrogenase complexes, all catalyzing the steps within the first half of the cycle. The second reaction product, the complex-bound thiyl radical, acts as affinity modifier inactivating the first component of the complexes. This is shown in Fig.5 by the negative regulation of the initial step of the overall process, exerted by this radical species (Bunik & Sievers, 2002; Bunik 2003). Thus, stimulation of the side route of catalysis by the 2-oxo acid dehydrogenase complexes is defined by the steady-state concentration of the dihydrolipoyl intermediate. This signal originates from the ratio of several network intermediates and indicators, such as 2-oxo acid, CoA,  $\text{NADH}/\text{NAD}^+$ ,  $\text{O}_2$  (Fig.5). The two types of secondary signaling molecules, i.e. the superoxide anion and thiyl radicals, are generated by the 2-oxo acid dehydrogenase complex as the complex response to sensing the ratio of its substrates and products. Importantly, that the complex senses not only a single metabolite, but an integral signal given by the ratio. One of the generated secondary signaling molecules, the superoxide anion radical, may affect the above mentioned enzymes and systems, i.e. act outside the active sites where it was generated. The other signaling molecule, thiyl radical, is bound to the complex and can therefore act only in its vicinity. In particular, it may prevent accumulation of the superoxide anion radical by inactivating the first component of the complex (Fig.5) so that the accumulation of the dihydrolipoyl intermediate is stopped. On the other hand, the thiyl radical of the complex-bound lipoate may interact with thioredoxin. The latter thiol-disulfide oxidoreductase was shown to be the potent thiyl radical scavenger (Hanine Lmoumene et al., 2000), owing to which it prevents the inactivation of the 2-oxo acid dehydrogenases by the thiyl radical as shown in Fig.6 (Bunik & Sievers, 2002; Bunik, 2000). Thus, generation of signaling molecules from the 2-oxo acid dehydrogenase complexes is coupled to the thioredoxin system. Dismutating the thiyl radicals of the complex-bound lipoate, thioredoxin prevents the inactivation of the first component of the complexes in the presence of 2-oxo acid and CoA (negative arrow in Fig.5, Fig.6), thus amplifying their generation of signaling molecules (side reaction inside the circle in Fig.5).

