PERSPECTIVE

Politics of a cell

Saubhik Sengupta

Abstract

The world is divided into countries, countries into states, states into districts, and districts into administrative blocks. The governance and success of a country also is defined by global affairs, the functioning of its neighbours, the inputs it receives from outside and how it accommodates and adjusts accordingly. Similarly, if we consider the human body as a country, it is constantly in exposure to the environment and its changes, and is built up of systems that are developed by organs and organs are subdivided into tissues and tissues are made up of cells. This complex degree of organization gives rise to the physiology and/or pathology or an individual.

Keywords: Gravitation, Interactome, Molecular interaction, Molecular pathway, System biology.

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Introduction

The world is divided into countries, countries into states, states into districts, and districts into administrative blocks. A country's governance and success are also defined by global affairs, the functioning of its neighbors, the inputs it receives from outside and how it accommodates and adjusts accordingly. Similarly, suppose we consider the human body as a country. In that case, it is constantly exposed to the environment and its changes and is built up of systems developed by organs and organs subdivided into tissues and tissues made up of cells. This complex degree of organization gives rise to an individual's physiology and/or pathology.

However, even cells are incredibly complex as individual units, and their cumulative behavior gives rise to organ phenotype. A cell has the primal ability to survive, protect itself from toxic chemicals or radiations, attach or adhere, migrate, invade other structures, and even undergo programmed death. A cell is made up of different molecules, viz., DNA, RNA, proteins, lipids, carbohydrates and minerals and a great organization of all these molecules contributes to its behavior in its lifetime. Based on these molecules, different omic studies have come up, viz., proteomics, lipidomics, and metabolomics, all of which contribute to making the cellular interactome.

This article is not a one-off article. Nor is it an article in the true sense. This article can be considered a conference of all scientists who are journeying to construct cellular behavior and functions and analyze associated pathways. Let us call this conference "The construction of a cell". Any structural biologist working with crystallography in analyzing a protein structure knows that a complete construction of a protein is not always possible. Therefore, once a crystal is generated, layers of solving the structure gradually complete the entire structure of the protein. Similarly, in this conference, we do not expect to construct a hypothetical cell in one go. Rather, this will be a continuous journey with contributions from all of you.

Why do we even need to make a cell? Construction of the possibilities of molecular workings in a cell can help us determine therapeutic strategies for many diseases, even by repurposing drugs. Understanding signaling pathways over the decades has generated newer drugs, more so after the publication of the human genome project in 2001.

Historically, the study of cell was done by biologists after the first cell was visualized under the microscope. Today, in addition to biologists, we have contributions from biotechnologists, engineers, mathematicians, physicists, and software professionals with knowledge of programming languages all contribute to the construction of a cell. Unfortunately, none of us speaks each other's language. While mathematicians have studied interactomes and have devised strategies to identify signaling pathway strengths using eigenvectors, biologists hardly have the training to understand it and apply it in their practice. Therefore, this will be the platform where all of us would be able to interact, understand each other's language and contribute to the construction of a platform that would lead the development of the first hypothetical cell.

From our student days, we have been taught about cell membranes, golgi bodies, endosomes, exosomes, lysosomes, mitochondria, nucleus, nucleoli and cytoplasm as different parts of a cell. Let us start thinking about them as different organizational structures comprised of different molecules that interact with each other and other molecules in the vicinity. Once the cell is constructed, we will be able to predict how outside influences like chemicals in tissue microenvironments contribute to the phenotypes of the cell. Thus, we will try to reach a condition of predictability, thereby helping mankind to improve the treatment of different diseases and helping convert pathology to physiology.

Just 100 years ago, ayurveda and western medical practitioners

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trusted their feelings through tactile, sound and other senses to diagnose and treat disease. Today, we are in the generation of personalized and evidence-based medicine, where in some cases, we can tailor a treatment based on the individual's physiology. Medical practice is evolving rapidly, and over the last couple of decades, the concepts of conversion from a pathological state to a physiological state have changed a lot. The construction of a cell, followed-up tissue and organ will catapult the journey to the new age of medicine.

