Hitting the sweet spot

A method for producing large carbohydrate microarrays allows high-throughput, sensitive detection of carbohydrate–antibody interactions.

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Carbohydrates are information-rich molecules vital to recognition processes ranging from host–pathogen interactions to the recruitment of neutrophils to sites of tissue damage[1]. The field has long awaited a means of rapidly and quantitatively assessing interactions between carbohydrates and proteins. In this issue, Wang *et al.*[2] take us a step in that direction. They describe the fabrication of a carbohydrate array, or "glyco-array", that can be used to identify specific carbohydrate epitopes that interact with antibodies. Such arrays show promise in applications such as detection of pathogens and tumor antigens.

Anyone who has ever had the flu—which is initiated when influenza virus adheres to host cells through cell-surface sialic acid residues—can appreciate the benefits of understanding protein-carbohydrate recognition. The challenge is to determine which carbohydrate epitopes are important for specific recognition events. This problem is compounded by the diversity of carbohydrate structures and the different contexts in which they occur. Moreover, carbohydrates often exhibit relatively low binding affinities for their binding partners[3]. Despite these difficulties, the pace of research in glycobiology is accelerating with the advent of new approaches.

Although carbohydrates are ubiquitous in both prokaryotic and eukaryotic cells, an appreciation of their varied functions is only beginning to emerge[1]. Carbohydrates exist in diverse forms. Some are attached to protein cores, as in glycoproteins and proteoglycans; others appear as lipids, as in microbial lipopolysaccharides and glycosyl phosphatidylinositol linkages; still others are found as polysaccharides, as in glycosaminoglycans and bacterial capsular polysaccharides. Additional structural complexity is achieved by carbohydrate branching, where multiple glycosidic linkages are made to a single residue. Each saccharide residue in a complex carbohydrate can possess up to five hydroxyl groups, and glycosidic linkages to each of them can be in one of two anomeric configurations. Although this structural diversity renders carbohydrates exquisitely suited to the transfer of information, it also underscores the need for methods that can analyze the interactions of proteins with many different types of glycan structures.

Much of what we know about carbohydrate function has been gathered from interdisciplinary studies involving methods from genetics, structural biology, biochemistry, organic and analytical chemistry, and cell biology. But high-throughput methods for characterizing protein–carbohydrate interactions have been lacking. For example, simple analogies to the powerful genetic methods[4] for characterizing protein–protein and protein–nucleic acid interactions do not hold. Because the biosynthesis of oligosaccharides involves the action of different enzymes to assemble different carbohydrate structures, it will be difficult to develop *in vivo* assays, such as two-hybrid-like screens, which have proven so effective in identifying protein–protein interactions. Thus, high throughput biochemical methods for analyzing protein–carbohydrate interactions may offer the greatest promise for deciphering the information embedded in carbohydrate structures.

Ideally, any method for characterizing protein–carbohydrate interactions would require only small amounts of material, allow analysis of many samples, and be compatible with the often weak interactions between carbohydrates and their target proteins. The microarray format addresses these issues. First, relatively small sample sizes can be analyzed. Second, it allows parallel screening of many samples simultaneously on a single chip. Third, the presentation of carbohydrates on the surface creates a multivalent display that binds avidly and specifically to carbohydrate-binding proteins.

Many of these benefits are well known in the context of DNA and protein arrays. DNA microarrays are widely used to investigate gene expression and mutations on a genomic scale[5]. Protein microarrays have been developed for the identification of protein–protein activities and for protein detection[6]. Similarly, the glyco-arrays of Wang *et al.*[2] are applied to detect carbohydrate-binding antibodies and to investigate the specificity of the antibodies for various carbohydrate structures.

Although carbohydrates and glycoconjugates have been immobilized on surfaces for binding studies[7,8], no methods for producing large glyco-arrays have been described. Wang *et al.*[2] take advantage of robotics technology currently used for making gene chips. Glycans (48 compounds) are adsorbed to single spots on the surface of nitrocellulose-coated glass slides and then air-dried. The carbohydrates and glycoconjugates used were derived mainly from biological samples—the collection included polysaccharides, glycosaminoglycans, glycoproteins, and semi-synthetic glycoconjugates. The authors note that the efficiency of immobilization depends on the molecular mass of the polysaccharide; consequently, other immobilization strategies may be needed to include low-molecular weight synthetic oligosaccharides in the

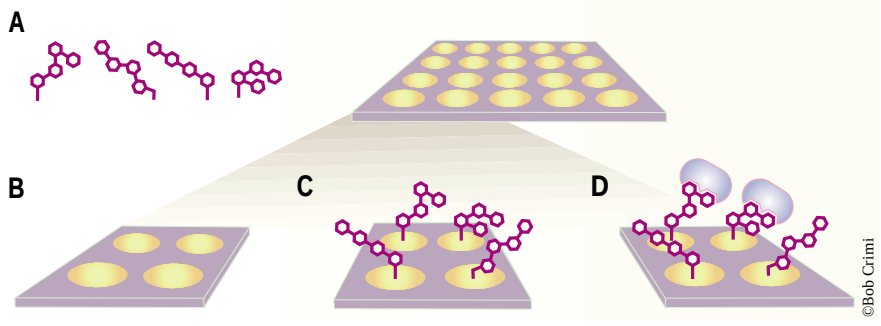

Figure 1. Glycan immobilization and epitope recognition on a microarray. A library of glycans (A) is spotted onto nitrocellulose coated glass slides (B). The glycans adsorbed to the surface (C) are stable and can be probed using tagged proteins that bind to specific epitopes (D).

