The challenges of duplicated prion proteins in fish

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The purpose of our work is to investigate,

a) whether fish might develop a Transmissible Spongiform Encephalopathy after having been exposed to Prions from a mammalian source, and

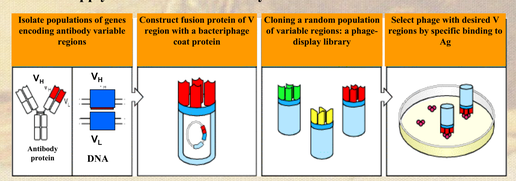
b) whether fish might serve as a potential source of infectivity, after having been fed with feed contaminated with BSE or Scrapie agent and therefore whether fish or fish derived food poses a potential risk for humans or animals

We have previously presented evidence for the existence of homologues to mammalian prion protein genes in fish (Oidtmann *et al.* 2003a and b, Oidtmann *et al*., 2004). The predicted proteins (based on cDNA sequences identified to date) show the main characteristic features described for mammalian PrPs, i.e. signal sequence, Gly-Pro-rich repeat region, a hydrophobic region, two cysteine residues potentially involved in the formation of an intramolecular disulfide bond, glycosylation site and a putative GPI-anchor site.

We have also demonstrated that expression may vary depending on fish species and which of the duplicated PrP genes is investigated.

Phage Display

We require antibodies to various prion proteins from a number of fish species for various applications, most importantly for in vitro conversion experiments.
Traditional methods for generating antibodies are time intensive, involve the use of animals and do not always result in the production of specific antibodies. Antibody Phage Display is a new method that does not require the sacrifice of animals, is simpler, rapid, more cost effective and results in a limitless supply of monoclonal antibody.

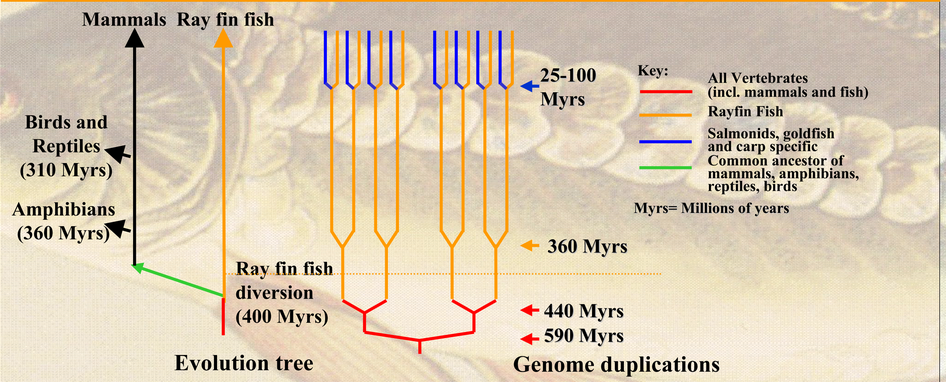


Advances in recombinant DNA technology have enabled the isolation of genes encoding antibodies by PCR from a simple blood sample. Phage display uses filamentous bacteriophage combined with plasmid vectors, which are genetically engineered to express foreign proteins on their surface. The genetic material for the expressed protein resides within the phage particle and if antibody genes are inserted the phagemids will display the antibody protein on the surface of the phage, thus coupling genotype and phenotype for antibody production.

The difficulties in answering the questions mentioned above lie in the following:

- **Fish genomes code for multiple homologues to mammalian Prion proteins**
- **The number of homologues existing in fish is yet unknown and is likely to vary with fish species.**
- **In order to come to a conclusive answer, it might be necessary to analyse all duplicated prion protein homologues in those species which are of highest importance for the consumer (e.g. salmon, rainbow trout)**

Genome duplications in fish

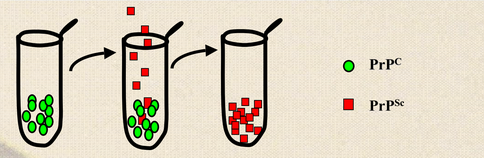


An important driver of evolution is the duplication of genes and entire genomes. Evidence is accumulating that during the evolution of vertebrates from early deuterostome ancestors entire genomes were duplicated through two rounds of duplications. The first genome duplication in chordate evolution is thought to have occurred about 590 million years ago, whereas the second is thought to have occurred around 440 million years back. Recent data suggest that later in the Devonian (around 360 million years ago), the fish genome was duplicated for a third time to produce up to eight copies of the original deuterostome genome. This last duplication took place after the two major radiations of jawed vertebrate life, the ray-finned fish (actinopterygian) and the lobe fin fish (sarcopterygian) lineage (which is including mammalian ancestors), diverged. Therefore the sarcopterygian line, which includes lungfish and all land vertebrates, tend to have only half the number of genes compared with ray-finned fish. Although many duplicated genes turned into pseudogenes, or even 'junk' DNA, many others evolved new functions particularly during development. The increased genetic complexity of fish might reflect their evolutionary success and diversity (Meyer und Schartl, 1999; David et al. 2003).

<u>phage display continued</u>
Antibody libraries comprising the whole antibody repertoire for certain species have been created. These antibody phage display libraries are then available for rapid screening (panning) against antigens *in vitro* by affinity selection. Bound phage are selected and the gene encoding the specific antibody transferred into bacteria expression systems to allow large-scale production of the relevant antibody protein.

Short synthetic peptides derived from fish PrP sequences and recombinant fish PrP proteins will be used in screening phage libraries to generate antibodies.
The selected clones, which express the antibodies can be frozen and stored indefinitely and used to produce more antibody protein, providing a continuous source of antibodies specific to PrP proteins of various species of fish

In vitro conversion of PrP^C to PrP^{Sc}



A central event in prion diseases is the conversion of the normal cellular isoform of the prion protein, PrP^C, into the abnormal pathological isoform, PrP^{Sc}. This conversion involves a substantial conformational change: PrP^C is a proteinase K (PK)-sensitive α-helical monomer, whereas PrP^{Sc} is an assembled multimer characterized by enhanced resistance toward PK-digestion and a higher content of β-structure.

In vitro cell-free conversion reaction experiments have been developed to assess the likelihood that an infecting prion from a donor species would potentially initiate the formation and propagation of pathogenic prions if it came in contact with normal prion protein from a recipient species.

We intend to use this method to investigate whether fish prion proteins can be converted by Prions from a mammalian source into a proteinase K resistant conformation.

Summary and Conclusions

- **The fact that fish genomes encode for multiple prion protein genes makes the task of answering the question of whether fish might serve as a potential source of infectivity due to conformation change of their own prion proteins after having been exposed to BSE or Scrapie agent more difficult**
- **The quickest way to obtain results on the question of possible conformation change of fish prion proteins into a proteinase K resistant conformation is to perform *in vitro* conversion experiments**
- **In order to detect whether conformation change of fish prion proteins has occurred, fish prion protein specific antibodies are required**
- **We intend to generate antibodies mainly by phage display, since this appears to be the quickest, most cost efficient approach, which is also in line with the aim to reduce animal experiments.**

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