EXPRESSION AND DETECTION OF RECOMBINANT

SHEEP PRION PROTEIN (RecShPrP^C) USING

HUMAN NEUROBLASTOMA CELLS

AND WESTERN BLOTTING

By

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CHAPTER I

INTRODUCTION

Over the decades, the food industry has faced repeated challenges with respect to food safety from a variety of sources including new and emerging pathogens that can pose serious threats to human health worldwide. The meat industry, especially the beef industry, has been especially vulnerable in this regard. Bovine Spongiform Encephalopathy (BSE) is one of the most recent and most fatal diseases that has affected the food industry, adversely. The first confirmed case of BSE, commonly known as mad cow disease in the United States, was reported in December 2003 in a Washington State dairy cow. This led to large scale culling of cow herds and bans on meat products that led to billions of dollars in economic loss.

Bovine Spongiform Encephalopathy belongs to a class of fatal neurodegenerative disorders called transmissible spongiform encephalopathy (TSE). These diseases affect both humans and animals and are caused by a unique group of infectious agents known as PRIONS (Prusiner, 1991a). Prions, unlike bacteria or other infectious agents, are glycoproteins that are highly expressed in neuronal cells (Thuring *et al.*, 2002). There are several other prion diseases which affect a variety of hosts like Scrapie in sheep and goats, Creutzfedlt Jakob disease (CJD), kuru, Grestmann-Straussler syndrome (GSS) and fatal insominia (FFI) in humans and encephalopathies in elk, deer, mule, cat and mink (Gajdusek, 1977, Masters *et al.*, 1981, Medori *et al.*, 1992).

The pathogenesis of prion diseases is a major area of research due to its impact on human health and due to the unique biochemical characteristics of prions (Harris, 1999). Over the last quarter of the twentieth century, a new variant of the Creutzfeldt-Jakob's disease that affects humans (vCJD), has been identified which has claimed more than 250 victims worldwide. This disease was transmitted to humans through meat that contained prion infected gonadotrophins (Aguzzi *et al.*, 2008). The United States government has taken necessary steps not only to prevent the transmission of the Specified Risk Materials (SRM) to the human food supply but also to control the transmission of the disease from animal to animal (Brown *et al.*, 2003).

Development of new detection methods and inactivation techniques to control the incidence of BSE in human food supply is gaining serious attention. In order to accomplish this aim, an in-depth understanding of the biochemical structure and processes underlying the conversion of normal prion protein (PrP^C) to abnormal prion protein (PrP^{Sc}) is of paramount importance. Therefore, apart from the availability of the abnormal form of prions, the availability of normal prions is also very important in conducting these studies. A major difficulty in prion research is the lack of availability of the PrP^C in sufficient amounts to conduct experiments. Efforts to purify PrP^C from multiple tissue sources have always met with minimum success. It has always been extremely difficult to obtain large amounts of the protein (Mehlhorn *et al.*, 1996). Scientists have produced the normal PrP^C of different species in various expression systems like virus and bacteria. They all faced problems in expressing and purifying the recombinant PrP^C due to the instability of the protein (Baron *et al.*, 1999). Scientists have successfully expressed the PrP^C in *E.coli* using the amino terminal fusion with

glutathione-S-transferase or GST and carboxy terminal fusion with hexa histidine sequence (Baron *et al.*, 1999). Previous research in our laboratory has also expressed PrP^C in a bacterial expression system. But expression of a protein like PrP^C which might need post translational modifications, in a bacterial system might not fully represent the actual protein in its structural entirety in vivo. A better approach would be to express and purify it in a mammalian system. Expression of PrP^C from scrapie susceptible sheep in a mammalian expression system and subsequent purification from it has not been done yet.

Scrapie is the encephalopathy that affects goats and sheep. We chose to work with the normal sheep prion protein for the expression studies for the following reasons: (i) Sheep prion protein is one of the most extensively studied and well understood among the prion proteins, and (ii) the sheep prion protein sequence differs from the human prion protein, implying a barrier for the transmission of Scrapie to humans. The objectives of this study are to overexpress the sheep normal prion protein as a fusion with the carboxy terminal hexa histidine tag using a mammalian tissue system and purify the protein using a Nikel affinity column chromatography. The mammalian cell culture system used to overexpress the prion protein was the Human neuroblastoma cells. The expressed protein was detected and confirmed using Western Blot assay.

