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A challenge to the riddle of the carbohydrate chain

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Abstract: Almost ten years have passed since the advent of glycobiology. It is now timely to reflect how this adventurous research field has so far progressed, what is its aims and scope, and where it is going. In this respect, several points may be raised for discussion.

1. The carbohydrate chains are characterized by their potentially enormous and unique molecular diversity which surpasses those theoretically being conceivable in two other major biopolymers, proteins and nucleic acid. But what are biological consequences or meaning of this diversity?

2. Which genetic and evolutionary mechanism are responsible for generating such diversity?

3. Are there unique signal transduction systems that are mediated by the carbohydrate recognition?

4. Is the carbohydrate chain or carbohydrate recognition involved in essential functions of the brain, in which the cell to cell interaction highly develops? What relevance to neuronal network formation, neuronal plasticity, learning and memory?

5. What and how much progression in the near future is anticipated in structural glycobiology?

6. When will molecular mapping at a ultramicro scale (or at a level of 105 cells) of carbohydrate chains and automatic machines for sequencing and synthesizing any carbohydrate chain be realized? It is utmost important to develop such technologies in order to make the door of glycobiology open to anonymous researchers who are not familiar with glycobiology.

Meanwhile, it should be emphasized that glycobiology is the biology of a socially ordered mass of cells, in which the self-organizing, form-forming form principle works. In other words, it is the biology of diversity, that forms a sharp contrast to the biology of unity so far being pursued in the world of nucleic acids and proteins.

COMMENTARY TO EACH INQUIRY

1 and 2:

Biochemistry and molecular biology have so far been principally concerned with the pursuit of the unity throughout bacteria to animals and have achieved a great success, culminating in the proposal of the so-called central dogma, "DNA $\leftrightarrows$ RNA $\rightarrow$ protein" (Fig. 1). Thus the unity is represented by two unique chain molecules, proteins and nucleic acids. Considering unsurpassed molecular diversity in carbohydrate chain, we may envisage the possible involvement of the carbohydrate chain in the diversity of living nature. A recent study on the relative abundance of species of animals and plants shows that we share the globe with fewer than 2 million species which are currently classified and that estimates of the total number including unclassified ones range from under 5 million to more than 50 million (R.M. May: *Science* **241**, (1988) 1441) (Table 1).

Presumably, the number of species that appears on our globe after the birth of living nature may be estimated to more than a few hundred million (>108). Such new forms are derived through cell to cell interactions, in which carbohydrate-mediated cellular recognition has been regarded to play an important role. Carbohydrate chain structures in animated world vary from species, individuals, organs, tissues, and up to cells. In the course of embryonic development starting from a simple fertilized gamete cell, the carbohydrate chain changes in a stage-specific manner, forming a new form of cell populations to result in the formation of different tissues and organs. In the processes of differentiation in such forming forms, cell

to cell interactions including adhesion, recognition and sorting have been known from of old as an important motive force of morphogenesis as well as differentiation.

Table 1 Carbohydrate diversity and diversity of the animated world

Diversity in carbohydrate and peptide chains

	isomers *	
	oligosaccharide	peptide
dimer	1116	400
trimer	119 736	8000
tetramer	18 601 680	150 000

* Possible isomers from 9 common monosaccharides and from 20 amino acids, respectively.

Diversity in species of animals and plants (R.M. May, 1988)

Classified	$\sim 2 \times 10^6$
Unclassified	$\sim 2 \times 10^7$
Total *	$10^8 \sim 10^{10}$

* Species appearing since the birth of life

Diversity in neuronal cell interactions

Potential quantity of information	
Human genome	Human brain
$\sim 3 \times 10^9 \sim 10^{10}$	$\sim 10^{14}$
	$\sim 1.4 \times 10^{10}$ neurons
	~ 1–3×10^4 synaptic contacts per neuron

