BIO IMAGINE

SYMPOSIUM AND WORKSHOP
affiliated to
PNS 2011 Meeting in Łódź

ABSTRACT BOOK

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POLISH NEUROSCIENCE SOCIETY

CAPACITIES

The Conference is supported by the EU FP7 Project BIO-IMAGINE:
BIO-IMAGing in research INnovation and Education, GA No. 264173

NUCLEAR ARCHITECTURE - AN EPIGENETIC MECHANISM FOR THE REGULATION OF NUCLEAR FUNCTIONS

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Increasing attention has been paid in the last years to the functionalrelevance of higher order chromatin arrangement as an epigenetic mechanism for specific gene regulation in different celltypes. Recent developments of 3D nanoscopy have provided newmeans to study nuclear architecture at nanometer resolution,which will help to bridge the gap from the molecular level to thelevel of higher-order structure. The segmental organisation inmetaphase chromosomes with regard to gene density is transposedinto a polar organization of chromosome territories ininterphase: interphase chromatin is spatially arranged in a radialpattern with the preferential localization of gene-dense chromatinin the nuclear interior and of gene-poor chromatin at the nuclearenvelope. The spatial proximity of genes in the nuclear interiormay facilitate the establishment of a chromatin topography, whichoptimally suits the structural requirements for transcription. Forhighly transcribed genes activation or silencing has been associatedwith nuclear repositioning. Yet, the influence of transcriptionalactivity per se has remained a matter of discussion. That anenrichment of (transcriptionally active) genes in the nuclear interioris not mandatory, was recently shown by the observation of an“inverted” chromatin pattern in rod cell nuclei of adult animalswith a nocturnal life style. This remodeling into an “inverted”pattern takes place during the postmitotic terminal differentiationof rod cells. In these cells chromatin poised for transcription, aswell as highly transcribed genes are located at the nuclear peripheryand gene-poor chromatin (heterochromatin) in the nuclearcenter. This unique organization suggests a functional significanceof the nuclear architecture in the retina of nocturnal animalsbased on physical properties of chromatin. An “inverted” chromatinarrangement is less diffractive to light and therefore providesan advantage for nocturnal life style.

METHODS OF NEURONAL CELL CULTURE

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Cell culture has proven to be a very powerful approach in addressing neurobiological questions. The hippocampus is a brain structure of pivotal role in many physiological and pathological responses including memory formation, epilepsy and ischemia. Understanding of these processes requires a good knowledge of hippocampal neurons. In vitro culture offers a tool to study these cells in a controlled environment. In order to optimize culture conditions several growth parameters were investigated in hippocampal neuronal cultures derived from rats of different age: 18-day old fetuses, newborns and 5-day old rat pups, maintained in either serum-containing or chemically defined media. Basic principles of neuronal cell culture and usefulness of particular culture conditions for special purposes is discussed.

MULTITASKING BY THE NEURONAL NUCLEOLUS:STRESS SENSING AND NEUROTROPHIC RESPONSES

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The ribosome is the nexus for all cellular protein translation.Critical steps of ribosomal biogenesis occur in the nucleolus,which is a nuclear subdomain that contains tandem repeats ofnucleolar rRNA genes (rDNA). Ribosomal biogenesis is initiatedby the RNA-Polymerase-1 (Pol1)-mediated transcription of thosegenes. That process is a primary site for the regulatory inputsadjusting ribosomal production to cellular needs. Althoughprominent nucleolar presence has been noted in neurons nearly200 years ago, studies that directly address significance of thatstructure for neuronal development and/or homeostasis started toappear only recently. Our recent work has demonstrated that Pol1serves as a sensor of neuronal DNA damage. Thus, DNA singlestrand breaks and/or DNA-protein adducts but not DNA doublestrand breaks inhibit Pol1 leading to disruption of nucleolar structure.Unlike developmentally-restricted apoptosis, such a nuclearstress response also occurs in adult neurons that are challengedwith DNA damage. In developing neurons, nucleolar stress leadsto activation of p53 and the p53-dependent apoptosis. Conversely,during normal development, Pol1 is major transcriptional effectorfor neurite outgrowth. The pro-neuritic neurotrophin BDNFincreases Pol1-mediated transcription in an ERK1/2-dependentmanner while Pol1 is both necessary and sufficient for the BDNF/ERK1/2-stimulated neurite outgrowth. Finally, studies of humancerebro-cortical samples from 33 Alzheimer’s disease (AD)patients and 24 age-matched controls reveal AD-associated epigeneticsilencing of rDNA as rDNA promoter becomes hypermethylated.Such a change in the epigenetic landscape of the AD corticalgenome appears reducing ribosomal biogenesis and stabilizingrDNA.