CHAPTER II

REVIEW OF LITERATURE

2.1 Prions: Overview

Prions are a group of infectious proteins that were discovered in 1991 by Dr Stanely B Prusiner. This discovery added a new and fatal disease causing entity to the list of infectious agents and has opened up a new frontier in infectious agent's research. Prions lead to fatal and irreversible neurodegenerative diseases that affect both humans and animals. Prions exist in two forms: normal and abnormal. The abnormal form is the infectious protein. The prion diseases are characterized and even identified by the intracerebral accumulation of the abnormal form of prions called PrP^{Sc}. The amino acid sequence of the abnormal isoform is identical to the normal, but PrP^{Sc} differs from PrP^{C} biochemically (Satoh and Yamamura, 2004).

Prions are highly resistant to physical and chemical treatments and have more similar properties to proteins than to viruses as they are too small (50-150 kDa) to be a virus (Alper *et al.*, 1966). The anonymity of prions was resolved by Prusiner who conducted enzymatic studies on both physical and biochemical aspects of the scrapie infectious agent. Prusiner coin the term prion (pronounced as pree-on) which was derived from proteinaceous and infectious, as they were diverse from plasmids, virus and viriods. From his experiments he concluded that the scrapie agent did not have any effect on the modification or destruction of nucleic acids and the procedures that were involved in the destruction or modification of inactivating proteins (Prusiner, 1982).

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PrP^C is a glycosylphosphatidylinositol (GPI) - anchored glycoprotein which is primarily expressed in neuronal cells. These proteins are also produced by astrocytes, oligodendrocyte, cells in lymphoreticular system (LRS) and follicular dendritic cells (FDCs) (Crozet *et al.*, 2007). The major functions of normal prions include cell signaling, survival, adhesion and differentiation. Studies have shown PrP^C induces polarization in the synapse development and also in neuritogenesis (Steele *et al.*, 2006). Apart from the neuronal cells, glial cells are also capable of expressing the prions and these proteins are accumulated in the cytosol and cell surface membrane (Kikuchi *et al.*, 2004). The prion protein is anchored to the cell surface by a GPI molecule. The C or N terminus associated membrane isoforms are located in the endoplasmic reticulum (Nieznanski *et al.*, 2005). The process of conversion of the normal form of prion protein to its infectious form is by post translational modifications causing changes in protein structures (Stahl *et al.*, 1993). It has been reported that mammalian PrP^C has two N glycosylation sites which appear as bi, uni or unglycosylated forms. Variabilities in structure are results from the attachment of both the N-glycans and GPI anchors. The post translational modifications (PTM) were found to have a higher impact on the variability in the prion protein structure. The exact role and mechanism of this is not known. Many scientists consider N glycosylation as the key to the conversion of PrP^C to PrP^{Sc}. Heterogeneity is thought to play a major role in determing the degree and severity of pathogenesis of Transmissible Spongiform Encephalopathies (Nieznanski *et al.*, 2005).

Prusiner in 1991 found that knockout mice with deficient prion gene (prnp$^{0/0}$) are resistant to the prion diseases. Bioassays of the brain extracts were conducted and found that there is no infectivity in the brains of prnp$^{0/0}$ mice. Studies in transgenic mice

indicated that the normal and abnormal forms of the prion protein play a crucial role in the pathogenesis and transmission of prion diseases (Prusiner, 1991b).

2.2 Terminology

Creutzfeldt-Jacob disease

Creutzfeldt - Jakob disease or CJD is the most common neurodegenerative disease in humans caused by prions. Patients with CJD show progressive dementia with myoclonus, and is diagnosed by Electroencecephalogram (ECG). This eventually leads to memory loss and death of the patient (Johnson and Gibbs, 1998). Incidence of CJD occurs at the rate of one in million people annually and the average age for the onset of the disease is found to be 60-65 years (Collinge and Rossor, 1996).

