INTERMOLECULAR INTERACTION OF PROTEINS AND SMALL MOLECULES

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This review presents the modern data on biomolecules involved in the formation of internal structures of cell, organ and organism as a whole. Almost every process occurring in the cell involves proteins and shows multifunctionality due to their ability to change the conformation of molecules in the interaction with ligands. Intermolecular interactions are crucial virtually in all fundamental biological processes, such as cellular regulation, pathways of biosynthesis and degradation, signal transmission, initiation of DNA replication, transcription and translation, the formation of oligomers and multimolecular complexes, the packing of the virus and the immune response. These interactions form the basis of any interactome of a living cell. Low-molecules act as ligands and are located in the cytoplasm of cells in the free state and form a pool of intermediate metabolites. Many of them are precursors of macromolecular synthesis and can regulate the activity of individual enzymes and entire enzyme cascades.

Keywords: small molecules, metabolites, intermolecular interaction, interactome

Millions of different molecules participate in the internal structures of cell building, as well as organs and an organism as a whole. Each molecule contains a characteristic set of chemical elements, has a specific structure and is capable of selective and specific binding with other molecules. Many biological molecules represent macromolecules, i.e. polymers with a molecular weight more than 5000. Thousands of different small and medium organic molecules that have a molecular weight from 50 to 500 dissolved in the cytoplasm of cells. All these molecules are water-soluble, polar or charged. A single set of small molecules in living cells is a manifestation of the universality and the evolutionary conservatism of metabolic processes [1].

Intermolecular interactions play a significant role in the implementation of vital cell functions, the organ and the organism as a whole. Universal carriers of interactions in living systems are biological molecules having different chemical structure and molecular weight. Protein-protein interactions are crucial in virtually all fundamental biological processes, such as cellular regulation, pathways of biosynthesis and degradation, signal transmission, initiation of DNA replication, transcription and translation, the formation of oligomers and multi molecular complexes, the packing of the virus and the immune response.

A natural process of metabolism is the coordinated activity of cells, in which the combination of all chemical reactions catalyzed by enzymes. Metabolism provides with many enzymatic reactions. It is known that the sequence of enzymatic reactions is called a metabolic pathway. Many metabolic pathways in the cell work simultaneously. In each reaction, which is also called the stage of the metabolic pathway, the specific change in the chemical composition is taken place. As a rule, these reactions consist of moving, adding or removing one atom or functional group; at the end of the reaction the intermediate products are formed, which are called metabolites. A central metabolic pathway performs the synthesis, breakdown or interconversion of the most important metabolites, and the accumulation of energy that allows us to grow, reproduce, maintain structures and respond to the environment. These metabolic pathways are remarkably similar for all living forms [2]. The understanding of these processes was the basis of metabolic maps creation. Metabolic maps allow us to get a full understanding of the specific metabolic pathway formed of intermediates and end products, the enzymes catalyzing the biochemical reactions of a given path, and can also serve as a reference to determine the location of known molecules in metabolism [3]. There is a huge number of works devoted to the study of the interaction of molecules with each other, in order to reflect all the interaction of the metabolome of a given organism [4].

The traditional approach to the compilation of the metabolic maps suggests the compartmentalization of metabolism in accordance with the main classes of compounds – metabolism of carbohydrates, metabolism of amino acids and proteins, lipid metabolism, nucleic acids metabolism, etc. In 2004, A.G. Malygin proposed a non-trivial approach to making the metabolic maps based on the symmetry found in the structure of the network of reactions in metabolism [5]. The number of carbon atoms and the number of COOH-groups in the skeleton of the chemical compound are displayed

in a two-dimensional coordinate system. All metabolites and their reactions are treated as full-fledged participants in the metabolism. Metabolic maps can be used as a key to databases associated with metabolic information, can help understand the metabolic processes in the conditions of norm and pathology, to identify reactions, inhibited or activated by various modulators. Currently, the main metabolites are already known; the list of intermediate of low molecular weight compounds and signaling molecules regulating metabolic fluxes is being updated actively.

