From the journals

By Nathalie Gerassimov, Jonathan Griffin, Dawn Hayward & Elizabeth Stivison

Testing the lipoprotein-blood clot link

Blood clots affect 600,000 people in the United States each year. In a natural process called fibrinolysis, the enzyme responsible for keeping clots together is inactivated by a protease. High levels of the lipid-containing complex lipoprotein(a), or Lp(a), are linked to cardiovascular disease. Lp(a) may inhibit protease activation; therefore, lowering its levels could break up dangerous blood clots, though this hypothesized connection has not been confirmed in humans.

Michael Boffa and colleagues at the University of Western Ontario and the University of California, San Diego, write in the **Journal of Lipid Research** that their recent study shows substantially lowering elevated Lp(a) levels in patients does not affect fibrinolysis when compared to similar patients receiving a placebo. The researchers found that clotting factor levels and clot lysis times at several time points after administration were not significantly different between the two patient groups. These results suggest that Lp(a) may not have an appreciable role in inhibiting fibrinolysis; studies with more patients are needed.
DOI: 10.1194/jlr.P094763

Unlocking the role of vaults

Within many eukaryotic cells, small noncoding vault RNAs, or vtRNAs, associate with proteins to form large barrel-shaped cytoplasmic organelles known as vaults. Researchers have yet to uncover the function of vtRNAs, but now Nikolay Kolev and a team of researchers at Yale University and Bar-Ilan University in Israel provide novel insights by using a permeabilized cell system for the sleeping sickness-causing parasite Trypanosoma brucei. They identified the previously discovered and abundant noncoding TBsRNA-10 as a vtRNA and showed that downregulating it disrupts mRNA splicing. These results, published in the **Journal of Biological Chemistry**, implicate vaults in RNA metabolism and pave the way for studies to further elucidate their function.
DOI: 10.1074/jbc.RA119.008580

How deadly bacteria survive and multiply

Francisella tularensis is extremely infectious; just 10 of the bacteria can cause tularemia, a life-threatening infection. The Centers for Disease Control and Prevention has classified F. tularensis as a bioterrorism agent, and scientists now are getting a grasp on how this bacteria lives. After being engulfed by white blood cells, F. tularensis is able to escape the phagosome inside the cell by using its type VI secretion system, or T6SS, to secrete its own proteins into the cell. This allows the bacteria to proliferate in the cytosol, causing the eventual death of the blood cell. Understanding this process can help us find ways to prevent it.

Jason Ziveri and colleagues at the Université Paris Descartes published a paper in the journal **Molecular & Cellular Proteomics** analyzing the change in the proteome and phosphoproteome during the assembly of a central component of the T6SS, the sheath. While the proteome remained unchanged, the team found one component of the sheath to be phosphorylated, the first evidence of phosphorylation in Francisella. More importantly, they found that when they mutated the phosphorylated residue to a non-phosphorylatable residue, the bacteria no longer could assemble the T6SS and escape the phagosome to grow in the cell, indicating that this phosphorylation event is essential to the bacteria's survival.
DOI: 10.1074/mcp.RA119.001532

When proteins misfold, how do cells react?
Protein aggregation is a pathological hallmark of many devastating neurodegenerative diseases. While useful, current cell-culture models fail to capture proteasomal changes with high temporal resolution. Colin Gottlieb, Airlia Thompson and a team of researchers from Stanford University and Harvard Medical School addressed this gap using chemical biology approaches to observe an increase in ubiquitylation within minutes of protein misfolding. This work, published in the Journal of Biological Chemistry, suggests that protein unfolding is sufficient to induce a stress response. This discovery may lead to important new therapeutic targets for the treatment of neurological disease.
DOI: 10.1074/jbc.RA119.009654

Why CD23 from only some species can bind glycans

Sugars or carbohydrates, also known as glycans when found as a polymer, are the underappreciated siblings of proteins, with which they often act in concert. Glycans play a critical role in cell-cell and cell-matrix interactions and the immune response. Despite their importance, relatively little is known about glycobiology, so glycans sometimes are referred to as the dark matter of the biological universe.