In the subsequent sections, let us discuss the gaps in knowledge we encounter.

The First Cell of the Human Body

The first cell that we encounter is the zygote formed from a sperm and an ovum that has the possibility to create a whole human system. Once a zygote is formed, it divides into the morula and blastula stages. At this point, cells start the process of differentiation. The question is, what signals are required for the cell to divide? What molecular reactions trigger the process of mitosis? How does the process of differentiation start leading to the development of an entire human body? And why are molecular mechanisms disturbed at certain times, leading to diseases? All these questions can be answered if we can identify all the possible molecular mechanisms in a cell, construct a hypothetical cell, and understand the physical and chemical interactions that take place so that the hypothetical cell can be reconstructed to understand a disease.

Molecular Interactions in a Cell

Gravitation

The first force that the molecules will experience in a cell is gravitational pull. The possible effects of the earth's gravitational field on biological systems have been studied from a quantitative point of view, focusing attention to a very simple system, a solution containing proteins, which biochemists might use in experiments. Gravity has been compared with other forces which are known to influence protein activity, including thermic agitation, weak electrostatic interactions, van der Waals forces and viscous dissipation. Comparisons have been described in terms of the energy of the interaction per mole, referring to some physically simple cases and substances of biological interest. This study shows that the earth's gravitational energy should be considered when considering the chemical behaviour of solutions containing substances with high molecular weight, such as a typical protein since its value is comparable to other weak interactions. Moreover, since solutions represent the basis of much more complex biological processes taking place inside cells, the influence of gravity should extend also to cellular biochemical behavior, especially in the presence of altered gravity, both in microgravity (such as on satellites orbiting around the earth), and in macrogravity (such as in a centrifuging biological system).[1]

For example, EGF-induced signal transmission from the plasma membrane to the nucleus has been studied in microgravity to understand the molecular mechanisms that effects of gravity on the growth of cells. Exposure of human A431 cells to microgravity strongly suppresses EGF- and PMA-induced c-fos and c-jun expression. In contrast, forskolin- and A23187-induced c-fos expression and constitutive beta-2 microglobulin expression remain unaffected. This suggests that microgravity differentially modulates EGF-induced signal transduction pathways. Since both EGF and PMA are known to be activators of PKC, which is not the case for forskolin and A23187, PKC-mediated signal transduction may be a cellular target for microgravity. Inhibition of EGF-induced c-fos expression by microgravity occurs downstream of the initiation of EGF-induced signal transduction, i.e., EGF binding and EGFR redistribution. In addition to PKC signaling, actin microfilament organization appears to be sensitive to microgravity. Therefore, microgravity's inhibition of signal transduction may be related to alterations in actin microfilament organization. The fact that early gene expression is affected by agents that alter the organization of the actin microfilament system supports this hypothesis. The decrease in c-fos and c-jun expression in microgravity may result in the decreased formation of the FOS and JUN proteins. Consequently, a short-term reduction in gene expression in microgravity may have a more dramatic effect over the long term since both the JUN and FOS protein families are required for normal cell cycle progression.[2]

Cellular response to mechanical loading has been well documented over the decades; however, the response that occurs when cells are placed under conditions of mechanical unloading remains in its infancy. The most apparent cellular changes that occur following exposure to a microgravity environment are alterations to cell shape, size, volume, and adherence properties.[3,4] Therefore, if we indicate any molecular interaction in a cell as Mi, then we can safely say that the interaction will be inversely proportional to the gravitational pull, no matter how small the amount is. We can write this as:

$$Mi \propto 1/G \ldots\ldots\ldots\ldots\ldots\ldots \text{(Equation 1)}$$

where G is the gravitational pull. For all practical purposes, we can keep this value as constant unless the cell is exposed to very low gravity or high gravitational pulls.

