Présentation des projets financés au titre de l'édition 2010 du Programme Blanc Inter SVSE 4

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Programme Blanc Inter SVSE 4

Edition 2010

Project title	HearDeafTreat – HEARING AND DEAFNESS: MOLECULAR MECHANISMS AND THERAPEUTIC APPROACHES

Abstract

Deafness, the most frequent sensory disorder, can happen at any age, with any degree of severity. The severe forms are sensorineural (SNHL). Among them, the early-onset forms (prelingual), impede oral language acquisition. The late-onset forms lead to social isolation and often to depression. These include the age-related hearing loss (AHL) or presbycusis, the most frequent cause of hearing impairment. In addition, noise-induced hearing loss (NIHL) affects a growing number of young people. Hearing impairment cost exceeds 90 billion € per year in the EU. There is a high demand for effective drug therapy whereas only prostheses, hearing aids and cochlear implants, are presently available. One of the main reasons for the underdevelopment of drug therapy is the insufficient knowledge regarding the basic molecular mechanisms of hearing and its impairment. Sensorineural hearing impairment is mainly due to defects taking place in the sensory organ (the cochlea) and its innervation by afferent auditory spiral ganglion neurons (SGNs). Defects in the efferent system may also be involved. The hereditary forms of deafness, which account for most of the early-onset forms in developed countries, are mainly due to defects of the sensory cells (hair cells; HCs). Aging, cochlear ischemia and noise trauma damage, HCs and SGNs. Mounting evidence points to reactive oxygen species (ROS) and the antioxidant system in the inner ear as a hub of sensorineural hearing impairment, possibly connecting the pathogenesis of several forms. This is substantiated by the increase of ROS in AHL, NIHL, ischemia-induced as well as in some monogenic forms of hearing impairment. Genetic susceptibility factors and causative genes encoding proteins involved in ROS homeostasis provide direct support to the involvement of ROS. Increased level of ROS, which serve both as signalling molecules and damaging agents of proteins, membrane lipids and DNA, injures both HCs and SGNs. However, the precise mechanism of ROS increase, their molecular targets and mechanisms of action in inherited and acquired SNHLs is still largely unexplored. Of note, recent results suggest that ROS may affect the

	mechanoelectrical transduction (MET) process operated by the HCs. The project HEARDEAFTREAT aims at deciphering the implication of ROS homeostatic defects in various deafness forms, at clarifying the underlying pathogenic processes, and at testing antioxidant drugs as potential preventive and curative agents. It first addresses the role of ROS in the MET process. To this purpose, it tackles as objectives the following prerequisite (i) the study of MET currents, that only the hemicochlea method allows to monitor at adult stage, (ii) the generation of conditional knockout mice with gene inactivation at mature stage thereby bypassing the earliest effect of the gene defect, and (iii) the elucidation of the structure/function of the MET machinery. The project also addresses the pathogenesis of monogenic forms of deafness likely involving dysfunction of ROS homeostasis, by studying the corresponding mouse models in vivo, ex vivo in the hemicochlea preparation, and through their comparison (including transcriptome analysis) with mouse mutants in genes encoding proteins known to be involved in ROS homeostasis. Hypervulnerability to sound and to oxygen-glucose deprivation (OGD) will be tested as a possible read-out of ROS homeostasis defect. The project also aims at studying noise preconditioning effect and the stimulation of the efferent system mediated by dopamine on ROS homeostasis. A screening of the effects of antioxidant drugs and dopamine release enhancer components will be performed on hemicochlea preparation and then in vivo in the appropriate mouse models. This project is based on the complementary skills of French geneticists and auditory neurobiologists with Hungarian neurophysiologists and neuropharmacologists, and takes full advantage of Sanofi-Aventis expertise in drug design.
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Titre du projet	RNAtrans – Visualizing single mRNA molecules and their topology to gain insight into RNP transport and local translation in neurons
Abstract	Translational control of gene expression is a key regulatory process that underlies many physiological functions of cells and organisms. This is probably best exemplified by the discovery of miRNAs, which are thought to regulate expression of a third of human genes at the translational level and have been shown to play key roles to control local translation in neurons. Despite this, there are currently no techniques that can distinguish translated from untranslated mRNAs at the cellular level by high-resolution microscopy. This limitation becomes a critical issue in the case of mRNAs translated locally, in particular sub-cellular compartments. Local mRNA translation plays an important role in a number of processes, such as the formation of the body axes, asymmetric cell division and cell motility. In neurons, localization of mRNAs at the synapse has been proposed to contribute to synaptic plasticity and thus to learning and memory. Furthermore, mRNA targeting and local protein synthesis critically contributes to axon guidance and nerve regeneration. Disruption of both processes causes diseases, e.g. fragile X mental retardation and spinal muscular atrophy. Messenger RNAs are the subject of numerous reactions before acting as a template in protein synthesis. In the nucleus, pre-mRNAs have to be spliced, processed at the 3'- end, and exported through the pores. In the cytoplasm, mRNAs can be kept silent, transported to specific sites, or translated. All these reactions require constant and dramatic modification of the mRNP composition. Similarly, the mRNA has to accommodate numerous changes in shape and structure during its lifetime. For instance, pre-mRNAs have to loop out introns to allow splicing, and the mature mRNA has to be circularized to initiate translation. In contrast, it has been suggested that mRNAs are elongated during passage of the pores and cross with their 5'-ends first. Thus, despite the small amount of data available, we can conclude that (i) mRNA molecules have a plastic shape, (ii) this shape is adapted to function, and (iii) dedicated factors control it.

The ultimate goal of this proposal is the use of super-resolution microscopy to determine mRNA topologies in neurons, and to correlate mRNA shape with its translational status. This will allow us to understand how well-known dendritically localized transcripts are kept translationally silent during transport and when and where relief of translation might be achieved upon synaptic activation in dendrites near synapses. Taking advantage of a very sensitive in situ hybridization (ISH) method involving sets of fluorescently labeled oligonucleotide probes, we propose to analyze the topology of endogenous and reporter mRNAs by detecting simultaneously their 5'-, middle region, and 3'-ends. This will determine whether mRNAs are found in a compact, elongated, or circulated state, and this will be correlated to their translational status. We have performed pilot experiments in HeLa cells showing that two ends of single mRNA molecules can be efficiently detected and distinguished, allowing for an unbiased, automated measurement of intra-molecular distances. With this proof of principle in hand, we would like to use HeLa cells and primary neurons to determine the topology and packing of candidate mRNAs during transport through the cytoplasm and to their final destination at synapses. This study will open the possibility to gain unprecedented insight into when and where a particular mRNA is silent and when it becomes translated. It will also determine of localization of single molecules of endogenous dendritic mRNAs at high resolution and in a quantitative manner, in a variety of conditions and with respect to a number of markers. Such experiments will therefore greatly advance our understanding of how individual mRNAs are transported, how and when they are translationally repressed and when translation might occur upon synaptic plasticity.

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