Lectins are proteins that bind to carbohydrates. One subtype is known as C-lectin because its calcium ion dependency plays a crucial role in immune response to fungal pathogens and other micro-organisms. Much remains to be learned about these interactions.

CD23 is a low-affinity immunoglobulin E receptor that also recognizes other ligands and is found in several cell types including B lymphocytes. Its extracellular domain contains a C-lectin-like domain that resembles a sugar-binding site, and previous studies reached conflicting conclusions on whether it can act as one.

In a paper published in the **Journal of Biological Chemistry**, Sabine A. F. Jégouzo and Hadar Feinberg, from the Imperial College London and the Stanford University School of Medicine, respectively, and colleagues showed that CD23 acts as a glycan receptor in cows and mice.

The research team used solid-phase binding competition assays, glycoprotein blotting experiments and glycan array analysis to show that CD23's lectin domain can bind several sugars (mannose, GlcNAc, glucose and fucose) alone or as part of the glycoprotein. They also provided a structural explanation of why this sugar-binding ability has been lost in humans and primates since their CD23 protein lost crucial amino acids required for sugar binding.

This research further elucidates CD23's role as a cell-surface receptor and provides structural and functional evidence for the glycan binding of its C-lectin-like domain. Because immunoglobulin E is an antibody isotope involved in allergy and resistance to parasites, these results are likely to influence future research into CD23's role as a receptor for potentially pathogenic micro-organisms.

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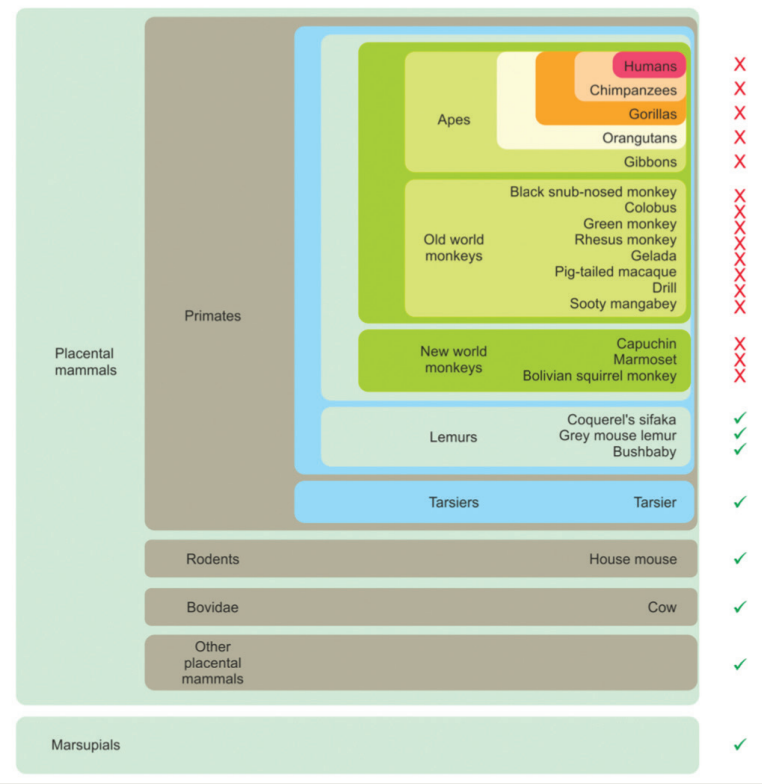
— Nathalie Gerassimov



JÉGOUZO ET AL./JBC

An evolutionary overview of the sugar-binding ability of CD23 based on the conservation of the primary sequence of its C-type lectinlike domain. Green check marks on the right indicate likely sugar-binding ability based on the prediction from cow and mouse data. Red X's indicated a loss of crucial sugar-binding amino acids.

Lipid rafts — the quintessential virus sailboat

Throughout a cell's plasma membrane are so-called lipid rafts. These small regions contain extra cholesterol and phospholipids and clusters of proteins involved in endocytosis and exocytosis for communication with the outside world. However, pathogens exploit lipid rafts to get through the membrane and into the cell. In a recent paper in the **Journal of Lipid Researc**h, Michael I. Bukrinsky and an international team reviewed this process from entry to exit.

