Background Information & Glossary

Im Folgenden finden Sie eine Zusammenfassung von einigen wichtigen wissenschaftlichen Veröffentlichungen der diesjährigen Ernst Schering Preisträgerin Carolyn Bertozzi aus den Feldern A) der chemischen Glykobiologie, also den biologischen Funktionen von Glykanen. B) des Sulfatmetabolismus` von Mycobacterium tuberculosis, dem Erreger der Tuberkulose.

A) Glykobiologie

1. Chemical Glycobiology. Carolyn R. Bertozzi and Laura L. Kiessling (2001). Science. Vol 291, pp 2357-2364.

Advances in understanding glycobiology have been slow to arrive compared with the revelations in other fields like protein or nucleic acid biochemistry.
Oligosaccharides and **glycoconjugates** like glycoproteins and glycolipids are often involved in complex cellular events. Their structural diversity far exceeds that of proteins and nucleic acids, which makes them key mediators of information for specific molecular recognition and to serve as determinants for protein folding, stability, and pharmacokinetics. **Glycosylation** is one of the most ubiquitous forms of **post-translational modification (PTM)**, the small number of human genes eventually detected in the sequencing of the human genome only underlining the importance of understanding the processes of PTM.
Oligosaccharides are assembled in a step-wise fashion, primarily at the **endoplasmatic reticulum (ER)** and **Golgi apparatus**. Exogenously supplied monosaccharides are taken up by cells and converted to "building blocks" (typically nucleotide sugars) inside the cell. The building blocks are imported into the secretory compartments where they are assembled by enzymes called glycolsyltransferases into oligosaccharides bound to a protein (or lipid) scaffold. Once expressed in fully mature form on the cell surface, the glycoconjugates can serve as ligands for receptors on other cells or pathogens. Chemical tools can be used to inhibit or control any stage of this process.
To understand biological functions of oligosaccharides however it is necessary to obtain homogenous and chemically defined glycoconjugates. Since the natural process in the cell is of such heterogeneous nature, harvesting cell lysates for glycoproteins and glycolipids is not a practical option. However they can be obtained from a number of processes ranging from chemical synthesis to enzyme-based routes. In chemical synthesis the appropriate building blocks are produced and assembled into oligosaccharides, and the oligosaccharides are assembled on proteins. Enzymatic approaches use enzymes in vitro to effect glycosylation with absolute regio- and stereo control. Combinations of these approaches are possible.
The discovery of diverse biological roles for oligosaccharides and glycoconjugates has sparked interest in the development of chemical tools that block their formation and/or their function. Two general types of inhibitors can be distinguished: those that block glycoconjugate biosynthesis and those that interfere with their recognition. Interfering with and altering the structure of glycoconjugates expressed on cell surfaces is important for understanding their biological functions. Using unnatural substrates can result in their incorporation into cell surface glycoconjugates. The result is the presentation of an unnatural epitope that might display different receptor binding properties than its natural counterpart. This then can be exploited for binding studies and to target probes to cells overexpressing unnatural sugar residues, a step toward diagnostic imaging of glycosylation in vivo.

2. Chemistry in living systems. Jennifer A Prescher & Carolyn R. Bertozzi (2005). Nature Chemical Biology. Vol 1, No 1, pp 13 – 21

Living systems are composed of networks of interacting biopolymers, ions and metabolites. These components drive a complex array of cellular processes, many of which cannot be observed when the biomolecules are examined in their purified, isolated forms. Therefore

researchers are constantly developing new methods for observing biomolecules in their natural environs.

Notably Green Fluorescent Protein (GFP) is the most powerful tool for imaging proteins within living systems. However GFPs size can be a significant structural perturbation and may therefore influence expression, localization or function of the tagged protein. Furthermore GFP variants cannot be applied to visualization of glycans, lipids, nucleic acids or the thousands of small organic metabolites amassed within cells.

In recent years, an alternative strategy for tagging biomolecules has emerged that blends the simplicity of genetically encoded tags with the specificity of antibody labelling and the versatility of small-molecule probes. This approach involves the incorporation of unique chemical functionality – a **bioorthogonal chemical reporter** – into a target biomolecule using the cells own biosynthetic machinery. Bioorthogonal chemical reporters are non-native, non-perturbing chemical handles that can be modified in living systems through highly selective reactions with exogenously delivered probes. Proteins, glycans and lipids have all been fashioned with an assortment of chemical reporters in living cells and subsequently ligated with reactive probes. The chemical reporter strategy has been applied to monitoring enzyme activities and tagging cell surface glycans in whole organisms.

Ideally, the chemical reporter should be integrated into the target scaffold without significant structural perturbation. This is accomplished by appending the reporter to substrates that can be used by the cell´s own metabolic machinery. For example, monosaccharides bearing bioorthogonal functional groups can be introduced into cell surface glycans by means of promiscuous enzymes. Once installed in a target biomolecule, the chemical reporter must be reacted with a probe bearing a complementary chemical moiety.

So far, only a handful of chemical motifs are known to possess the requisite qualities of biocompatibility and selective reactivity to function as bioorthogonal chemical reporters in living cells. This elite group comprises peptide sequences, cell surface electrophiles, azides, and terminal alkynes. The most versatile of the above mentioned moieties are the azides, which are abiotic in animals and absent from nearly all naturally occurring species. Azides do not react appreciably with water and are resistant to oxidation. Azides are prone to decomposition at elevated temperatures, but they are quite stable at physiological temperatures. While the azide anion is used as a cytotoxin, organic azides have no intrinsic toxicity.