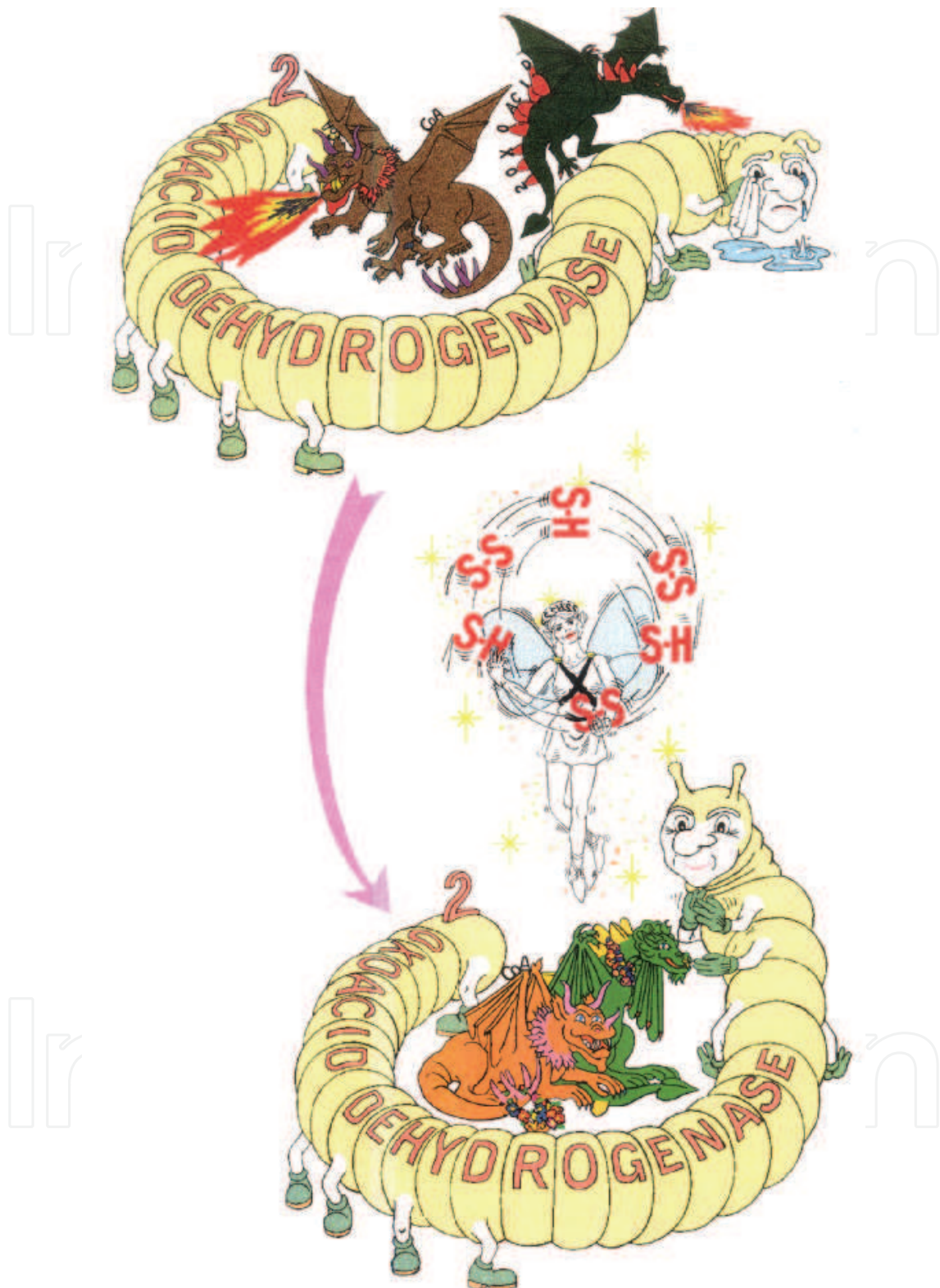


Fig. 6. Inactivation of the 2-oxo acid dehydrogenase complexes (depicted as a caterpillar) by their substrates 2-oxo acid and CoA (depicted as dragons) is prevented by a thiol-disulfide oxidoreductase thioredoxin (depicted as a fairy). Thioredoxin thus reconciles all the system components. The signaling implications of the reconciliation are discussed in the text. (The picture created by Dr. J. Schütz)



#### 4. Contribution of biological context to chemical networking

Living systems widely use compartmentation of the medium, where metabolic networking occurs. Membrane-surrounded intracellular compartments, forming different organelles, represent an advanced compartmentation mechanism, separating different modules of the metabolic network in eukaryotic cells. Although this type of compartmentation is absent in prokaryotes, they do have a specific compartment of periplasm where the oxidizing conditions prevail, in contrast to the reducing conditions of cytoplasm. Development of intracellular imaging of high resolution strengthened the view that spatial organization is crucial for intracellular communication, because it provides for local interactions between and the density fluctuations of the system components (Dehmelt & Bastaniens, 2010). Indeed, as discussed in Section 2.3, such local changes are essential for the network coordination through the disulfide formation in signaling proteins, which may occur transiently under thermodynamically unfavorable conditions of generally reducing cytoplasmic milieu. However, there is a need to also have the stable structural protein disulfides. This is controlled by the enzymes oxidizing the neighboring cysteine residues of the nascent polypeptide into a disulfide and further isomerising this disulfide to its correct position in the final polypeptide after all cysteine residues have already been included. Function of this part of cellular network, which comprises many component proteins with the redox active thiols, is intensively studied, although not completely understood as yet (Thorpe & Coppock, 2007; Appenzeller-Herzog et al., 2010). However, it is well known that these reactions require separate cellular compartment where oxidizing conditions prevail to enable the disulfide formation. In bacteria this occurs in periplasm, while in eukaryotes the system is localized to the endoplasmic reticulum. Remarkably, that although the oxidized glutathione plays important role in the redox homeostasis of the endoplasmic reticulum, significant part of the redox equilibrium in this compartment is defined by the thiols and disulfides of the catalysts themselves (Appenzeller-Herzog et al., 2010). Obviously, this is required to make possible the dynamic equilibrium of the isomerization process, which includes both the reduction and oxidation of the disulfides under generally oxidizing conditions of the compartment. To achieve this, mammalian endoplasmic reticulum contains the protein disulfide isomerase at nearly millimolar concentration (Thorpe & Coppock, 2007). This example demonstrates benefits of comparable concentrations of the catalyst and its substrate, which may thus be employed by biological network, in contrast to the common chemical catalysis.