Viscosity or Density of a Solution Where a Molecular Interaction is Happening

It is important to understand that the density of a solution is important for a protein to float around in a system, viz., cytoplasm, plasma membrane, nucleus, or other cellular bodies. It is also important to understand the drag force of a molecule in a cell through the mesh-like actin structure that keeps the cell in shape. In simple terms, we can think of a fishing net where there are several fishes (Figure 1). Let us imagine that the fishes in the net are molecules and the net is a mesh of actin filaments that are holding the cell. While the fishes are very close to each other, they will not be able to come in contact with another fish unless they are smaller

Figure 1: Fish in a fishing net

in size than the mesh or the mesh opens up, allowing the proteins to interact. Therefore, we can imagine that any molecular interaction or Mi will be inversely dependent viscosity of the area and the mesh size of the actin gel in the cell. We can write this as

$$Mi \propto 1/V \quad \text{(Equation 2)}$$

$$\text{And } Mi \propto 1/Z \quad \text{(Equation 3)}$$

Where V is the viscosity where the reaction is happening, and Z is the mesh pore size. As temperature would decrease the viscosity and the mesh pore size the reaction will also depend on the temperature of the environment where the reaction is happening in the cell. However, the temperature factor will be taken care of in the viscosity equation.

Molecule to Molecule Interactive Forces

Structure-derived statistical potentials have been widely applied not only in protein structure prediction and design but also in protein complexes studies, such as protein–ligand affinity prediction (the ligand can be protein, peptide, DNA, RNA, or other molecules), mutation-induced changes in protein stability, and rational drug design.[5,6] In those approaches, the potential is extracted by statistically analyzing known three-dimensional structure data of biomolecules. Therefore, they were also termed knowledge-based potentials. One kind of them, potential of mean force (PMF), is derived from the statistical mechanics of simple liquids, which converts particle pair distribution of distance into distance-dependent potential function. PMF has been frequently used in affinity prediction and structure scoring because its physical meaning and function curve are similar to those of the "true" energy potential, which in principle can be derived from fundamental analysis of the forces between particles, such as quantum chemical calculations. Therefore, PMF was also called as energy-like potential or quantity.

Volume correction must be considered when PMF is applied in protein systems. It is one of the key factors that can improve the precision of prediction and the reasonableness of potential function. Since PMF was introduced into the studies of protein systems, the understanding and the application of volume correction (or frequency correction) have undergone a series of development.

Therefore, it is now known that while calculating molecule to molecule interactions (Mi), PMF is an important force that needs to be taken into account. The calculation of PMF can bypass the use of Lennard Jones statistics[7] as well as the Stokes –Sutherland – Einstein statistics. Therefore, as an equation, we can write

$$Mi \propto PMF \quad \text{(Equation 4)}$$

Where PMF is the potential mean force of molecule to molecule interactions.

Based on all four equations we can write

$$Mi \propto [PMF] / [G \times V \times Z] \quad \text{(Equation 5)}$$

Where Mi is Molecular Interaction, PMF is the potential mean force experienced between atoms where they are interacting, G is gravity imparted on the molecule, V is the viscosity of the area where the reaction is happening and Z is the hydrodynamic drag imparted by the pores of the mesh.

This equation is in stark contrast to the old age equations of the Michaelis – Menten equation and the subsequent calculation of Vmax as the Michaelis – Menten equation do not take into consideration all the different forces that are imparted on a molecule during its process of actual interaction.

Why is this Calculation Important?

For the longest time, biochemists have appreciated that the speed of the reaction is important for a molecular reaction to occur in a cell. The importance is greatly aggravated because in a particular cell, thousands of molecules are present simultaneously and the probability of a reaction happening will depend on the above-discussed parameters. Thermodynamically speaking, whichever reaction will spend the least energy and will have the fastest reaction time. A human genome houses around 30000 genes that give rise to proteins and an additional 50000 genes that give rise to different types of RNAs like LncRNA (long non-coding RNAs) or SncRNA (small non-coding RNAs) that gives a cell total control of its molecular activities.