PRINCIPLES OF SUPER-RESOLUTION MICROSCOPY

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Super-resolution microscopy is a form of light microscopy. Due to the diffraction of light, the resolution of conventional light microscopy is limited as stated by Ernst Abbe in 1873. A good approximation of the resolution attainable is the FWHM (full width at half-maximum) of the point spread function and the precise confocal microscope with high numerical aperture objectives and visible light usually reaches a resolution of ~250 nm. Super-resolution techniques allow the capture of images with a higher resolution than this diffraction limit. Several techniques have been developed over the past few years for super-resolution fluorescence microscopy. Among them stimulated emission depletion microscopy (STED), photoactivated localization microscopy (PALM), fluorescence photo activation localization microscopy (FPALM), stochastic optical reconstruction microscopy (STORM) and ground state depletion microscopy (GSD). They have shown great promise for biological and medical research even if having some individual strengths and weaknesses.This presentation will outline some variations on the theme in optical nanoscopy and super resolution microscopy. Since both these fields are comparatively new, scientific discussion would help in focusing on new outstanding goals.

QUANTITATIVE ANALYSIS OF NEURONAL SUBSTRUCTURE

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Even though the molecular mechanisms of gene-expression in neurons are well described in the literature, little is known about the relationship between these processes and the architecture of the neuronal cell nucleus. To study cell nuclei in neurons of the hippocampus, the brain region involved in both learning and epilepsy, one needs to quantify properly fluorescent signals in three-dimensional stacks of confocal images. An essential prerequisite to any quantification is a segmentation of the neuronal nuclei which are typically tightly packed within the cell layer, and frequently appear to overlap, due to limitations in microscope resolution. Therefore, it was necessary to establish an algorithm based on continuous boundary tracing criterion aiming to reconstruct nucleus surface and to separate adjacent nuclei. The rough position of nuclei is determined by the harmonic analysis and serves as an input to trace the boundary. Whilst subsequent slices are being analyzed a certain set of conditions is checked in order to determine whether the recognized outline is acceptable, needs an iterative retracing or becomes discarded. The algorithm does not use a rigid threshold what makes it robust against variations in image intensity and poor contrast. In the aftermath the reconstructed surface is used to identify objects in the interior of the nucleus and to study their morphology and spatial arrangement. This program is an efficient segmentation tool for crowded and overlapping objects in 3D space. It allows us to study quantitatively the architecture of the neuronal nucleus using confocal-microscopic approach.

EPIGENETIC REGULATION OF MATRIXMETALLOPROTEINASE GENE EXPRESSION IN THERAT BRAIN

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Matrix Metalloproteinase-9 (MMP-9) is an extracellularly operating enzyme involved in the synaptic plasticity, hippocampal-dependent long term memory and neurodegeneration. Previous studies have shown its upregulation following seizure-evoking stimuli. Herein, we show that in the rat brain, MMP-9 mRNA expression in response to pentylenetetrazole-evoked neuronal depolarization is transient. Furthermore, we demonstrate that in the rat hippocampus neuronal activation strongly induces JunB expression, simultaneously leading to an accumulation of JunB/FosB complexes onto the -88/-80 bp site of the rat MMP-9 gene promoter in vivo. Surprisingly, manipulations with JunB expression levels in activated neurons revealed its moderate repressive action onto MMP-9 gene expression. Therefore, our study documents the active repressive influence of AP-1 onto MMP-9 transcriptional regulation by the engagement of JunB.

ARCHITECTURAL CHANGES IN THE NEURONALNUCLEUS DURING EPILEPTOGENESIS

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Synaptic plasticity is the ability of neurons to change the strengthof their synaptic connections according to the demands of thechanging environment. The phenomenon underlies cognitive functionslike learning and memory, and, in its aberrant form, playsimportant pathogenic role in brain disorders, especially in epilepsy.It is now firmly established that long-lasting synaptic plasticityinvolves dramatic changes in neuronal gene expression. Themechanisms of these changes are quite well understood at the levelof cis- and trans-acting regulatory factors. In contrast, the potentialrole of higher-order nuclear architecture in genetic regulationof synaptic plasticity and epileptogenesis has not been explored.Therefore, we have examined the structure of the nuclei in theneurons of the rat hippocampus at different time points after acuteseizures, using high-resolution morphological techniques andthree-dimensional quantitative analysis. Our results indicate thatthere is prominent reorganization of the neuronal nucleus uponseizures, involving movements of highly expressed genes andchromosomal gene clusters. Such reorganization may lead to formationof molecular factories, in which transcription, splicing, and(possibly) quality control/export of pre-mRNA occur in concert.

Microscopy approaches to study protein-protein interactions

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We review the current fluorescence microscopy based methods for protein-protein interaction study. Among many, we will discuss methods based on: Bimolecular Fluorescence Complementation, Acceptor Photobleaching, Fluorescence Lifetime Imaging. Particularly, we focus on Förster Resonance Energy transfer based methods both intensity based and time-resolved approaches. Additionally, a novel method for spectral analysis of Förster resonance energy transfer (FRET) signals (luxFRET) will be presented, taking into consideration both the contributions of unpaired donor and acceptor fluorophores and the influence of incomplete labeling of the interacting partners. We discuss the advantages and disadvantages of currently used methods and provide examples of biological applications, focusing on interaction between matrix metalloproteinase with their inhibitors.