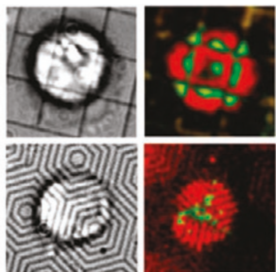
CLEARING OUT GLYCOPROTEINS

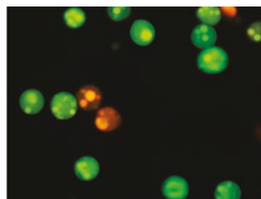
In certain cases, glycan moieties mark glycoproteins for removal from the circulation. However, in many cases the precise ligand-receptor interactions involved in glycoprotein delivery are unknown. A BSA neoglycoprotein bearing sialic acid-(α2,6)-*N*-acetylgalactosamine (Siaα2,6GalNAc) as its terminal disaccharide was found to be rapidly cleared from the circulation in rats, but the receptor responsible for this clearance had not been identified. Park, Baenziger and co-workers now show that this terminal disaccharide is an endogenous ligand of the asialoglycoprotein receptor (ASGP-R). Located in mammalian liver parenchymal cells, ASGP-Rs are traditionally known to recognize glycoconjugates with terminal galactose (Gal) and *N*-acetylgalactosamine (GalNAc) residues, quickly removing them from the circulation. The authors demonstrated that BSA neoglycoproteins containing terminal Siaα2,6GalNAc bound to the carbohydrate recognition domains of the ASGP-R, and the Siaα2,6Gal-neoprotein also bound to ASGP-R, although more weakly. The authors then showed that glycoconjugates containing terminal Siaα2,6GalNAc were cleared much less quickly in ASGP-R–deficient mice than in wild-type mice. Their results suggest that glycoconjugates with terminal α2,6 sialic acid are *in vivo* ligands for the ASGP-R. Most serum glycoproteins end with a terminal sialic acid, and future studies can now begin to define which of these circulating glycoproteins are regulated by ASGP-R–mediated clearance.
(*Proc. Natl. Acad. Sci. USA* **102**, 17125–17129, 2005) *GW*

Patterning T cell activation


Jay T. Groves

Cell-mediated immune responses are initiated by interactions between T cells and antigen-presenting cells (APCs) at an interface called the immunological synapse. This synapse is composed of the central supramolecular activation cluster (c-SMAC), organized by the interaction of T cell receptors (TCRs) on T cells and major histocompatibility complex (pMHC) proteins of APCs. The architecture of the c-SMAC seems to be important for regulating signaling pathways involved in T cell activation, but how the spatial distribution of TCR-pMHC interactions organizes this process has remained unclear. To explore this question, Groves, Dustin and coworkers used patterned surfaces to probe how geometric confinement of proteins would affect assembly and function of the immunological synapse. Patterned barriers spatially confined pMHC on a APC model bilayer surface. When APCs were patterned in boxed grids, microclusters of TCR and pMHC that gradually gravitated toward the center of the c-SMAC domain were observed. These observations suggested that formation of the synapse is initiated by engagement of the TCR with pMHC molecules, followed by clustering and transport of the microclusters to form the c-SMAC domain. Using markers of cellular signaling, the authors showed that mechanically trapping TCR-pMHC clusters in the radial periphery of the synaptic interface showed enhanced signaling duration compared with clusters that were allowed to translocate into a c-SMAC domain. This data supports a model in which spatial control of T cell–APC interactions is a central feature of immunological synapse formation, and this approach provides a system to determine the contribution of individual interactions in immunological synapse assembly.
(***Science*** **310**, 1191–1193, 2005) ***TLS***

Probing calcium-binding proteins


Varda Shoshan-Barmatz

Calcium (Ca^{2+}) mediates many signaling events involved in important cellular processes such as apoptosis and differentiation. Although many calcium-binding proteins (CBPs) that participate in these events have been identified, there are numerous others that remain elusive. Ca^{2+} binding agents that monitor intracellular Ca^{2+} concentrations during signaling events have been studied extensively. However, there are few reagents that can bind selectively to the calcium binding sites in proteins. In a recent study, Israelson, Shoshan-Barmatz and colleagues describe a new probe that binds to the calcium binding site in proteins, whereupon photoactivation renders the binding irreversible. The authors developed the azido ruthenium (AzRu) probe, in which the ruthenium moiety can compete for the Ca^{2+}-binding site of proteins, and the azide allows for photoactivation. In both ***in vitro*** and ***in vivo*** studies, AzRu was shown to inhibit only Ca^{2+}-triggered activity of several known functionally different CBPs. AzRu protected against apoptotic cell death of U-937 cells induced by staurosporine or overexpression of the voltage-dependant anion channels, which is mediated by Ca^{2+}. To label mitochondrial CBPs the authors irradiated mitochondria in the presence of radioactive $Az^{103}Ru$, revealing two familiar and two unknown mitochondrial CBPs. These studies provide a new inhibitor of Ca^{2+} signaling and AzRu could be used to identify unknown CBPs.
(***Chem. Biol.*** 12, 1169–1178, 2005) ***GW***

YOU SAY SUCROSE, I SAY SUCRALOSE

Whether or not mammals prefer certain tastes depends on the chemosensory receptor variants they express on their tongues. Humans can taste a larger 'range' of sweets than mice. For example, whereas both mice and humans find sucrose sweeter than glucose, humans, but not mice, are sensitive to cyclamate. The G protein–coupled receptor (GPCR) family contributes many of its members to this diversity. One GPCR, T1R3, accounts for up to 80% of variation in sweet taste preference, at least in mice, but the basis of this is unknown. T1R3 can heterodimerize to other GPCRs, including T1R1 and T1R2, to give different specificities and preferences in taste. Nie, Munger and coworkers recently described a new approach to help understand how variation in taste preference occurs. The authors first decoupled the downstream signaling events from the upstream ligand-binding events by focusing on the N-terminal domains of mouse T1R2 and T1R3, which had been implicated in the binding of some sweeteners. By fluorescence spectroscopy, they monitored ligand binding to receptors and found that those that were preferred by both mice and humans (such as sugars) could bind both subunits. These binding events led to measurable changes in receptor conformation. T1R3 containing a point mutation corresponding to the *Sac* (saccharin preference) phenotype described in mice showed reduced ligand affinities and an absence of ligand-dependent conformational changes. The decreased binding suggests a way to further modulate taste preference and helps to explain phenotypic variations in sweet taste.
(*Curr. Biol.*, **15**, 1948–1952, 2005) *MB*

Research Highlights written by Mirella Bucci, Terry L. Sheppard and Greg Watt.

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