##### 4.1 Protein-based compartmentation for the network coordination

Both pro- and eukaryotes employ still another compartmentation type, which does not require the membrane-separated compartments, but is created through the protein-protein interactions. This type of compartmentation may be called as microcompartmentation to distinguish it from the membrane-afforded organellar compartmentation. However, independent of the scale, already the homo- or heterooligomerization of catalysts, i.e. their assembly from several identical or different subunits, respectively, could create a compartment with certain advantages for the network function. The advantages are implied by the fact that many enzymes are built of at least two identical subunits, with the active sites formed at the interface between the subunits, even if each of the subunits possesses all

the residues needed to perform catalysis (Branden & Tooze, 1999). One of the advantages of the enzyme function as dimers or tetramers is the ensuing opportunity of the cooperative interactions between the active sites which enables the information transfer (Fenton 2008; Branden & Tooze, 1999; Keleti 1986). In particular, this makes possible a steeper (positive cooperativity) or a slower (negative cooperativity) increase in the enzymatic activity in response to an increase in the substrate concentration, compared to the hyperbolic saturation of an enzyme with substrate in the absence of cooperative interactions. In case of heterooligomers with different active sites the cooperative effects may enable the information transfer between these sites so that the second active site would not be working if the first one lacks its substrate (Nagradova, 2003; Mouilleron & Golinelli-Pimpaneau, 2007), thus preventing a useless substrate consumption. Strong regulation of the catalytic process within the heterooligomeric multienzyme complexes is also evident from the fact that their component enzymes may even lose the capacity to catalyze the physiological reaction outside the complex. An example is provided by the 2-oxo acid dehydrogenase complexes. Their first components, the 2-oxo acid dehydrogenases, catalyze the 2-oxo acid oxidative decarboxylation reaction, reducing the second substrate, lipoamide, only when it is covalently bound to the second enzyme of the complex. Catalytic efficiency (kcat/Km) of the 2-oxo acid dehydrogenase with free lipoamide is 20000-fold less (Graham et al., 1989; Graham and Perham, 1990). The microcompartmentation may also serve protection to surrounding medium by creating channels through which the toxic intermediates are transported from one catalytic site to another (Doukov et al., 2008; Mouilleron & Golinelli-Pimpaneau, 2007). Another opportunity from the catalyst oligomerization is forming the reaction chambers. This may be needed for processing large protein substrates as in case of chaperones (Hofmann et al., 2010). However, reaction chambers are also useful to cage a common substrate so that its local concentration is increased only in a relevant part of a network, i.e. in the vicinity of the active sites where this substrate is required. This is true for the acyl carrier protein in the fatty acid synthetases and lipoic acid in the 2-oxo acid dehydrogenase complexes (Perham 2000; Jenni et al., 2007, Maier et al., 2006). Furthermore, the whole pathways may be compartmentalized. In this case, supramolecular structures, so called metabolones, are assembled, which comprise the catalysts belonging to the same pathway (Velot & Srere, 2000; Ovádi & Srere, 2000, Ovádi & Saks, 2004). It is reasonable to suggest that local concentration of the pathway intermediates within such metabolones may increase, because their diffusion into the bulk solution is hindered due to molecular crowding inherent in a cell containing many macromolecules and solutes. As a result of the hindered diffusion, the intermediates would rather bind to the vicinal enzymes belonging to the pathway metabolone, which enables a more efficient transformation of substrate and coordination of the pathway enzymes.

Why are some sequences of reactions catalyzed by relatively unstable metabolones, whereas others – by isolatable as a whole unit, i.e. rather stable, multienzyme complexes or even multifunctional enzymes? In the latter case, the genes for different enzymes catalyzing successive steps of a substrate transformation, merge to code for a single polypeptide with many active sites, but even in multienzyme complexes the overall reactions mostly occur only when the full structure is self-assembled. One may suggest that the degree of the evolutionary stabilization of such supramolecular structures depends, in particular, on the criticality of the metabolic process for the coordination of a given network and on the employed mechanisms to achieve such coordination.