Gene

A gene is the fundamental functional and physical unit of heredity, composed of an ordered sequence of nucleotides positioned on a particular chromosome which encodes a specific functional product (Human Genome Project, 2003)

Glycosylphosphatidiyl Inositol (GPI)

Glycosyphophatidyl inosotol is a glycolipid which will get attached to the C-terminus of the protein during post translational modification (Prusiner, 1991a).

Meat and Bone Meal (MBM)

Meat and Bone Meal is a rendered product which contains 50% of mammalian protein used in animal feed to improve the amino acid profile of the feed (Adedokun and Adeola, 2005). It does not conatin any blood meal or other animal by-products. The United Kingdom posed a ban on the use of MBM in the year 1998, due to the incidence of BSE in cattle (Taylor *et al.*, 1994).

Open Reading Frame (ORF)

An open reading frame is a section of an organism's genome which potentially encodes for a protein or poly peptide (Collinge and Rossor, 1996). ORFs are located between a start or initiation codon (Methionine) and a stop or termination codon.

Plasmid

Plasmid is an extrachromosomal DNA which is capable of self replicating if in a suitable host. It is composed of circular DNA molecules different from the normal bacterial genome that are not essential for cell survival (Human Genome Project, 2003). They are generally found in bacteria and vary in size from 1 – 200 kilo base pairs (Holmgren and Lefers, 2004).

Prion protein

Prion proteins are glycoproteins that are resistant to proteases. They were first identified from Syrian Hamaster brain enriched for Scrapie infectivity and is designated as PrP 27-30. The prion protein exists in two forms. The normal cellular isoform and is about 33-35kDa where as the abnormal isoform is 27-30kDa (Prusiner and McKinley, 1987).

Prion protein gene

The prion protein gene has been identified in a variety of organisms including humans (Chesebro *et al.*, 1985). The prion gene is designated as prnp.

Post translational modification (PTM)

The chemical modification occurring in a protein after its translation is called a post translational modification. It involves addition of various chemicals like glycosyl groups, phosphate groups, etc (Polevoda and Sherman, 2003).

Scrapie

Scrapie is the transmissible spongiform encephalopathy disease affecting goats and sheep caused by the infectious agent prions. Scrapie is also charatcterized by its long incubation periods and includes ataxia and pruritis. Like other prion infections, Scrapie also leads to death of the animal (Prusiner, 1991a).

Specified Risk Materials (SRM)

Specified Risk Materials are the BSE-infected tissues in cattle that have shown to have the infectious agent. Tonsils and distal ileum of the small intestine are the SRMs in cattle (Turk *et al.*, 1988).

Transformation

Transformation is the process of introduction of an exogenous DNA into a cell, causing it to acquire a new phenotype.

Variant Creutzfeldt-Jacob disease (vCJD)

In the year 1994, the United Kingdom saw the incidence of a new form of Creutzfeldt-Jacob disease which had distinctive characteristics from the classical CJD. The prions identified in BSE and vCJD have the same physical and chemical characteristics, which indicates that they have common sources. For this reason, it was believed that vCJD is caused by infected beef products (Hill *et al.*, 1997).

Vector

A vector is a DNA molecule that replicates autonomously in the host cell, to which a segment of the DNA may be attached for replication.

Viriod

Viriods are short infectious pathogens composed of low molecular weight RNA. They are so far found only in plants and are capable of using the host's enzymatic machinery for its replication (Prusiner, 1994).

Virus

Viruses are small infectious agents composed of one or more nucleic acids and are coated with a protein. Some are found to be coated with lipids and carbohydrates. Viruses replicate by its nucleic acid using the host's enzymatic machinery (Prusiner and McKinley, 1987).

2.3 Scrapie

Natural TSE in sheep is termed as Scrapie. The complete ovine prion gene has been characterized and is found to localize in chromosome 13q17/q18. There are three exons identified for the gene, and the complete ORF for prion protein is encoded in the third exon (O'Neill *et al.*, 2003, Weissmann and Flechsig, 2003). Similar structures in prion gene were identified in mouse, cattle and rats. Several polymorphisms associated with the prion protein genes were characterized. They are thought to play a role in disease susceptibility and are mostly seen in codons 136, 154 and 171(O'Neill *et al.*, 2003).