Recently, several bioactive glycoconjugates with differentiation-inducing activity were discovered. Also, involvement of carbohydrate-mediated cell adhesion was definitely proved in the mechanisms of inflammation and lymphocyte recruit. It is now known that bacterial cells and certain viruses utilize carbohyrate-mediated cell recognition and adhesion as an initiation step for infection. Thus, anti-adhesion therapy recently has started. Cancer cells, malignantly transformed cells, always give rise to a characteristic change in the carbohydrate chain. Such a change has been thought to be closely associated with uncontrolled cell growth as well as metastasis. Recent gene targeting technology showed that targeting of particular glycosyltransferases results in triggering abnormal course of morphogenesis and finally death of the embryo. It is likely that cells which were isolated and cultured in vitro seemed to be not so much changed from the normal except for behaviors as well as recognition of the cells. Meanwhile, some cultured mutant cells deficient or greatly reduced in glycosphingolipids (mouse melanoma cells) seemingly did not show little change of cell biological parameters like cell growth but not for cell behaviors such as cell attachment onto substratum. Thus, very recently, disruption of cell to substratum adhesion was found to be caused by complete removal of sphingolipids. All these observations suggest that the cell either in a particular cell group in vivo like a tissue or in an isolated cultured situation is different from each others and that the essential function of the carbohydrate chain is realized in such grouping of cells or the society of cells. Thus, glycobiology is aiming at finding the principle of cell sociology or of the self organizing multi-cellular system. Perhaps, in order to achieve such an aim experimentally, a strategy for establishing efficient three-dimensional cell culture system may be importantly necessary.

Up to the present numbers of minor components of glycosphingolipids with a novel structure have been found mainly using of carbohydrate-specific monoclonal antibody technology and their number is still increasing. For example, the estimates of the total number of gangliosides range around more than 130.

Some of them are good cell marker molecules useful to identify cell type, to analyze cell lineage in development and differentiation , etc. Others were shown to be involved in a variety of cell functions. Remaining most, however, are unknown in terms of biological roles. Besides the number of glycosyltransferase genes so far cloned is estimated to be around 120 and is still increasing. It seems still premature to conceive a unitary machinery for generating diversification of carbohydrate chain on the basis of molecular genetics as well as evolutional mechanisms.

3 and 4:

It has been known that lectin or other carbohydrate-binding ligands such as antibodies and bacterial toxins not only specifically bind to cells, but activate or suppress cell growth. Particular molecular species of gangliosides were found to modulate growth factor-dependent cell growth and sometimes to induce cell differentiation. As a consequence a notion of bioactive gangliosides was born. The molecular mechanisms underlying such carbohydrate-protein or carbohydrate-carbohydrate interactions are not known. Recently, GM1 ganglioside was shown to enhance nerve growth factor (NGF)-dependent neuritogenesis by its specific colocalization to NGF receptor (tyrosine receptor kinase, trk) in plasma membranes and consequently activating dimerization of the receptor protein unit to result in the promotion of tyrosine autophosphorylation. Importance of such carbohydrate-mediated oligomerization of membrane receptor in complying with subsequent activation of signal transduction pathway is recognized also in other glycoconjugates, for instance, relationship between proteoglycans and HGF (or b-FGF) receptor.

There are another bioactive glycoconjugates that show a different mode of action. A tetrasialoganglioside, GQ1b, can induce and promote neuritogenesis in human neuroblastoma cells. The action is initiated by highly specific carbohydrate recognition of cell surface receptor and subsequent coupling with phosphorylation of cell surface proteins (ecto phosphorylation) which is catalyzed by a specific ecto-protein kinase in the presence of extracellular ATP. This type of receptor is called glycoreceptor that should be different from lectin devoting to carbohydrate binding alone. Isolation of glycoreceptor, however, has not been successful as yet. The transfection of the neuronal cell line deficient in GQ1b with cDNA of GQ1b synthase (a2,8-sialyltransferase) also could induce neuritogenesis, interestingly accompanying induction of acetylcholine esterase. This is the first indication that glycogene belongs to one of neuro-differentiation genes.