#### **4.2 Advantages for the network coordination of the random coupling vs channeling between the active sites of a supramolecular structure**

It is important to note that the structure-function relationships in the multienzyme complexes established up to date do not support simplistic view that such complexes are created only to achieve efficient catalysis through intermediate channeling. Indeed, as mentioned above, the catalytic efficiency is greatly increased and the channeling is indeed important and occurs in case of toxic intermediates, such as, e.g., ammoniac, which is channeled between the active sites of the synthetase complexes using this intermediate (Nagradova, 2003; Mouilleron & Golinelli-Pimpaneau, 2007). However, in many multienzyme complexes multiple active sites are randomly coupled to each other rather than channeling intermediates through the defined routes. It thus seems that - unless there is a need to protect from toxic intermediates - increasing the local concentration of intermediates in the enzymatic assemblies is preferred by the nature to the intermediate channeling. An obvious explanation would be that the most efficient catalysis employing channeling is unable to provide the equally efficient network coordination, because the channeling excludes the chemical information transfer between the channeled intermediate and the network. In contrast, the locally increased concentration of an intermediate could be a compromise between the needs to achieve both the efficient catalysis and network coordination. To demonstrate how this occurs, an example of the 2-oxo acid dehydrogenase multienzyme complexes may be considered. These complexes are formed around the core of a cubic or dodecahedral structure, which is built by 24 or 60 subunits of the dihydrolipoyl acyltransferase component of the complex, respectively (Izard et al., 1999). Like in other multienzyme complexes (Jenni et al., 2007), the microcompartment made by the complex core forms an internal cavity, connected with the surrounding medium by pores. In case of dodecahedral complexes the cavity diameter reaches 12 nm, and the pore size is 5 nm. The structure suggests that certain intermediates may be accumulated/compartimentalized in the cavity with the regulatory consequences. The core-forming component also has extensions outwards of the core, represented by the flexibly connected lipoyl-bearing domains of an extended structure (Fig.7). Dependent on the species, each subunit of the acyltransferase contains from one to three such domains which extrude from the core structure outwards. Thus, the 2-oxo acid dehydrogenase complexes are cellular microcompartments of the covalently bound lipoic acid. The number of the lipoic acid residues included in the complex varies between the different complexes (Perham, 2000). For instance, the cubic pyruvate dehydrogenase complex of *Escherichia coli* includes 72 molecules, with each of the 24 subunits of the core possessing the three lipoyl-bearing domains. The mammalian pyruvate dehydrogenase complex of dodecahedral structure contains more than 100 lipoate residues, dependent on the ratio of the isoforms of the acetyl transferase component including either two or one lipoate residues (Vijayakrishnan et al., 2010). As a result, increased local concentration of lipoic acid is available. However, the amount is excessive for the catalysis, as more than half of the lipoyl moieties of the dihydrolipoyl acyltransferase oligomer (Hackert et al., 1983; Danson et al., 1978; Collins J.H., Reed, 1978), or two of the three lipoyl-bearing domains of the acetyl transferase (Guest et al., 1990; 1997), may be removed without significant change in the overall activity of the complexes. In spite of no catalytic disadvantage, the physiological behaviour of such mutants is impaired. Indeed, bacteria with a decreased number of lipoyl domains in their pyruvate dehydrogenase complex have decreased growth rates and are washed-out from the mixed population of the mutant and wild type cells [Guest et al., 1997; Dave et al., 1995].

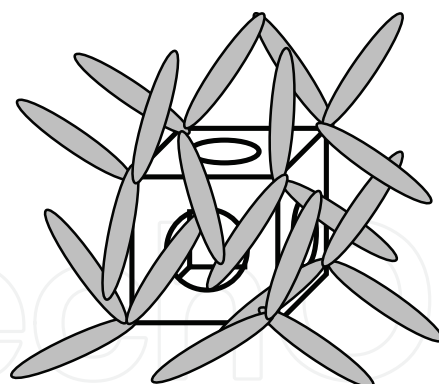


Fig. 7. Schematic presentation of the cubic core of a 2-oxo acid dehydrogenase complex (white) with the extruding lipoyl-bearing domains (grey). Catalytic domains of 24 subunits of the dihydrolipoyl acyltransferase forming the complex core are arranged in trimers at the eight cube corners. The lipoyl-bearing part of these 24 subunits shuttles between the active sites of the complex core and peripheral enzymes (not shown). This extended part may include up to three domains, with each carrying one lipoate residue.