Viruses, such as Influenza A, make contact with specific receptors clustered in the lipid raft; the influenza virus rolls over many sites on the lipid raft until the precise host receptor protein is located. Other viruses gain entry by making pores in the membrane or hijacking the endocytic pathway to fuse virus with cell. To exit, the viruses can siphon a host pathway where they are released outside the cell.

Although bacteria are larger than lipid rafts, they can bind host receptors and sometimes disrupt raft composition and gain entry. Other pathogens can benefit from lipid rafts without ever entering the cell; for example, by getting rid of cholesterol, the bacterial stomach bug Helicobacter pylori can bypass the immune response in intestinal macrophages.
DOI: 10.1194/jlr.TR119000391

Salvage pathway not enough to make DNA

Deoxyribonucleoside triphosphates, or dNTPs — the building blocks of DNA — are formed in mammalian cells via either de novo synthesis or salvage of deoxyribonucleosides. To uncover to what degree cells can rely on the salvage pathway alone for DNA production, Phong Tran and a research team at Umeå University in Sweden developed a mouse model with a heart and skeletal muscle-specific deletion of ribonucleotide reductase — the enzyme that catalyzes the first step of DNA synthesis. These knockout mice exhibited aberrant production of DNA and proteins and underwent heart failure after the first postnatal week, which indicated that the salvage pathway on its own is inadequate in supporting DNA production. This work was published in the **Journal of Biological Chemistry**.
DOI: 10.1074/jbc.RA119.009492

Golgi protein plays key role in virus maturation

Pathogenic phleboviruses can cause a range of diseases from mild to fatal. To find treatments for those infected, researchers must understand the virus lifecycle. One critical step that remains poorly understood is how these viruses bud and exit host cells. Zina M. Uckeley, Rebecca Moeller and a research team from Germany and Sweden published a paper in the journal **Molecular & Cellular Proteomics** on work that took a proteomics approach to elucidating this step.

The researchers examined the Uukuniemi virus of the phlebovirus family as it infected human cells. They performed a pull-down of the virus and used mass spectrometry to determine what host cell proteins came out with it. They found 39 cellular protein partners, picked the 12 candidates with the best likelihood of having a role in the virus lifecycle and knocked each down with siRNA. One gene, GBF1, decreased infection by 50% when knocked down, indicating that it plays an essential role. GBF1 resides in the Golgi and participates in the secretory pathway of cells, which viruses hijack. The researchers then examined other viruses that rely on the secretory pathway, finding that GBF1 plays a key role in the lifecycles of the Flaviviridae, Coronaviridae, Rhabdoviridae and Togaviridae familes as well. GBF1 may be a promising antiviral target for many viruses that replicate in the cytoplasm of cells.
DOI: 10.1074/mcp.RA119.001631

Where do FOGs come from?

O-glycosylation is a key protein modification in numerous essential cellular processes. Although free O-glycans, or FOGs, have been detected in the extracellular space, little is known about their origin. In research published in the **Journal of Biological Chemistry**, Hirayama Hiroto and team of researchers in Japan found that the yeast Saccharomyces cerevisiae, using mannose as a carbon source, produced FOGs similar to those attached to glycoproteins. They also deleted the general transcriptional repressor Cy8, which resulted in FOG accumulation and strong growth defects. These results revealed a novel mechanism of FOG removal from yeast and prompt the question of whether similar pathways exist in higher eukaryotes.
DOI: 10.1074/jbc.RA119.009491

Blocking the SNARE-priming stage

Membrane fusion is required for vesicle trafficking and cellular homeostasis. Soluble NSF attachment protein receptor, or SNARE, proteins facilitate the fusion

Fatty livers and hormones

When too much fat is deposited in the liver, typically due to poor diet, a person can develop nonalcoholic fatty liver disease, or NAFLD. The hormonal system regulating the blood pressure renin-angiotensin-aldosterone system, or RAAS, may play a significant role in NAFLD presentation, although studies have yielded conflicting results. Some researchers have observed that blood pressure-lowering drugs such as beta blockers appear to affect weight gain associated with lipid accumulation, while others have not — or have found that blockade of a different pathway prevents weight gain.