Almost every process occurring in the cell, involves proteins, showing the inexhaustible variety of functions. Proteins are multi-functional due to their ability to change the conformation of molecules in the interaction with ligands. Proteins can interact with almost all types of molecules, from small organic compounds, metals, sugars, fatty acids, phospholipids of cell membranes to macromolecular proteins and nucleic acids [6]. The systematic analysis of binary interactions of proteins was begun in the1990s; it showed us some complex interactions in large macromolecular protein complexes such as DNA polymerase, and a simple interaction, for example, in complex enzyme-inhibitor, enzyme-substrate [7]. Protein-ligand interactions can be classified as fixed or stable, for example, cytochrome oxidase forming protein aggregates of the 13 proteins or ATP-synthase is temporary or reversible that is a characteristic of most proteins involved in biochemical cascades [8]. Numerous studies of protein-ligand interactions allowed us to identify hundreds of thousands contacts, information about which was collected in specialized databases, which can be divided into two groups. The first group includes the primary database, where the existence of protein-ligand interactions was established experimentally. For example, Biomolecular Interaction Network Database (BIND), Biological General Repository for Interaction Datasets (BioGRID), Human Protein Reference Database (HPRD), IntAct Molecular Interaction Database, Molecular Interactions Database (MINT) (Ceol, 2009). The second group includes databases of forecasting, which consists of mainly predicted protein-ligand interaction obtained with the use of computer and computer-based methods; they are sometimes supplemented by experimental data. For example: Online Predicted Human Interaction Database (OPHID), Known and Predicted Protein-Protein Interactions (STRING) and Unified Human Interactome (UniHI). In the UniHI database contains information about 350.000 molecular interactions of more than 30,000 human proteins [9]. For better understanding the interactions of proteins with ligands, you can use software visualization in three dimensions, such as RasMol, Jmol and Protein Explorer First Glance.

The main processes in living cells are largely controlled by macromolecular interactions and among them protein-protein interactions play a crucial role. Violation of interactions between proteins is the basis of many diseases [10, 11]. Many key cell functions such as DNA replication, signal transmission, immune response are regulated by the complexation of proteins. Functioning, activity and specificity of such complexes depend on the nature of protein-protein interactions. In addition, in the genomic era, the study of protein networks provides an insight look into molecular evolution, the reaction of cells to external and internal stimules and the elucidation of protein functions [12]. The combination of protein-protein interactions for the given organism is called interactome. The term "interactome" was proposed by a group of French scientists headed by B. Jacques in 1999 [13]. It is established that the size of the interactome of S. Cerevisiae, varied from 10–17 thousands to 25–35 thousands protein-protein interactions according to the different studies [14]. It is assumed that the size of the human interactome can be formed about 650 thousands of protein interactions [15]. About 39 thousand protein interactions were recognized in human cells [16]. Thus, the number of interacting pairs of human proteins is 10 times more than Drosophila melanogaster.

These data help us to make the assumption that the size of the interactome depends on the level of complexity of the organism. Determination of interacting pairs of proteins was a step to make an interactome card. Such maps are graphs consisting of nodes showing the binary contacts. Creation of maps are useful for understanding the functioning of proteins to determine the role and interaction of individual proteins in causing pathological conditions of an organism, their diagnostics, and also possible targets for the action of different modulators [17, 18]. Currently, a large number of studies devoted to the analysis and clarification of data interaction of proteins, using various experimental and computer methods are already available. Thus, for example, according to Janin et.al. (2008) detailed structural analysis of the sites responsible for the interaction between proteins (interface) at constant heterodimeric complexes shows in

average 204 atoms belonging to 57 amino acids (mainly glycine, asparagine and lysine, to a lesser extent, methionine, phenylalanine, tyrosine and tryptophan) [19].

