TALKS BY INVITED SPEAKERS

A BIOPHYSICAL DISSECTION OF NEUROTRANSMITTER RELEASE AT A GLUTAMATERGIC SYNAPSE

Erwin Neher
1991 Nobel Laureate in Physiology or Medicine and Max Planck Institute for Biophysical Chemistry, Department of Membrane Biophysics, 37077 Goettingen, Germany

The unique capabilities of our brain as an information processor are critically dependent on the correct function of some 10 billions of neurons, each of which is connected to about 10 000 other neurons by way of synapses. Unlike in electronic computers these connections are not rigid but adapt their coupling strengths in response to the information flow in the system – a phenomenon called synaptic plasticity. A dissection of the process of synaptic transmission as well as of the mechanisms underlying plasticity is essential for understanding some of the major neurological diseases. It has been known since the early fifties, that synaptic transmission is initiated by the release of a signalling substance, the neurotransmitter, from the presynaptic neuron. This, in turn, is triggered by an influx of Calcium ions (Ca^{++}) into the nerve terminal. The neurotransmitter, once liberated, induces an increase in the conductance of the postsynaptic membrane. When synaptic strength changes during 'plasticity' this can be a consequence of changes in any of the steps of this complicated process. Unfortunately, most nerve terminals are very small and not readily accessible to detailed investigation, such that usually it is very difficult to assign a given change to one of these molecular mechansims. Quite recently, however, it was discovered that a specialized synapse in the auditory pathway, the 'Calyx of Held', has presynaptic terminals, which are large enough that quantitative biophysical techniques can be applied. Particularly, the postsynaptic current can be measured precisely, while the presynaptic calcium concentration ($[Ca^{++}]$) can be increased or decreased – either by opening and closing of Ca^{++} channels or by releasing Ca^{++} from a chemically caged form by photolysis. Furthermore, $[Ca^{++}]$ can be measured by introducing fluorescent Ca^{++} indicators into the terminal. Using these experimental possibilities, we have studied the role of Ca^{++} and other second messengers in short-term changes of synaptic strength. We found that there are two steps, which are strongly modulated: i) action potential waveform and Ca^{++} influx is modulated in multiple ways by second messengers ii) during ongoing activity new synaptic vesicles have to be recruited, to replace those that have undergone exocytosis. This step of recruitment is also modulated strongly by $[Ca^{++}]$, cAMP and other second messengers. The release process itself – although steeply dependent on $[Ca^{++}]$ – is relatively immune to other forms of modulation.

IMMEDIATE-EARLY GENES AS MASTER SWITCHES IN DISEASE

Levon Khachigian
Centre for Vascular Research, University of New South Wales, and Department of Haematology, The Prince of Wales Hospital, Sydney, Australia