Angiotensinogen, or AGT, an RAAS component primarily produced in the liver, is converted to the hormone angiotensin I by renin and angiotensin II by a different enzyme. Among its effects, angiotensin II increases blood pressure. This suggests that reducing AGT production could have an effect on NAFLD.

To study this question, Xin-Ran Tao and colleagues at the Zhejiang University School of Medicine engineered mice that did not produce AGT in the liver, placed some on a high-fat diet and investigated the effects. These mice gained less weight on the high-fat diet than mice with normal AGT. Also, fatty acid synthesis in the liver and liver steatosis, a precursor to NAFLD as fat accumulates, both were reduced in the non-AGT mice on a high-fat diet.

The researchers found that the pathway responsible for activating the transcription factor that controls fatty acid synthesis in the liver was reduced in the mice without AGT, linking AGT to fatty acid synthesis and suggesting why these mice had less weight gain on a high-fat diet.

This study, published in the Journal of Lipid Research, shows that hormonal systems may play a significant role in the lipid accumulation that occurs in NAFLD, and depletion of AGT may attenuate the effects of a high-fat diet.
DOI: 10.1194/jlr.M093252

— Dawn Hayward

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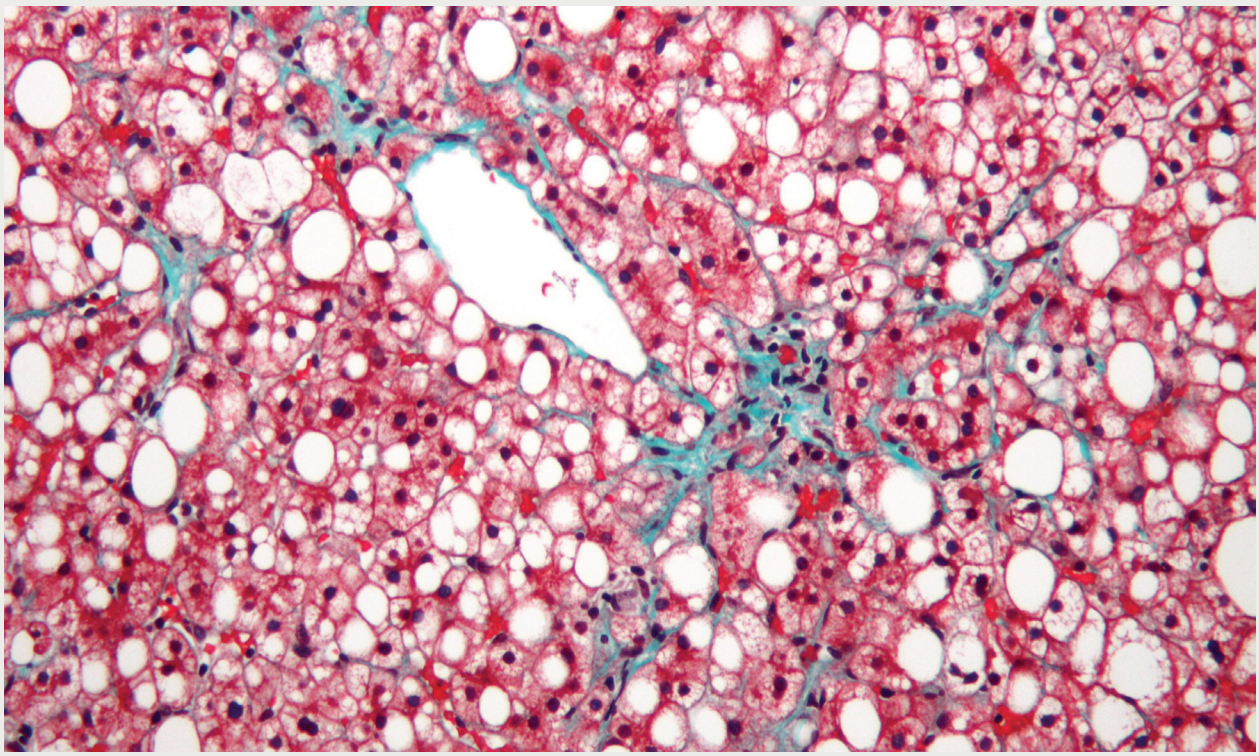


In this micrograph of nonalcoholic fatty liver disease, the liver has a prominent retention of fat (white) and mild fibrosis (green). The hepatocytes are stained red.