Usefulness of specific inhibitors is also proved in this case. Rat cortical neurons in primary cultures were added with a specific inhibitor D-threo-PDMP (D-thereo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) for glucosylceramide synthase to extensively deplete glycosphingolipid content, since glucosylceramide synthase (UDP-Glc: N-acylsphingosine b-D-glucosyltransferease, EC 2.4.1.80) comprises an initial step of synthesis of almost all glycosphingolipids in animal cells. In these cells showing a remarkable decrease in glycosphingolipids, synapse formation decreased in a dose-and time-dependent manner and subsequent addition of GQ1b but not other gangliosides recoverd synapse formation. Thus, this type of assay should be useful in general to disclose biological activity of glycoconjugates. Development of many different types of inhibitor for particular step of glyocconjugate metabolism including gene knockout or anti-sense strategy is to be expected.

There is little knowledge about the presence and role of glycoconjugates in cell nuclei except pore sites of nuclear membranes. Synthetic sialylcholesterol has a potency to promote neuritogenesis. Most of exogenously added and uptaken sialylcholesterol is quickly transported into cell nuclei resulting in remarkable transcriptional activation of RNA, suggesting that cell nuclei-localized sialyl cholesterol may play a key role in neuritogenesis.

4:

Perhaps, involvement of the carbohydrate in brain functions is more interesting. 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 of which activity and specificity are under the influence of 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 is sharply contrasted with the template-dependent mechanism of the synthesis of nucleic acids and proteins, that is a rigorously

controlled mechanism. It is of interest to note that the potential quantity of information contained in human genome is around the order of 3x109-1010, whereas that of information in brain is estimated to be the order of more than 1014, based on the facts that human brain contains around 1.4x1010 neurons and that single neuron in human brain is usually connected by around 1 to 3x104 synaptic contacts to other neurons (Table 1). This unique situation makes one of the basis of the plastic function of brain. A great structural diversity of the carbohydrate chain and the fuzzy character of its cellular expression may fulfill a condition for the plasticity of brain. Thus, the elucidation of functional roles of the carbohydrate chain in brain should be one of the most interesting challenging targets of glycobiolgy and glycotechnology.

The texture of life is woven by the interplay of the unity as the warp and the diversity as the weft of living nature. I like to mention Erwin Chargaff's warning, "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" (E. Chargaff: Heraclitian *Fire-Sketches from a Life before Nature*, 1978). The carbohydrate chain as the third chain of life next to proteins and nucleic acids solely concerns with the diversity as the weft of living nature (Fig. 1). And glycobiology is the biology of a socially ordered mass of cells, in which the self-organizing, form-forming form principle operates. In the other words, it is the biology of diversity that forms a sharp contrast to the biology of unity so far being pursued in the world of nucleic acids and proteins.

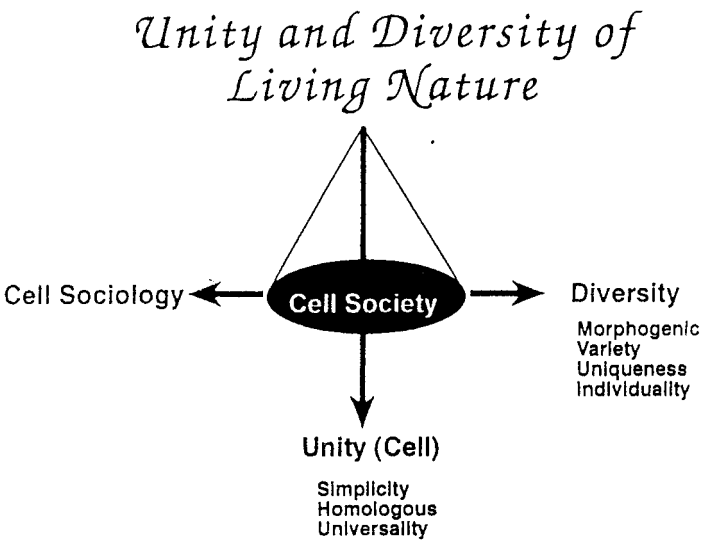

Fig. 1