Scrapie has been identified as a slow progressive neurologic disorder which has been affecting sheep and goats for several years. The disease has long incubation periods and exists in different forms in different mammals. The abnormal isoform of scrapie infectious agent when composed of the single glycosylated protein has a molecular weight of 27-30 kDa. The molecular weight of the native form is 35-37 kDa (Aiken and Marsh, 1990). Scrapie was first diagnosed in the United States in 1947 in a flock of sheep

in Michigan. The genetic makeup of the animal also has high impact on the development of disease. The susceptibility for the disease can be predicted, if the gene sequence of the individual animal is identified. This is because the prion protein gene exhibits mutations in three different codons in the ORF at codons 136, 154 and 171 (Aiken and Marsh, 1990). In United States, mutations in codon 171 are thought to cause the Scrapie infection. The animal is highly susceptible to develop Scrapie if the codon 171 corresponds to the amino acid glutamine (Caughey *et al.*, 1991).

Scrapie is a disease which has no treatment, and is fatal. Early symptoms of Scrapie include weight loss and behavioral changes in animal. Clinical signs of infection appear only after 2 to 5 years after the animal was infected (O'Rourke *et al.*, 2000). The spread of the disease can be through vertical transmission (i.e. from mother to off-springs) and by horizontal transmission (i.e. by environmental factors). There is no evidence of any live animal test to detect the presence of the infectious PrP^{Sc} and it takes several months to years for the symptoms to appear and this depends on the strain of scrapie infectious agent.

2.4 Chemical properties of PrP and recombinant PrP

The prion protein gene is approximately 256 amino acids long, and has N and C terminal signal peptides that are 24-AA and 22-AA respectively. As stated earlier the prnp gene is encoded by a single exon (Riesner, 2003). It was found that after proteolysis of the abnormal prion protein, the amino terminus gets truncated to form PrP 27-30, which is composed of around 142 amino acid sequences (Prusiner, 1982).

During the trafficking of the protein out of the cell once it is formed, the signal peptide which is 1-22 amino acids long gets cleaved and a glycosyl-phosphatidylinosotol

(GPI) anchor is added on the cell surface (Turk *et al.*, 1988). The asparagine residues at 181 and 197 have highly branched glycosylation with the sialic acid substitution. This can be visualized on a Western blot assay followed by SDS-PAGE separation of prion protein. Three forms of glycosylation can be observed based on the glycosylation pattern - with two glycosyl groups, one glycosyl group and with no glycosyl group (Turk *et al.*, 1988, Prusiner, 1991b).

The complete coding region of the sheep (Ovis aries) prnp gene is shown in Appendix 9. The translation product is a 256 amino acid peptide with both N and C terminal signal peptides. The C terminal signal peptide will later be replaced by the GPI anchor. The final processed form of prion protein will thereby contain amino acids from 23-231 (Riesner, 2003).

2.5 Prions protein genetics and expression

2.5.1 Prion protein gene structure

The prion gene that codes for the prion protein is found to be present in all mammals and is highly conserved among species (van Rheede *et al.*, 2003). The entire ORF of the prion gene is present as a single exon, which is similar to mouse and hamster prion gene (Saeki *et al.*, 1996). The human and mouse prion protein is located in the locus 2 and 20 on the chromosome where as the rat prion protein is on chromosome 3 (Saeki *et al.*, 1996). In case of eukaryotes, the presence of TATA box (Goldberg-Hogness box) helps the promoter to bind to RNA polymerase and start transcription. A comparison study of mouse prion protein and Syrian Hamster prion protein found that they are rich in G+C content in the promoter regions and both lack the TATA box. In this

case, these G+C regions may serve as a binding site for the polymerase and the transcription factor Sp1 (Basler *et al.*, 1986).