Remarkably, the physiology is not perturbed, if the reduced number of lipoyl groups is not accompanied by the reduced length of the lipoyl-bearing protein molecule. That is, the mutant strain with the dihydrolipoyl acyltransferase bearing the three lipoyl domains with only the outermost one lipoylated behaved similar to the wild type under experimental conditions, although such a mutant complex catalyzes pyruvate oxidation 25% less efficiently than the complexes which are unable to provide the normal growth rates, i.e. those with dihydrolipoyl acyltransferase containing one or two fully lipoylated domains (Guest et al., 1997). Hence, the physiological advantage of the three lipoyl domains seems to depend on how far away the lipoyl group is able to protrude from the inner core rather than the catalytic requirements. This feature suggests that it is the interaction of the lipoyl residues with the surrounding medium beyond the peripheral enzymes of the complex, which provides the physiological advantage. Such interaction is experimentally supported by the thiol-disulfide exchange reactions between the complex-bound lipoate and cellular thiols (Bunik 2000; Applegate et al., 2008) and by other modifications of the complex-bound lipoate residues. For instance, both in vitro and in vivo the complex-bound lipoate is modified with 4-hydroxy-2-nonenal (Millar & Leaver, 2000; Humphries & Szwedda, 1998; Moreau et al., 2003). Furthermore, such interaction is obvious from the thioredoxin-dependent scavenging of the complex-bound thyl radicals of the dihydrolipoyl intermediate, discussed in the previous Section 3. Thus, apart serving the catalytic function, the complex-bound lipoate communicates with the surrounding medium, with the outcome of this communication significantly affecting the key reactions of energy metabolism, where lipoate participates as the cofactor. Another advantage of the high local concentration of the lipoate residues within the microcompartment created by the complexes (Fig.7) is the efficient and thioredoxin-dependent regulation of their superoxide anion radical production through the self-inactivation by the intrinsic thyl radical (Fig.5). Enabling the electron migration between the neighboring lipoate residues, the lipoate microcompartment stabilizes the thyl radical, thus promoting its interaction with the surrounding medium including the first component of the complex and/or thioredoxin.

Thus, the protein structure-based compartmentation may be used not only for the catalytic, but also for the network coordination purposes, which may explain the wide occurrence of this type of compartmentation in the living systems. For instance, up to 16 stable and most abundant large multiprotein complexes with the mass more than 400 000 Da were characterized in a soil bacterium *Desulfovibrio vulgaris* (Han et al., 2009). Remarkably, the authors of this work conclude that the subunit composition and quaternary structure of these complexes vary to a great extent between different organisms. This view is supported by other data on the multienzyme complexes studied. For instance, even the limited number of organisms from which the 2-oxo acid dehydrogenase complexes were isolated, showed significant structural differences, which do not correlate with the catalytic performance. The variations include the different architectures known for the complexes (Izard et al., 1999; De Kok et al., 1998), their different lipoate content and stoichiometries of catalytic components and different localization of the lipoate residues. Those may be incorporated not only in the established lipoate holder, the core-forming dihydrolipoyl acyltransferase component of the complexes, but also in the peripheral components (De Kok et al., 1998; De la Sierra et al., 1997). As discussed in Section 2.1.1, the catalytic selectivity of the complexes may differ between the species as well (Niebisch et al., 2006). For the fatty acid synthase systems the three different architectures are known, from the dissociated system of individual monofunctional proteins in *Escherichia coli*, through the heterododecamer  $\alpha_6\beta_6$  including multiple copies of the two multifunctional polypeptides in yeast to the dimer of the identical multifunctional polypeptides comprising all active sites plus acyl carrier protein in mammals (Maier et al., 2006; Jenni et al., 2007). Although evolution from the dissociated bacterial system comprising individual enzymes to the mammalian multifunctional dimer is clearly of catalytic importance, the difference between the fungal dodecamer and mammalian dimer, as well as the different structures known for the 2-oxo acid dehydrogenases are not interpretable from the catalytic viewpoint. In view of the role of the protein-based cellular microcompartments for the network coordination through the mechanisms discussed above, the absence of structural conservation in the multienzyme complexes is plausible to consider from the viewpoint of the species-specific significance of the microcompartmentation for the network coordination.