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arrays. The convenience of the immobilization procedure employed here, however, is an advantage.

Wang *et al.*[2] are the first to test the specificity and sensitivity of carbohydrate microarrays. Antibodies with affinity for carbohydrate epitopes were used to probe the specificities of the glycans by fluorescence spectroscopy (Fig. 1). For example, spots containing internal α(1,6)-glucose linkages (found in dextrans) are easily visualized using the internal α(1,6)-glucose specific 4.3F1 antibody. Alternatively, spots containing terminal (non-reducing) α(1,6)-glucose linkages are recognized by the 16.4.12E antibody. This procedure can rapidly determine the presence or absence of specific carbohydrate epitopes.

In this particular investigation, most of the detected antibody–glycan binding interactions were consistent with known specificities. However, the 4.3F1 antibody (anti-terminal-α(1,6)-glucose) was observed to interact with one unexpected spot: chondroitin sulfate B (CS-B), prepared commercially from pig intestine. The CS-B sample would not be expected to react with this antibody because the structure of this glycosaminoglycan is not known to contain the terminal α(1,6)-glucose epitope. Treatment of tissue sections from the intestine of pigs and mice both confirmed that the 4.3F1 antibody stained cell-surface components. Although the specific epitope recognized is unknown, this result demonstrates that the carbohydrate microarray was able to detect a native epitope present in the CS-B sample. It also suggests that glyco-array technology could be used to discover unexpected and biologically relevant antibody specificities.

The detection of carbohydrate-binding antibodies from human serum could provide insight into what pathogens an individual might have encountered. A glyco-array is well suited to this application because most pathogens possess unique cell-surface carbohydrates. Wang *et al.*[2] illustrate the strengths of glyco-arrays for this application. Serum samples from individuals were screened using the glyco-array and a labeled secondary antibody, and antibodies that bound to polysaccharides of pathogenic strains of *Escherichia coli* and *Pneumococcus* were detected. This experiment may illustrate the most direct application of the method: rapid detection of pathogen exposure. Another potential use is in cancer research, as tumor cells often possess different patterns of glycosylation than do normal cells.[1]

The future utility of large glyco-arrays depends critically on the development of methods for obtaining diverse glycans for immobilization. New methods for the chemical, enzymatic, and chemo-enzymatic synthesis of oligosaccharides continue to emerge. As these synthetic strategies mature[2,9], they could enable the creation of a vast array of glycans unavailable from natural sources. Engineered cell lines can be used to produce glycoproteins with specific complex carbohydrate epitopes, which will be useful for characterizing the impact of protein glycosylation on protein recognition. Application of a range of methods should allow access to the diversity of glycans required for large-scale implementation of carbohydrate arrays.

Although the construction of such arrays will be challenging, the study by Wang *et al.*[2] suggests that it will be worth the effort. Their findings that specific carbohydrate–antibody interactions can be identified rapidly provide the impetus. Although this initial report focuses on detecting antibody specificity, glyco-arrays could also be used to identify novel carbohydrate-binding proteins of other classes. Integration with mass spectrometric technologies developed for high-throughput protein characterization[10] may provide the means to identify proteins with affinities for particular glycan structures. By interrogating either the glycan library or the components in solution or both, we should be able to gain information on multiple aspects of protein–carbohydrate recognition.

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Guided genes for tumor warfare

T cells have been recruited to both produce and deliver vectors to specific tumors in an animal model.

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Many military campaigns rely on the success of designing different guidance mechanisms to target bombs precisely to enemy facilities. These involve directing planes to the enemy's location, firing the bombs or missiles in the general vicinity of the target, "painting" targets with lasers to guide the incoming bombs, and triggering explosions based on the depth of penetration of these bombs into the target. Very often, analogous concepts are used in the design of therapeutics to mediate molecular battles against tumor cells. Along these lines, a report in this issue by Chester *et al.*[1] describes an ingenious—yet complex (because of the many different targeting tactics)—strategy to guide and deliver gene therapy to a tumor.

The first use of gene therapy for cancer involved the engineering of mouse fibroblasts (designated as "producer or packaging cells") to produce retroviral vectors that carried an anticancer cDNA, such as the herpes simplex thymidine kinase (*tk*) cDNA (ref. 2). Because of the relative instability of these vectors, grafting of the producer cells within the tumor mass was carried out first in animal models and then in a multitude of clinical trials in humans. When tissue was available for analysis from these trials, it was shown that the injected producer cells remained stuck in close proximity to the injecting needle and *tk* cDNA transfer by the retroviral vector was limited to a few cell layers away[3,4]. These "bombs" not only lacked guidance mechanisms, but also did not leave the plane's cargo bays. It was evident that gene therapy's "war" on cancer could not be won with such technology.

In recent years, several creative strategies have been reported for engineering cells other than mouse fibroblasts as vector-producing cells[5–8]. It was hoped that cells of the immune system, stem cells, or even endothelial cells could provide a better delivery system for the retroviral vectors and thus allow better targeting to tumors. An important advance was provided by the

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