In homodimer protein interface there are twice or more hydrophobic because it contains more aromatic and aliphatic amino acid residues. Analysis of the interfaces may be conducted in the group of proteins involved in specific disease. It was found that the interfaces of protein-protein complexes in cancer were smaller and more densely packed [20]. It is important to estimate the number of possible types of protein interaction which is, according to some sources, about 4000 [21]. In the literature, there are data on similar interfaces and templates in interactome's interaction [22]. Some researchers try to define classes of protein-protein interactions to connect low-molecular ligands easily. Here we wonder if small molecules can contact multiple protein complexes. It is assumed, that the binding site of the protein with low molecular weight ligands must be small and narrow [23]. The most important target among protein molecules – enzymes, receptors and ion channels, usually have a concave binding site with small molecule as a pocket [22]. Some researchers noted the marked differences between the interfaces that determine the nature of protein-protein interactions and protein-ligand interactions. The binding sites with small ligands typically have from 3 to 5 amino acid residues and are found in proteins that bind to components on the surface of the cell membrane [25]. Such binding sites are called the "anchor" [26]. Separate interfaces can dynamically adapt to the upcoming sequestration and have transition states that may occur the binding with the ligand [27].

Based on the literature information, we would like to mention the necessity of studying the nature of interaction between small molecules and large biomolecules or their complexes, accompanied by a conformational change of the latest. The ability to enter into parameterizes interaction defines a wide range of biological effects of small molecules, low molecular weight intermediates, the specific mechanisms which require detailed study [28].

The number of studies of interactions of protein – small molecule (metabolite) increased significantly. However, the study of these interactions according to Wiley Online Library in 2011, the number of publications lags far behind research into other types of interactions [29].

From the biochemical point of view, the majority of biological systems work through the implementation of their proteins of diverse functions. Due to the revolutionary progress in the study of genomics and proteomics a more accurate idea of the amount of proteins synthesized in the body has developed currently, but there is little understanding about how much of the metabolites are formed in the body and what kind of proteins interact with them. To decipher the mechanisms of protein binding and various types of molecules metabolic networks of molecular interactions were constructed. As a model organism, the yeast is very convenient for constructing a metabolic network having a simple genome and evolutionary preserved the basic biological mechanisms [30]. It should be noted, that the use of model organisms based on the fact that all living organisms have a common origin and retain key mechanisms of storage and realization of genetic information. The number of protein-ligand interactions in yeast cells, according to the project, Model Organism ENCyclopedia Of DNA Elements (modENCODE Consortium, 2011) can range from 3,5 to 43,7 million, it is the sum of the maximum theoretical protein-DNA, protein-RNA, protein-protein and protein-small molecule (metabolite) interactions involved in various biological processes. Information was obtained from the database of a yeast genome and databases of metabolites [31]. We can assume, that much more is theoretically possible protein-ligand interactions in higher organisms with more complex structure. Given that the second largest group is theoretically possible protein-small molecule interactions, it becomes clear the relative scarcity of relevant studies.

Metabolic pipeline contains compounds having a wide range of types and targets of influence, depending on the needs of the cell and the external environment. Special role is played by molecules with a molecular weight of from 100 to 1000. Low-molecular compounds are found in the cytoplasm of the cell in the free state and form a pool of intermediate metabolites. Many of them are precursors for macromolecular synthesis and can regulate the activity of certain enzymes and enzymatic cascades. Small molecules are very dynamic, computer databases contain now information about more than 90,000 known interactions of small molecules [32]. This diversity is determined by the ability of small molecules quickly and easily diffuse through the cell membrane. Currently, significant research efforts directed to the discovery of small and middle molecules, that specifically bind to specific proteins or proteins that have

distinctive functional properties, which determine the cellular phenotype [33].