A powerful feature of chemical reporters is their applicability to labelling not just glycans but many classes of biopolymers. In the field of glycobiology, ketones and azides have already proven to be useful markers for visualizing glycans in their natural environs. At the cellular level, changes in glycosylation are known to correlate with malignant transformations and the development of a chronic inflammatory state. Visualization of these changes at the level of glycan structures would add a new dimension to our understanding of the underlying pathology.

B) Mycobacterium tuberculosis

1. Sulfate Metabolism in **Mycobacteria**. Michael W. Schelle Carolyn R. Bertozzi (2006). ChemBioChem 7, 1516 – 1524

One major challenge in the treatment of tuberculosis is that the bacteria can persist in a nonreplicative state for decades within the **eukaryotic** host. Identifying drug targets that are critical for survival in the persistent state could greatly enhance the efficacy of tuberculosis therapy and decrease the time required to eliminate the bacteria from the patient. Many enzymes and metabolites involved with sulfate metabolism in the human pathogen Mycobacterium tuberculosis (M. tb) are important for virulence in persistent infections.

Pathogenic bacteria have developed numerous mechanisms to survive inside a hostile host environment. M. tb is thought to control the human immune response with diverse

biomolecules, including a variety of exotic lipids. One M. tb-specific sulfated metabolite, termed sulfolipid-1 (SL-1), has been correlated with virulence, its specific function however remains unknown. Sulfated metabolites as mediators of interactions between bacteria and plants suggest that **sulfation** is a key modulator of extracellular signalling between prokaryotes and their hosts. Furthermore studies of M tb. mutants with defects in sulfate assimilation indicate that the fate of sulfur in M. tb is a critical survival determinant for the bacteria during infection and suggest novel targets of tuberculosis drug therapy.

2. A sulfated metabolite produced by *stf3* negatively regulates the virulence of Mycobacterium tuberculosis. Mougous et al. (2006). PNAS. Vol 103, No 11, pp4258-4263

A wide variety of organisms use sulfated molecules to control extracellular events. In bacteria, sulfated glycolipids have been shown to serve as extracellular signalling molecules (see above in 1. Sinorhizobium meliloti and M. tb).
Sulfotransferases are the enzymes responsible for installing sulfate esters on metabolites by transfer of the sulfuryl group from the universal donor molecule 3´-phosphoadenosine-5´-phosphosulfate (PAPS). Because the sulfate ester is generally a determinant of function, these enzymes are attractive targets for the perturbation and study of sulfated molecules. Pathogenic mycobacteria have a complex lifecycle, involving several stages of interaction with their host that could be mediated by small molecules. Many lipid-based metabolites have shown to be virulence determinants, but the sulfated components of the mycobacterial metabolome remain essentially uncharacterized.
In this report genetic evidence is presented that S881 is a sulfated compound that requires PAPS and a specific M. tb sulfotransferase (*stf3*) for its biosynthesis. It is further demonstrated that S881 localizes to the outer envelope of M. tb, a position consistent with a role in cell-cell communication. Finally it is shown that deletion of *stf3* causes a hastened progression of tuberculosis in mice. These data indicate that S881 is a negative regulator of virulence and implicate sulfated metabolites as modulators of human host-pathogen interactions. The authors believe that S881 possesses a lipid character.

Glossary:

(from Wikipedia, the free encyclopedia)

Bioorthogonal chemical reporter: non-native, non-perturbing chemical handles that can be modified in living systems through highly selective reactions with exogenously delivered probes. It has been used to enrich proteins and to conduct proteomic analysis.

Endoplasmatic reticulum: an organelle found in all eukaryotic cells that is an interconnected network of tubules, vesicles and cisternae that is responsible for several specialized functions: Protein translation, folding, and transport of proteins to be used in the cell membrane, or to be secreted from the cell (e.g., digestive enzymes); sequestration of calcium; and production and storage of glycogen, steroids, and other macromolecules.

eukaryotic: Animals, plants, fungi, and protists are eukaryotes, organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane bound structure is the nucleus. This feature gives them their name, also spelled "eucaryote", which comes from the Greek ευ, meaning good/true, and κάρυον, meaning nut, referring to the nucleus.

Glycoconjugates: the general classification for carbohydrates covalently linked with other chemical species. Glycoconjugates are very important compounds in biology and consist of many different categories such as glycoproteins, glycopeptides, peptidoglycans, glycolipids, and lipopolysaccharides. They are involved in cell-cell interactions, including cell-cell recognition, and cell-matrix interactions.

Glycosylation: the process or result of addition of saccharides to proteins and lipids. The process is one of four principal co-translational and post-translational modification steps in the synthesis of membrane and secreted proteins and the majority of proteins synthesized in the rough ER undergo glycosylation.

Golgi apparatus: (also called the Golgi body, Golgi complex, or dictyosome) is an organelle found in typical eukaryotic cells. The primary function of the Golgi apparatus is to process and package macromolecules synthesised by the cell, primarily proteins and lipids.

Green fluorescent protein (GFP): a protein, from the jellyfish Aequorea victoria that fluoresces green when exposed to blue light. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. This has triggered the development of highly automated live cell fluorescence microscopy systems which can be used to observe cells over time expressing one or more proteins tagged with fluorescent proteins. Analysis of such time lapse movies has redefined the understanding of many biological processes including protein folding, protein transport, and RNA dynamics.

Mycobacteria: a genus of Actinobacteria, given its own family, the Mycobacteriaceae. The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis and leprosy.

Oligosaccharides: a saccharide polymer containing a small number (typically three to ten) of component monosaccharides, also known as simple sugars.

Post-translational modification (PTM): the chemical modification of a protein after its translation. It is one of the later steps in protein biosynthesis for many proteins.

Sulfation: a biotransformation process that uses its cosubstrate 3'-phosphoadenosine-5'-phosphosulfate (PAPS).