Once these calculations are in place, we will be at a position to predict the pathways that will activate a signaling cascade.

Formation of a Protein in a Cell

While several mathematicians and bioinformatics specialties are working on the mathematical equations above, the role of biologists is significantly low. This is because we as biologists have been trained to understand reactions in a cell based on experiments that do not tell the whole story. For example, when a protein is produced in the cell after activation of the specific gene, the protein undergoes several steps of post-translational modifications and several other interactions to reach the area of the cell where it is supposed to work.

Cell Signaling – A Personal Experience

After years of hard work on cell signaling mechanisms in ovarian cancer, we demonstrated that 18:1 lysophosphatidic acid (LPA) is up-regulated in both blood and peritoneal

ascites of women suffering from the disease and successfully demonstrated that LPA is released from the ovarian cancer cells once it sits on laminin 10/11 of the extracellular matrix protein complex in the peritoneal mesentery (Figure 2 and 3).[8] This released LPA binds to LPA receptors on ovarian cancer cells in an autocrine fashion. Once this is accomplished, the LPA receptors starts sending signals to cells to survive and migrate by activating PI3Kinase through the G proteins in the cells. In the Akt pathway that leads to cell survival, p38 MAP kinase plays an important role and TIMPs control the cPLA2 pathway. Actual experimentations achieved these works by inhibiting the intracellular proteins with small molecule inhibitors, siRNAs and several functional assays. Baudhuin et al demonstrated in her PhD thesis that the pathway from LPA to Akt can be written as LPAR – Gi – PI3Kinase – MEK – Erk – MKK6 – p38 MAP kinase to PDK2 – Akt.[9]

In today's world, if we put all these molecules in www.string-db.org and set the interactors level at 50, then we get something like this, as shown in Figure 4. This means that now we have a plethora of information that is validated by scientists all over the world and instead of fishing around for experimental evidence, we can easily dig deep into the experiences of different scientists and collate their work to get a network.

The signaling pathways we see in Figure 4 are actually probabilities of reactions happening in a cell at the moment. The bigger question is, are all the reactions demonstrated are correct? Are the directions of the reactions correct? Are there no other intermediate molecules in each of these reactions? These reactions can only therefore be dealt as probabilities and unless the calculations from each of the interactions, as discussed in the previous sections, are performed, there can be little surety.

Whenever we create an interactome like this, we come across thousands and thousands of pathways in all possible combinations and permutations. Signor database has listed a few thousand reactions to absolute certainty. Looking at these reactions, can we identify the critical pathways going on in a cell? To achieve this, we will need to calculate the strengths of the pathways and identify the strongest pathways that are happening in a cell. This is where mathematicians have come up with the concept of centralities.

Centralities of a Network in a Cell

CentiScaPe is generally used in open-source platforms like Cytoscape to calculate network centralities. The main usage of CentiScaPe is to rank the nodes of a network depending on their topological and experimental relevance. The numerical results are saved as node, edge or network attributes in the Cytoscape attributes browser, depending on the kind of parameters, so all the Cytoscape features for managing attributes are supported; after the computation the centralities are treated as normal Cytoscape attributes. CentiScaPe can be used in undirected, directed, and weighted networks.[10] Herein, important terms like 1. Closeness, 2. Betweenness, 3. Eigen Vector, 4. Radiality are calculated. Each has a mathematical meaning as well as a biological meaning. However, using these parameters to understand which pathways are sitting at the network's core can be hugely oversimplified.

