

Since the budget of 2006 for NF OTKA proposals was drastically reduced, my NF proposal received support of about just 20% of that was planned (that is close to an F type proposal without PD grant). This support was not sufficient to keep any of the aims of the original proposal. Therefore, we had to revise our research plan at the start of the research project. Despite all these, we, I think, managed to use effectively the support we gained. The research group as well as key techniques and critical model systems were successfully established, as follows:

Earlier studies have suggested a few PrP segments that are involved in the pro- or anti-apoptotic effects of PrP, however, a more accurate and detailed mapping of these segments meets with substantial technical difficulties. Basically, the answer to this question requires the introduction of mutant and wild type PrP-s to cells (or animals) and then, the measurements of the extent of the apoptosis that are induced. Transgenic animal experiments are restricted by high cost and long time investments. Since wild type PrP rescues the pro-apoptotic phenotype, and thus, considerably moderate the experimental outcome, PrP-knock out cell line has to be used in these studies. Furthermore, although transient transfections would be preferable over the generation of stable cell lines, the low transfection efficiency that is associated with the neuronal cell lines used here makes difficult it to discern the effects of the mutation due to the background caused by the non-transfected cell population. The another drawback of the transient expression of mutant PrP-s is the very limited time-window for executing the experiments. In the last years with the help of this young OTKA grant we managed to build our lab, set up the needed techniques and develop several new methods and approaches. Some of these developments make it possible to overcome on above difficulties. By now, we have PrP-knock out mice (a gift from prof. Agguzi) and neuronal cell lines (Hpl, Zpl, generated from the hippocampus of PrP knock out mice) addition to the commercially available SH-SY5Y cells expressing no detectable amount of PrP. We also have set up the *sleeping beauty* transposon/transposase system (SB100) of Zsuzsa Izsvák for the transfection of above cells that offers several advantages. The transposase ensures a high integration efficiency of the target gene to the genom of the cells (about 70% of the transfected cells express the target protein at high level). We envisaged that this high percentages of stable integration allowed us to use a transfected cell population for an extended time period without the tedious generation of individual clones. Furthermore, since the transposase inserts the entire sequences between the two transposon arms, a practically 100% percent coupling can be established between the expression of the target and a marker protein, such as GFP. This allowed us to significantly increase the signal to noise ration since the non-transfected cells do not contribute to the background when only the

green cells population are selected for the measurements. These high percentages coupling were experimentally demonstrated by using a *sleeping beauty* transposon vector where a puromycin and a cherry protein expression cassette were inserted between the transposon arms. After a transfection of Zpl cells, the puromycin selected clones were analysed for red fluorescence. We also set up systems (using annexin or Propidium Iodide) for the measurement of apoptosis by FACS in Zpl and SH-SY5Y cells transfected by above SB vector system.

The team made considerable effort to establish an appropriate cell model system for TSE that is not available currently on the field. One of our attempts to generate a neuronal cell line with a tight inducible expression of PrP-3F4 has failed, since the available inducible expression systems were found to be either leaky or the expression can not be turned off sufficient rapidly if once it was turned on.

In another approach we set up an expression system in PrP<sup>-/-</sup> neuronal cell line for the expression of mutant PrP and PrP paralogues, such as doppel and shadoo. There is an increasing body of evidences that there is a connection between the apoptotic neuronal cell death induced by the conformational conversion of PrP during TSE-s and the physiological role of PrP that is likely to include a pro- and/or anti-apoptotic function. Shadoo that is an analogue of the N-terminal part of PrP has been shown to have an anti-apoptotic function. Doppel that is an analogue of the C-terminal part of PrP has been shown to have a pro-apoptotic function. The team uses these protein variants for delineating the mechanism of cell death during the progression of TSE. There are evidences that PrP molecules form dimers interacting directly. We also proposes that both shadow and doppel forms heterodimers with PrP. To study and demonstrate these interactions CFP and YFP fusion of these proteins were generated along with YFP-GPI and CFP-GPI, as well as YFP-CFP-GPI constructs for negative and positive controls, respectively. A bleach out protocol was set up with the use of confocal microscopy to measure FRET.

It is not clear if post-translation modifications contribute to the initiation of the conformational transition of PrP. We took part in developing methods for the measurments of the extent of post-translation modification such as deamidation in proteins. Thus, we have a tool now for following such modifications in various mutant PrP molecules.

We needed also methods for the effective folding of various mutants of PrP. To this end, a generally-applicable method was developed in our lab in collaboration with two research groups at Cornell for effective screening of oxidative folding conditions that promote the regeneration of disulfide-bond-containing proteins. The method is so powerful that could be effectively used in high throughput applications and was published in Nature Biotechnology.