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REVIEW

GLYCOBIOLOGY IN THE 21ST CENTURY: Coming developments in glycobiology

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The texture of life is woven through an interplay between unity as the warp and diversity as the weft of nature. Erwin Chargaff, who in 1949 formulated the concept of base-pairing of DNA (Chargaff's rule), the most important single piece of evidence for the double helical structure of DNA proposed by James D. Watson and Francis H. C. Crick in 1953, said "The edifice of the animated world rests on two pillars; one is the unity of nature, the other is its diversity. To pay attention only to the unity, as is usually done, completely distorts our vision and condemns us to the kind of analogy research that fills our journals" [1]. Biochemistry and molecular biology have so far been principally concerned with the pursuit of unity throughout the animal world, from bacteria to high order animals, and have achieved a great deal of success, culminating in the proposal of the so-called central dogma, "DNA → RNA → protein."

Unity in nature is represented by two unique chain molecules, nucleic acids and proteins. Based on this fundamental recognition of life as living matter, gene technology started to develop in the 1970s and now information technology has been introduced in the forms of bioinformatics and computational biology, which deal with living things as systems. As for diversity, however, there remains a great gap to bridge between genotype and phenotypic expression in living nature, as evidenced by advances in molecular evolution and molecular phylogeny. Recent molecular phylogenical approaches to phenotypic diversity among living things strongly suggest that the explosive diversification of species that is said to have occurred in the Cambrian period of the early Paleozoic era, for example, was achieved by versatile reutilization of genes in new combinations, rather than by the creation of new genes [2]. It should be added that there may be functional ranking among the genes utilized, as is revealed in the case of the *eyeless* gene of *Drosophila* [3–5]. In this connection, it is interesting to note that galactosyl ß1-1 ceramide, typical of brain myelin membranes, was replaced by glucosyl ß1-1 ceramide, apparently without anomaly of myelination and function, when the galactosylceramide synthase gene was disrupted [6]. François Jacob, discussing the diversity of living things as evolutionary tinkering said: "Evolution does not produce novelties from scratch. It works on what already exists, either transforming a system to give it new functions or combining several systems to produce a more elaborate one." And further, "Small changes modifying the distribution in time and space of the same structure are sufficient to affect deeply the form, the functioning, and the behavior of the final product—the adult animal. It is always a matter of using the same elements, of adjusting them, of altering here or there, of arranging various combinations to produce new objects of increasing complexity. It is always a matter of tinkering" [7].

Meanwhile, a recent study on the relative abundance of plant and animal species and their number shows that we share the globe with a total number of species estimated variously from five million to more than 50, with fewer than two million species currently classified [8].

There is a third unique chain molecule in living nature, the carbohydrate chain. The most prominent characteristic of this chain is its unsurpassed structural diversity. For example, a trimer of nine common sugar units from the human body theoretically gives rise to 119,736 different structural isomers, a striking contrast to 8,000 tripeptides utilizing twenty different amino acids. From this situation we may envisage the possible involvement of this third chain in the diversity of living nature. In fact, carbohydrate chain structures in the animated world vary between species, individuals, organs, tissues and even cells.

This can be observed typically in the case of blood group substances. Natural carbohydrate chains are usually found in

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combination with proteins (glycoproteins and proteoglycans) or lipids (glycolipids). Numerous minor components of glycolipids with novel structures and functions have been found, and monoclonal antibody technology has been utilized to show unique distributions of such glycolipids in different areas of some organs and tissues. Furthermore, the development of cancer cells or malignant cell transformation always causes characteristic changes in the carbohydrate chain. Such changes have been thought to be closely associated with uncontrolled cell growth and with metastasis. The carbohydrate chain is thus a molecule analogous in the human to its face: that by which it is recognized by others.

In the course of embryonic development starting from a single fertilized gamete cell, the carbohydrate chain changes in a stage-specific manner, creating new forms of cell populations that result in the formation of different tissues and organs. In the process of differentiation that brings about these disparate forms, cell to cell interaction—adhesion, recognition, sorting, cell migration, etc.—has long been known as a motive force in morphogenesis as well as differentiation. Cell surfaces are largely covered with various types of carbohydrate chains that are involved in this interaction. There have recently been discovered bioactive glycoconjugates with differentiation-inducing activity in coupling with receptor-mediated signaling, and the involvement of carbohydrate-mediated cell-to-cell adhesion in the mechanism of inflammation and lymphocyte recruitment has been proven. It is also well known that bacteria cells and certain viruses utilize carbohydrate-mediated cell recognition and adhesion as an initial step to infection. Recent gene targeting or gene expression-controlled technologies have revealed that targeting or suppression of a particular glycosyltransferase frequently results in triggering abnormal morphogenesis and sometimes death of the embryo, making it evident that glycobiology is intimately related to developmental biology.