Calculation of Pathway Strengths

Cell signaling pathways are sets of directed interactions between biological molecules initiated by a particular signal (e.g. a ligand binding to a receptor) and result in the realization of certain target processes (*e.g.* transcription of genes). Pathways can be represented as graphs, with nodes as biological entities (proteins, other biomolecules, chemical compounds, other pathways), and edges as physical or

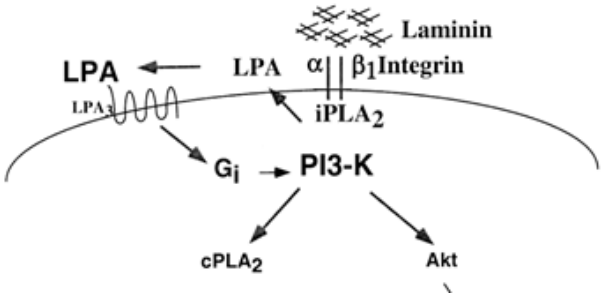

Figure 2: Lysophosphatidic acid is produced by cancer cells due to interactions with extracellular matrix proteins.[18]

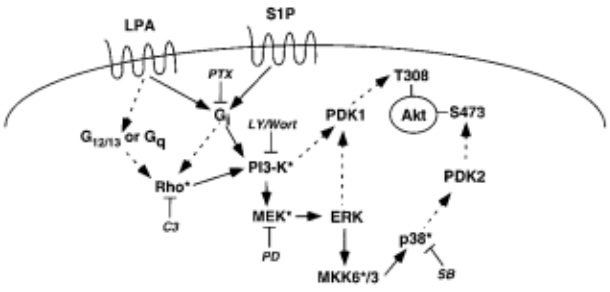

Figure 3: Pathways leading from LPA receptors to cell survival.

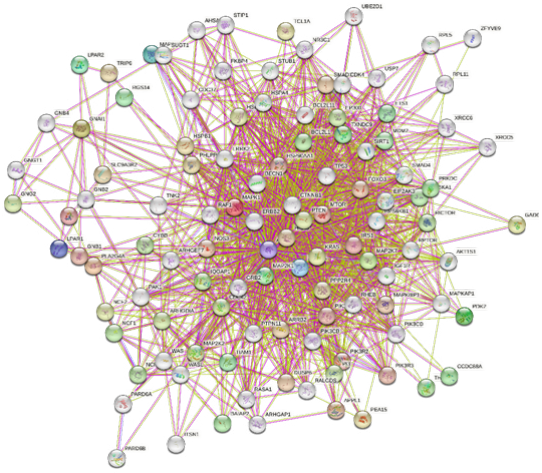

Figure 4: Interactome created in string database.