## 5. Conclusion

Already primary metabolic networks which consist of catalysts only, have all pre-requisites for participating in the network coordination. Independent of the network complexity, the network coordination mechanisms are based on the regulation of critical enzymes by the network intermediates of signalling importance. In addition, cellular networking widely uses benefits of the organellar and/or protein-based compartmentation, intracellular macromolecular crowding and in some cases comparable concentrations of the enzyme and its substrate. This allows for local subsets of the signal generation, transduction and response, which are different from those expected in a homogeneous medium. The coordinating function of catalysts may interfere with achievement of the highest catalytic efficiency and substrate specificity, because the network coordination requires the catalytic site to respond not only to its substrate, but also to the network regulator(s). A better compromise between the coordination and catalysis may be therefore found through development of specific regulatory binding sites and/or purely regulating catalysts, as observed in the networks of high complexity. The exact mechanisms of the coordination and

the critical enzymes themselves may differ, dependent on the network. Remarkably, different coordination mechanisms may be involved in the transduction of the same signal through different enzymes, and each enzyme may respond to a variety of signals, some of them already integers of several critical parameters of the network function. This feature underlies a high interconnectivity of the network coordination mechanisms. Functional outcome of the catalytic activity adjustment (enzyme activation or inhibition) is less obvious in a network, than in a single pathway, owing to which genetic manipulations of living organisms often do not lead to desired changes in metabolism. To efficiently regulate metabolic network, we thus need to increase our knowledge on the metabolic state indicators, the enzymatically generated signaling molecules and molecular mechanisms of their regulatory action on enzymes catalyzing metabolic reactions.

## 6. Acknowledgement

V.I.Bunik greatly acknowledges the long-standing support of her work within the program RUS-1003594 by the Alexander von Humboldt Foundation (Bonn, Germany) and current financial support by grants from Russian Foundation of Basic Research (09-04-90473 and 10-04-90007)

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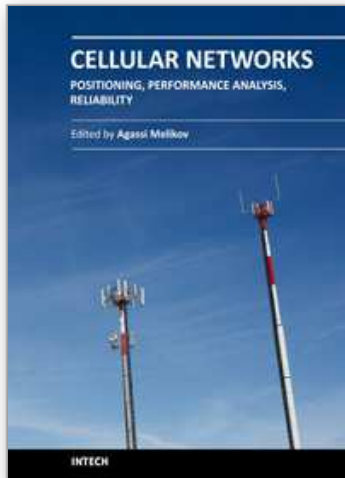
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## **Cellular Networks - Positioning, Performance Analysis, Reliability**

Edited by Dr. Agassi Melikov

ISBN 978-953-307-246-3

Hard cover, 404 pages

**Publisher** InTech

**Published online** 26, April, 2011

**Published in print edition** April, 2011

Wireless cellular networks are an integral part of modern telecommunication systems. Today it is hard to imagine our life without the use of such networks. Nevertheless, the development, implementation and operation of these networks require engineers and scientists to address a number of interrelated problems. Among them are the problem of choosing the proper geometric shape and dimensions of cells based on geographical location, finding the optimal location of cell base station, selection the scheme dividing the total net bandwidth between its cells, organization of the handover of a call between cells, information security and network reliability, and many others. The book focuses on three types of problems from the above list - Positioning, Performance Analysis and Reliability. It contains three sections. The Section 1 is devoted to problems of Positioning and contains five chapters. The Section 2 contains eight Chapters which are devoted to quality of service (QoS) metrics analysis of wireless cellular networks. The Section 3 contains two Chapters and deal with reliability issues of wireless cellular networks. The book will be useful to researches in academia and industry and also to post-graduate students in telecommunication specialitiies.

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Victoria Bunik (2011). Metabolic Networking through Enzymatic Sensing, Signaling and Response to Homeostatic Fluctuations, Cellular Networks - Positioning, Performance Analysis, Reliability, Dr. Agassi Melikov (Ed.), ISBN: 978-953-307-246-3, InTech, Available from: <http://www.intechopen.com/books/cellular-networks-positioning-performance-analysis-reliability/metabolic-networking-through-enzymatic-sensing-signaling-and-response-to-homeostatic-fluctuations>

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