aminoacid sequence homologies with other lectins of similar size, e.g. murine CBP35/Mac2/IgE-binding protein. Unlike most secreted proteins these lectins contain no signal sequence and we report that drugs, such as brefeldin A and monensin, that inhibit the intracellular transport of classical secretory (glyco)proteins do not block secretion of CBP30 from BHK cells. Secretion is inhibited by methylamine and serum starvation and is increased by heat shock and calcium ionophore A23187, treatments known to block or stimulate exocytosis respectively. Immunofluorescence and biochemical analysis shows that CBP30 is distributed throughout the cytoplasm of sub-confluent BHK cells where it turns over with a half-life of about 30 h, and small amounts are also deposited on the cell surface and substratum. At confluency, the CBP30 assembles into patches that eventually appear to underlie the plasma membrane and extracellular deposits become more numerous. In filter-grown confluent monolayers of Madin-Darby Canine Kidney (MDCK) cells the lectin is secreted from and expressed at the apical domain of the polarized cells whereas laminin is secreted from the basal domain and becomes incorporated into the matrix between cells and substratum.

1. Foddy, L., Stamatoglou, S. C. and Hughes, R. C. (1990) *J. Cell Sci.*, **97**: 139 – 148.
2. Sato, S. and Hughes, R.C. (1992) *J. Biol. Chem.*, **267**: 6983 – 6990.
3. Hughes, R. C. (1992) *Biochem. Soc. Trans.*, **20**: 279 – 284.

S8.20

Structure and Activity of the Carbohydrate Chains in Acrosome Reaction-Inducing Substance of Starfish Eggs

T. Okinaga and M. Hoshi
Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 227, Japan.

Acrosome reaction is an exocytic event of spermatozoa in response to egg signals, which is indispensable to fertilization. Upon the acrosome reaction they expose and/or release lysin to penetrate the egg coat and expose the membrane to fuse with egg plasma membrane. In several animals egg coat glycoprotein are identified as the acrosome reaction-inducing substance, though the information on the role and the structure of their carbohydrate chains is much limited. We have studied the structure and the function of the carbohydrate chain(s) of the acrosome reaction-inducing substance (ARIS) in the starfish, *Asterias amurensis*. ARIS is a sulfated glycoprotein of a huge molecular size ($>10^7$). Sugar composition of ARIS was Fuc: Gal:Xyl:GalNAc:GlcNAc = 2.37:1.00:0.87:0.21:0.32. An oligosaccharide mixture obtained from ARIS by mild acid hydrolysis (10 mM H_2SO_4, 100°C, 1 h) had some activity of ARIS, though the specific activity decreased significantly. It was fractionated by Bio-Gel P-2 gel-filtration and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Some of the anionic oligosaccharides obtained were Fuc(SO_3^-), Gal(SO_3^-)1→Fuc, Xyl1→3Gal1→(SO_3^-)3,4Fuc, Xyl1→3Gal1→$Fuc_2(SO_3^-)$ and Xyl1→3Gal1→$Fuc_3(SO_3^-)$. They had a fucose residue at the reducing terminus and a sulfate group linked to fucose or galactose. These results indicate that the oligosaccharides liberated from ARIS retain some of the activity and their structures are rather unusual, for the presence of xylose and internal fucose.

S8.21

Cell-Cell Recognition as a Prelude to Mating in *Saccharomyces cerevisiae*

C. Cappellaro, K. Hauser and W. Tanner
Lehrstuhl für Zellbiologie und Pflanzenphysiologie, Universitätsstr. 31, 8400 Regensburg.

Sex specific agglutination of *S. cerevisiae* is mediated by a heterophilic 1:1 interaction of 2 glycosylated cell surface proteins. Both proteins (*a*- and *α*-agglutinin) have been purified and the corresponding genes have been cloned.

The *α*-agglutinin is a 250 kDa highly *N*-glycosylated protein; the saccharide components are not required for activity. The protein moiety without the signal peptide is 631 amino acids long. By deletion analysis and by site directed mutagenesis the active region of *α*-agglutinin has been localized between amino acid 171 and 371. This part contains the essential histidyl residue H273 [1].

The *a*-agglutinin is a 18 kDa *O*-glycosylated protein. The molecule is bound to a cell wall component by two cysteins. Preliminary experiments indicate that the sugar portion has only a stabilizing effect on *a*- and *α*-agglutinin interaction. The central non-glycosylated part of *a*-agglutinin is not responsible, however, for activity, as had been postulated previously [1]. This has been shown by site directed mutagenesis as well as by testing proteolytically obtained fragments. The active part of the molecule was found in a 27 amino acid V8 peptide (S43-F69). The corresponding non-glycosylated synthetic peptide is going to be tested.

[1] C. Cappellaro, K. Hauser, V. Mrsa, M. Watzele, G. Watzele, C. Gruber and W. Tanner (1991) *The EMBO Journal*, **10**, 4081 – 8088.

S8.22

The Binding of Hamster Oviductin to Spermatozoa and its Inhibitory Effect to *In Vitro* Fertilization

H. Kimura, J. Matsuda, A. Ogura, T. Asano and M. Naiki
Dept. of Vet. Science, National Institute of Health, Tokyo, Japan.

Hamster oviductal protein(oviductin) is a mucin-like glycoprotein which has a GalNAc residue at its non-reducing carbohydrate terminal and blood type A activity. After ovulation, the antigenicity and lectin specificity of zona pellucida are changed by the addition of the oviductin in oviducts. Since it is known that carbohydrates play important roles at fertilization such as sperm-egg recognition, we examined the biological function of the oviductin during gamete interaction. The indirect fluorescence technique revealed that the oviductin has a binding ability to hamster spermatozoa, especially the head region. Furthermore, the oviductin-sperm binding resulted in the inhibition of *in vitro* fertilization of oviductal and ovarian oocytes. Because the fertilization of zona-free oocytes was not affected, it indicates that the oviductin interfered with sperm-zona binding, not sperm-egg membrane fusion. The question why fertilization occurs *in vivo* where the oviductin is present was answered in the experiment using cumulus oophorus enclosed oocytes. The