In the literature, considerable attention is paid to proteins responsible for transport of endogenous and exogenous metabolites. For example, human serum albumin due to the numerous binding sites and conformational lability, has a large number of ligands [34]. Studying the interactions of albumin with endogenous metabolites, it was found that fatty acids significantly inhibit the binding of the albumin-metabolite, which is required for its operation. Fatty stearic and myristic acids have the greatest influence on the binding of albumin lactate, significantly reduce binding to phenylalanine and pyruvate, and do not affect the binding with citrate [35]. For most metabolites, competition for binding to albumin is absent due to the large number of binding sites [36]. In another experiment using the method of mass spectrometry integrated with equilibrium dialysis (MIDAS), it was determined that palmitic acid binds with glucokinase and glycogen phosphorilase, suppressing the activity of both enzymes, thereby providing a mechanism of reservation of carbohydrates in the body [37].

Metabolic pathways are extremely dynamic and intertwined, which is convenient for their regulation [38]. Endogenous metabolites are the numerical majority of the cellular molecules and protein-metabolite interactions is a widespread phenomenon in the cell [39]. Metabolites can act as not only substrates and products of enzymatic reactions but also serve as regulators of signaling pathways and modulators functioning [40]. Medium and small-molecule metabolites act quickly and their contact is reversible, which allows a high degree of modulating the function of biomolecules [41]. This is the classic example of such regulation in bacteria: lactose binds with the protein-repressor stop the transcription process and the modulation of the activity of pyruvate kinase with different concentrations of the metabolites, ribose-5-phosphate, glucose-6-phosphate, AMP, ATP [42]. The tricarboxylic acid cycle is the central intersection of many metabolic pathways in the body, an important source of precursor molecules, from which in the course of other biochemical reactions that synthesize these important metabolic compounds such as amino acids, carbohydrates, fatty acids. The intermediate metabolite TCA, α – Ketoglutarate identifies as a new ligand-binding β-subunit of ATP synthase, also known as complex V of the mitochondrial chain of electron transfer. The respiratory chain is highly conservative, generating energy in the cell. In the experiments of M. Randall et.al. (2014) found that α-Ketoglutarate inhibit the activity of ATP-synthase, which leads to a reduction of ATP content [43]. Partial suppression of the activity of ATP synthase increases the lifespan of Caenorhabditis elegans [44]. This inhibition is also easy to detect in living mammalian cells, for example, α-Ketoglutarate inhibits the activity of ATP synthase of mitochondria bovine heart. Physiological increase in the level of metabolite was registered in yeast, bacteria and birds during fasting, and a man – after exercise [45, 46]. It can be assumed that the biochemical basis of the increase in the level of α-Ketoglutarate is the activation of glucose synthesis in the gluconeogenesis process. The identification of a new protein target for α-Ketoglutarate, showed that key metabolites is mediated by regulating cell energy metabolism and metabolic networks is arranged in a much more difficult way. Moderation of ATP synthesis probably is an evolutionary mechanism to ensure the efficiency of the body in response to the presence or absence of nutrients.

Central part of the regulation of metabolism of carbohydrates is pyruvic acid because it is the end product of glycolysis and the main substrate of the citric acid cycle in the mitochondria. It has been suggested that diffusion of pyruvic acid across the mitochondrial membrane is the transport-mediated [47]. In studies of D.K. Bricker et al. in 2012 identified two protein mitochondrial pyruvate carrier 1 and mitochondrial pyruvate carrier 2, required for the transport of pyruvate into mitochondria of yeast, Drosophila and mammals. Proteins function as a single heterodimer complex (150 Mm Kd) in the inner membrane of mitochondria [48]. Inhibitoring effect on the transport function of mitochondrial pyruvate carriers α-cyanocinnamate – derived acetic acid shows. Identification of the mitochondrial pyruvate carrier, understanding the mechanism of regulation of their functions, provides the basis for understanding a new level of metabolic control of catabolic and anabolic processes, the key point of which is pyruvate [49, 50].

We can notice a growing number of studies dedicated to the problem of understanding the protein-protein interactions. Many of the reported interactions were “unexpected” and had a profound influence on the understanding of cell signaling. Now the molecular mechanisms of action and biochemical targets of low weight molecules as well as their physiological effects are on the foreground of the scientific interest.

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