Immunological responses are essentially composed of cell-to-cell interactions, in which carbohydrate chains may play important roles. For example, natural killer T (NKT) cells are activated by synthetic galactosyl α1-1 ceramide (α-GC) in submicromolar amounts. The reaction is mediated by the monomorphic CD1d molecule as a receptor that specifically recognizes α-GC as a ligand [9]. In this way α-GC prevents tumor cell metastasis as well as rejection *in vivo* [10] and also autoimmune diseases such as experimental autoimmune encephalomyelitis [11] and autoimmune Type 1 diabetes [12,13].

The cultured mutant cells deficient or greatly reduced in glycolipids that have been examined so far displayed little change, however, in cell biological parameters, except in cell behaviors such as cell attachment. This suggests that the cells of either a particular cell group *in vivo*, such as in a living tissue, or in an isolated cultured situation are different from one another, and that the essential function of the carbohydrate chain is accomplished by the grouping of cells or the society of cells. More succinctly, a carbohydrate chain may constitute a unique morphogenic field in which the cells as a group conform in

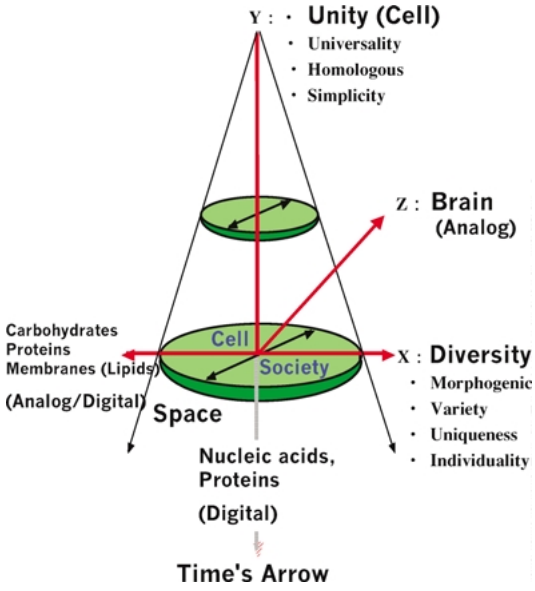

Figure 1. Unity and diversity of living nature.

the development of a particular, socially integrated functional system. Glycobiology aims to find the principle of cell sociology, or, in other words, the principle of the self-organizing multi-cellular system. The biological systems are harmonious and hierarchical.

It is interesting to note that Thomas H. Morgan, the father of chromosome genetics, started his scientific career with developmental biology and then turned his interests to genetics because of the highly complicated nature of morphogenesis, attacking the problem through logical and analytical approaches. Now that molecular genetics armed with versatile technologies is opening a new path to the analysis of morphogenesis at the molecular and gene levels, glycobiology is expected to play an important role in this process. Biological forms are derived either specifically or nonspecifically from cell to cell interactions. It is likely that the potential diversity of carbohydrate chains is concerned with such a diversified process for the creation of forms. However, we do not know at present how the glyco-genes responsible for the diversified expression of carbohydrate chains have evolved or how they behave during the forming processes. Recent studies on Notch signaling and associated genes, for example, have shown that glycosylation plays an essential role in the receptor-ligand interactions required for morphogenesis of *Drosophila*, and further that the gene products are specific glycosyltransferases [14,15].

Perhaps, involvement of the carbohydrate chain in brain functions is most interesting. A novel ganglioside was found to have powerful potency to promote neurite outgrowth in human neuroblastoma cells and its carbohydrate signal is uniquely transduced at the cell surface through the specific protein phosphorylation catalyzed by a new type of protein kinase named ecto (cell surface)-protein kinase, indicating possible involvement of the carbohydrate in the formation of the neuron network

[16]. In fact, it is known that synapses are enriched in glycoconjugates. The biosynthesis of a carbohydrate chain is catalyzed step-by-step by specific glycosyltransferases whose activity and specificity are influenced by several environmental parameters (ions, salts, temperature, concentration and competition of sugar donors and acceptors, etc.), giving a certain fuzzy nature to the biosynthetic mechanism. This sharply contrasts to the template-dependent mechanism of the synthesis of nucleic acids and proteins, a mechanism which is rigorously controlled. It is of interest to note that the potential quantity of information contained in the human genome is around the order of 3×10^9–10^{10}, whereas that of the brain is estimated to be on the order of more than 10^{14}, based on the facts that human brain contains around 1.4×10^{10} neurons and that one single neuron in the human brain is usually connected by around 10^4 synaptic contacts to other neurons. This unique situation represents one of the bases of the plastic function of brain. The great structural diversity of the carbohydrate chain and the fuzzy character of its cellular expression may fulfill a condition for the plasticity of the brain. Genome sequence analysis has shown that there is only 1.23 percent difference between human and chimpanzee. Further analysis, however, focusing on the level of gene expression and its products, proteins, revealed that distinct differences were found in the brain in particular, but not so much in other tissues like blood cells and the liver and kidney [17]. It may be anticipated that the carbohydrate chain is involved in a mechanism for making up such a difference. Thus, elucidation of the functional roles of the carbohydrate chain in the brain should be one of the most important targets of glycobiology and glycotechnology in the coming generation of scientific research.

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