

## SUMMARY OF FINAL REPORT

### Investigations into the cellular function of ataxin-2 by the use of ssRNA aptamers/intrabodies

**Principal researchers: Dr Sylvia Krobitch and Dr. Zoltán Konthur; Max Planck Institute for Molecular Genetics, Berlin, Germany**

1 September 2005 – 22 September 2008

#### **Background and aims:**

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant inherited neurodegenerative disease that is caused by an expansion of CAG repeats in the *SCA2* gene resulting in an enlarged polyglutamine stretch in its gene product, ataxin-2 (ATXN2). Since the cellular function of ATXN2 remains unknown, the project was designed to explore the molecular function of ATXN2 and the pathways contributing to SCA2 pathogenesis by (i) exploiting the yeast-2-hybrid system for the identification of novel protein interaction partners and (ii) by developing screening techniques to isolate blocking molecules that would assist the functional characterisation of protein-protein interactions in the ATXN2 network.

#### **Results:**

In the course of the project we have generated and validated a protein-protein interaction network for ATXN2 using the yeast-2-hybrid system with further follow-up of different approaches in mammalian cell lines. With these approaches, we revealed several pathways in which ATXN2 is implicated in, such as cellular mRNA metabolism, transcriptional regulation, and cell signalling. Moreover, we discovered that some of the identified interaction partners were found to be present in stress granules after treatment of cells with arsenite as reported for ATXN2. Interestingly, these cellular structures represent cellular sites of mRNA triage. Moreover, a few interaction partners have been found associated with promyelocytic leukaemia (PML) bodies, nuclear domains of unknown function that are found in most normal cells.

In order to further functionally characterise the ATXN2 interactions, we set out developing and applying a novel technique based on *in vitro* and *in vivo* selection techniques. Originally we started looking for ssRNA aptamers, however during the project we widened the scope to include intrabodies, antibody fragments that are active within the cell. With this approach we were able to identify intrabodies with interfering properties; however we were not able to identify blocking ssRNA aptamers with these methods. Further experiments on these intrabodies showed that they can be used to detect endogenous proteins and are therefore useful tools for research. Currently, we are evaluating the blocking capability of the isolated intrabodies in the cellular read out systems such as stress granules and PML bodies integrity.

**Lay summary of the results:**

We know that the ataxin-2 protein (ATXN2) is mutated in SCA2 and that this is contributing to the disease. The aim of this project was to gain insight into the role of ATXN2 in the cell and how SCA2 progresses. Specifically, the interactions of ATXN2 with other proteins (protein-protein interactions) were investigated and molecules with interfering properties have been isolated.

We have identified proteins that interact with ATXN2 and used this to generate a protein-protein interaction network for ATXN2. This network is a useful tool for the identification of molecules that block ATXN2's interactions with other proteins.

In parallel, we used novel screening approaches to try and identify small molecules (ssRNA aptamers and intrabodies) that would block ATXN2 interactions within the cell. Using these methods, we were able to identify intrabodies with blocking properties. We are currently evaluating the blocking capability of these intrabodies using the cellular read out systems developed within this project.

In summary, these studies tell us more about the interactions of ATXN2 with other proteins and how this is altered in SCA2. This knowledge will help reveal potential targets for SCA2 therapy in the future.

**Publications arisen from this project:**

There are three manuscripts containing aspects of the funded work in preparation.

**Conferences/ meetings where this research has been presented:**

Inhibitory molecules to explore molecular pathways in neurodegenerative disorders (oral presentation). Ligand Binders against the Proteome Meeting. Krusenbergl / Uppsala, Sweden, June 2005.

Combining SELEX and reverse Yeast-2-Hybrid system: A novel *in-vitro* / *in-vivo* method for the generation of ssRNA aptamers with specific inhibitory potential towards characterized protein-protein interactions (oral presentation). GBM Fall Meeting, Berlin, Germany, September 2005.

Expression of *in vivo*-biotinylated proteins for the development of recombinant antibodies by phage display (poster presentation). Protein Expression Europe, Prague, Czech Republic, October 2007

Expression of *in vivo*-biotinylated proteins for the development of recombinant antibodies by phage display (poster presentation). National Genome Research Network Meeting, Heidelberg, Germany, November 2007

**For more support or information please contact: Ataxia UK, Lincoln House, Kennington Park, 1 – 3 Brixton Road. London SW9 6DE**

**Website:** [www.ataxia.org.uk](http://www.ataxia.org.uk).

**Helpline:** 0845 644 0606 **Tel:** +44 (0)20 7582 1444 **Fax:** +44 (0)20 7582 9444

**Email:** [helpline@ataxia.org.uk](mailto:helpline@ataxia.org.uk)