A fast new way to ID bacteria in infections

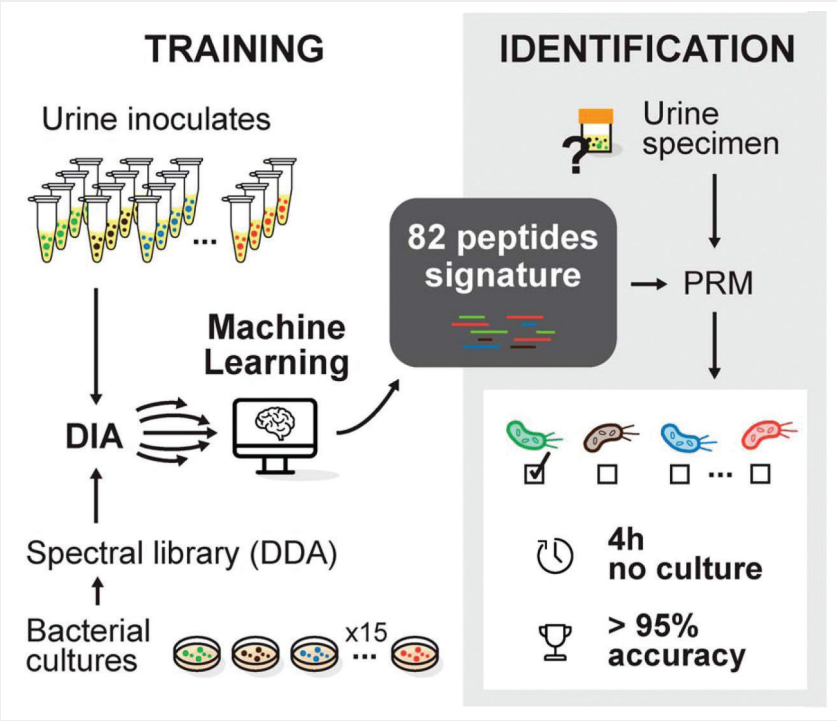
When a patient with an infection needs treatment, doctors often will take a sample of urine or blood to analyze and prescribe broad-spectrum antibiotics for a few days until the bacterial culture results come back from the lab. However, treating with broad-spectrum antibiotics is not very effective for some infections and has many unintended results including contributing to antibiotic resistance. Using more species-specific antibiotics is the best route, because it lessens the number of bacteria unnecessarily exposed to antibiotics; however, current methods of determining what bacteria are present take a few days. Speeding this up would give the patient accurate, effective treatment more quickly.

Florence Roux-Dalvai and colleagues at the Université Laval Research Center in Quebec aim to address this problem by creating a new method to identify bacterial species present in patient samples in under four hours. They recently published a paper on their work in the journal **Molecular & Cellular Proteomics**.

The researchers used liquid chromatography tandem mass spectrometry, or LC-MS/MS, of bacterial peptides and machine learning to identify peptide fingerprints of each bacterial strain. First, they grew pure bacterial cultures of 15 species commonly found in UTIs and digested the proteins to create many short peptides. These peptides then were analyzed by LC-MS/MS to create a library of thousands of peptides. Next, they inoculated healthy urine with bacteria and tested 190 samples to create lists of peptides, using the libraries created from the pure cultures as a reference. These lists of peptides then were submitted to the machine-learning program, where peptide signatures for each strain were determined. This resulted in a signature of between five and 26 peptides for each bacterial species. They then validated this algorithm by testing patient urine and successfully identifying the pathogens. This work holds real promise for improving the treatment of UTIs and many other infections.

DOI: 10.1074/mcp.TIR119.001559

— Elizabeth Stivison



This schematic shows the workflow used to find peptide signatures for identifying bacterial strains in patient samples.