Immediate early genes are genes that share the characteristic of having their expression rapidly and transiently induced upon stimulation without dependence on de novo protein synthesis. Our studies over recent years have demonstrated that restenosis, angiogenesis and inflammation can be suppressed using novel "anti-gene therapeutic" strategies targeting certain immediate-early genes. We targeted the basic-region leucine zipper protein, c-Jun with a catalytic DNA molecule, Dz13, a 34-bp oligonucleotide capable of cleaving both murine and human c-Jun transcripts at position G967 or G1311 respectively. Since both angiogenesis and inflammation depend on vascular permeability, we investigated whether the DNAzyme, Dz13, could suppress retinal neovascularisation in a murine model of proliferative retinopathy (ROP). We demonstrated that knockdown of c-Jun by Dz13, inhibited retinal neovascularisation. The control DNAzyme, Dz13scr, a size-matched molecule retaining the catalytic core, but with scrambled hybridizing arms was unable to influence neovascularisation. This led us to investigate whether Dz13 could influence a number of inflammatory processes. Dz13 suppressed vascular permeability and transendothelial emigration of leukocytes in murine models of vascular permeability, acute inflammation and collagen antibody induced arthritis (CAIA), whereas its scrambled counterpart, Dz13scr had no influence over these processes. Treatment with Dz13 reduced vascular permeability due to cutaneous anaphylactic challenge or VEGF administration in mice. Dz13 also abrogated monocyte endothelial cell adhesion in vitro and abolished leukocyte rolling, adhesion and extravasation in a rat model of inflammation. Dz13 attenuated neutrophil infiltration in the lungs of mice challenged with endotoxin, a model of acute inflammation. Dz13 also reduced joint swelling, inflammatory cell infiltration and bone erosion in a mouse model of rheumatoid arthritis (CAIA). FITC-conjugated DNAzyme localised within the tissue and was still catalytically-active 1 hr following intradermal injection. We demonstrated a reduction in c-Jun immunoreactivity in Dz13-treated joint (CAIA), lung (sepsis) and retina (ROP). Further, we showed that Dz13 blocks cytokine-inducible endothelial c-Jun, E-selectin, ICAM-1, VCAM-1 and VE-cadherin expression but has no effect on JAM-1, PECAM-1, p-JNK-1 or c-Fos. Previous studies by our group demonstrated that Dz13-mediated inhibition of c-Jun leads to suppression of SMC proliferation and wound repair *in vitro*, the reduction of neointima formation following a rat carotid artery injury model, and markedly reduced intimal hyperplasia and increased lumen size in balloon-injured segments in rabbits. Our recent findings thus implicate c-Jun as a useful target for anti-inflammatory, anti-angiogenic and anti-restenotic therapies.

NEGATIVE REGULATION OF SIGNAL REGULATORY PROTEIN ON CANCER SIGNALING

Hong Yang Wang
International Cooperation Laboratory on Signal Transduction, Eastern Hepatobiliary Surgery Institute/Hospital Shanghai, SMMU

SIRPα1 is a member of the signal regulatory protein (SIRP) family that undergoes tyrosine phosphorylation and binds SHP-2 tyrosine phosphatase in response to various mitogens. The expression levels of SIRPα1 were decreased in human HCC tissues, as compared with the matched normal tissues. Exogenous expression of wild-type SIRPα1, but not of a mutant SIRPα1 lacking the tyrosine phosphorylation sites, in SIRPα1 negative Huh7 human HCC cells resulted in suppression of tumor cell growth both in vitro and in vivo. Treatment of Huh7 transfectants with EGF or HGF induced tyrosine phosphorylation of SIRPα1 and its association with SHP-2, which were accompanied by reduced ERK1 activation. Expression of SIRPα1 significantly suppressed activation of NF-κB and also sensitized Huh7 cells to TNFα or cisplatin-induced cell death. In addition, SIRPα1-transfected Huh7 cells displayed reduced cell migration and cell spreading in a fashion that was dependent on SIRPα1/SHP-2 complex formation. In conclusion, these results suggest a negative regulatory effect of SIRPα1 on hepatocarcinogenesis through, at least in part, inhibition of ERK and NF-κB pathway. The heightened sensitivity of cells restoring SIRPα1 function could be exploited in the development of therapeutic regimens which may potentiate the antineoplastic effect of conventional cytokines or chemotherapeutic agents.

TALKS ON SPECIAL TOPICS

EPIGENETIC REGULATION OF GENE EXPRESSION BY LINKER HISTONE H1

Yu Hong Fan
Georgia Cancer Coalition Distinguished Cancer Scholar, School of Biology and the Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, IBB 2313, 315 Ferst Drive, Atlanta, GA 30332-0363, USA

H1 linker histones play a key role in the folding of chromatin into higher order structures. Mice contain at least eight H1 subtypes that differ in expression during development. Our previous studies showed that mice develop normally when any one of six different H1 genes is inactivated homozygously, whereas mice lacking three H1 subtypes, H1c, H1d and H1e, generated by three rounds of gene inactivation in ES cells, die by mid-gestation with a broad range of defects. To further understand the role of H1 in chromatin structure and