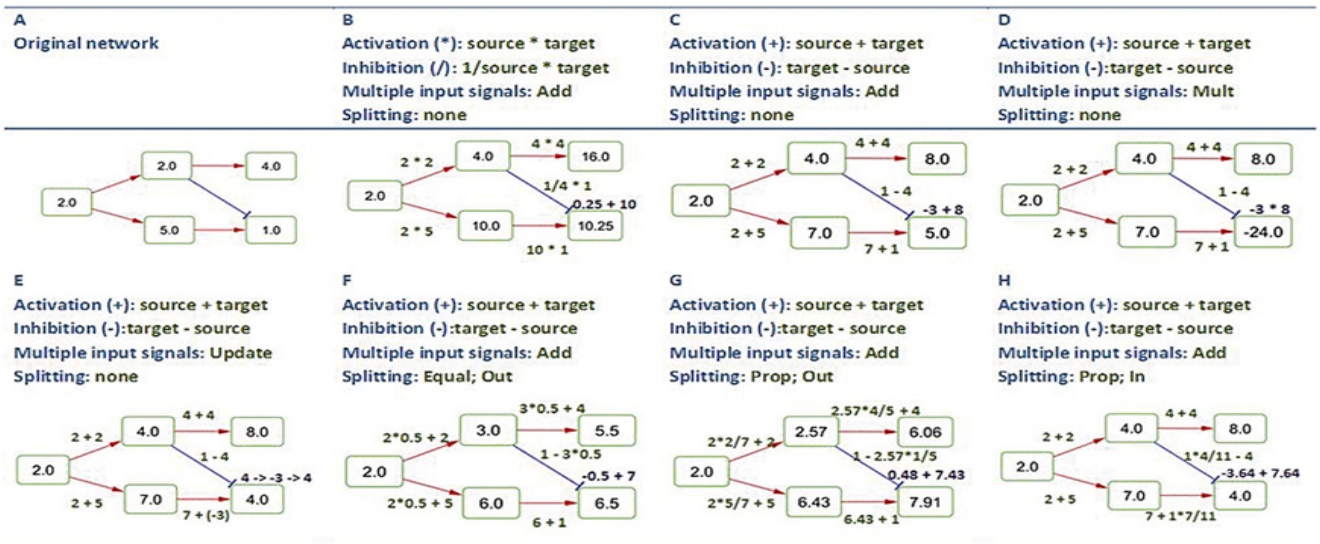

Figure 5: Demonstration of PSF computation on a sample network with different flow propagation rules. Figure copied from Nersisyan L, Johnson G, Riel-Mehan M *et al.* PSFC: a Pathway Signal Flow Calculator App for Cytoscape [version 2; peer review: 2 approved]. F1000Research 2017, 4:480.

regulatory interactions between them. In contrast to protein-protein interaction networks, biomolecular pathways have directionality, input nodes, intermediate nodes and branches, and output or sink nodes.

Pathway Signal Flow (PSF), or perturbation, is the flux generated by the propagation of the signal starting from input nodes, flowing through intermediate nodes in branches and accumulating at sink nodes. Thus, PSF can be an indicator of the pathway activity state. Assessment of changes in pathway activity is of major interest for identifying processes involved in forming certain phenotypes (healthy and diseased states), and assessing cell response to drugs and other stimuli. First attempts to globally evaluate the pathway activity changes were performed in parallel with the appearance of high-throughput gene expression measurement experiments. Pathway involvement is typically analyzed by over-representation analysis (ORA)[11] or gene set enrichment analysis (GSEA).[12] The major drawback of these widely used approaches is that they operate on gene sets involved in the pathway but do not account for the pathway topology and ignore the interactions between the nodes.

A number of techniques and tools have recently emerged, aimed at determining pathway activities based on topological information of pathways and gene expression/protein activity levels. One of the pioneering papers in this direction was the Pathway Impact Analysis algorithm, which combines GSEA with gene position in the network.[13] Other approaches apply specific rules to model flow or signal propagation through the pathway and evaluate the amount of the signal reaching the sink nodes.[14-16]

Figure 5 demonstrates how the pathways can be calculated using the PSFC algorithm. Nodes are the proteins or can be the molecules interacting in the cell.

While trying to calculate pathway strength in our laboratory, we identified every individual protein as Node (N). We rationalized that the Node strength (strength of the source protein contributing or interacting with the target protein defined as NS) need to be identified to calculate pathway strength. Therefore, NS will vary according to the expression of the source node (E). NS will vary inversely to the number of output paths (O). In biological terms this means the probability of a node or protein sending signals to different target proteins. This can be written as

$$NS \propto E \text{ and } NS \propto \frac{1}{O} \text{ or } NS \propto \frac{E}{O} \text{ or } NS = k.\frac{E}{O} \quad \text{.....(Equation 6)}$$

Where k is the constant.

Path length is denoted as PL and Path Strength (PS) can be calculated as the sum of Node strengths divided by path length.

Path strength can then be denoted as $\Sigma\frac{NS}{PL}$ ….. (Equation 7)

Using these approaches, we have demonstrated that a drug used in hematological oncology can be used for treating carboplatin treatment failure solid tumor oncology (paper under review). We have started clinical trials to prove this point.

Conclusions

We have discussed in detail that if we succeed in creating a virtual cell with all the molecular possibilities happening in the cell, we would be able to then able to reconstruct a disease state. Once a cell is constructed, we can start constructing tissues and, ultimately, an entire organism and plan how to convert a pathological state to a physiological state. Let us join forces from all science disciplines, including physiology, biochemistry, microbiology, bioinformatics, mathematics, and software programming and build a cell.

Using this platform, we can all contribute and build up a database for all the parameters that go into making a cell.

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