The prion gene ORFs for many mammals and other organisms have been sequenced and they all encode for a protein of ~250 amino acids (Kretzschmar *et al.*, 1986). Prusiner in 1989 found that the C terminal region of the prion gene ORFs codes for a hydrophobic peptide which is later anchored by a GPI and the N terminal has a glycine rich region. The C terminus has two consensus sites for glycosylation of cysteines and asparagines.

2.5.2 Prion protein expression in eukaryotic cells

The mRNA of PrP^C is highly expressed in the brain and in other cell types of all mammals (Basler *et al.*, 1986). It was found that during the developmental stages of the disease, the prion gene mRNA and choline acetyl transferase levels are increased. Also in some parts of the brain the PrP^C levels are increased during early stages (Basler *et al.*, 1986).

The level of expression of the PrP^C in brain can be determined by Immunohistochemical studies. Immunostaining of PrP^C in the Syrian Hamster brain showed that the prion protein was largely present in the amygalda region where as the expression of PrP^{Sc} was much lower. Similar patterns were observed in the other regions of brain like stratum radiatum and stratum oriens of the hippocampus.

The normal scrapie prion protein is protease sensitive and acts as a template for the infectious agent in the conversion process from PrP^C to PrP^{Sc}. It is known that the conversion of PrP^C to PrP^{Sc} is due to the post translational modification and this occurs several hours after the mature PrP^{Sc} is formed (Borchelt *et al.*, 1990). In most cases of

prion diseases, the route of infection is through peripheral infection. Current evidence

suggests that the neuro invasion and prion replication involves the lymphoid and

peripheral nervous system. In a study which used transgenic mice for expression of ovine

prion protein with a neuron specific enolase promoter, it was seen that the protein was

expressed only in the brains of mice and not in any non-nervous tissues. The use of

neuron specific promoters helps in the suitable detection of scrapie infectious agent and

also to study the trafficking of the infectious agent between intracerebral and

extracerebral compartments. Also the use of neuron specific enolase promoter helps in

expression of only the infectious form of prion that helps in its specific detection (Crozet

et al., 2007).

Not many studies have been conducted that resulted in the successful production

of normal scrapie prion protein using human cell lines. Several cell lines are known to

expresses the infectious form of scrapie prion protein like mouse neuroblastoma cells,

mouse hypothalamic neuronal cells, mouse schwann cells and rat pheochromocytoma

cells. These cell lines were used to identify the conversion mechanism and sub cellular

localization of the protease resistant scrapie prion protein. The T98G (Human

glioblastoma cells) cells expresses the normal prion protein mRNA and produces a high

level of PrP^C in G_1 phase. Another study successfully found out that the T98G cells can

convert the normal prion to abnormal form after reaching a high passage number. The

conversion took place at the cell surface or in endocytic cellular compartments (Kikuchi

et al., 2004).

Infectious prions induce neurotoxicity in the brain cells as a result of

mitochondrial dysfunction or alteration of inflammatory responses. This leads to

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activation of microglial cells and release of cytokines. It was reported that the PrP^{Sc} 106-126 induces neuronal death, even in the absence of microglia. The expression of the PrP^C was high during the neuronal differentiation process both in *in vitro* and *in vivo* studies. In *in vivo* studies, PrP^C is found at a higher amount in mature neurons (Steele *et al.*, 2006).

Cell lines expressing larger amounts of normal prion protein are important for biochemical studies of both PrP^C and PrP^{Sc} as theoretically PrP^C plays a role in the conversion reaction (Sakudo *et al.*, 2008). One of the major concerns using the *E.coli* expression systems, is the production of misfolded or inactive proteins due to high expression of the gene and lack of post translational modifications. Initial attempts to produce the prion protein using *E.coli* yielded very low expression levels due to the insoluble and unstable nature of the protein. Also attempts to fuse the prions with proteins to express in the periplasm were ineffective in producing larger amounts (Riesner, 2003).

2.6 Conversion reaction in prion protein

The abnormal form of the prion protein, PrP^{Sc}, is present in the cell membrane and is attached by a GPI anchor (Stahl *et al.*, 1993). The PrP^C and PrP^{Sc} are chemically similar but differ in their structural conformation. Even though they are coded by the same gene, due to the post translational modifications in PrP^C they exhibit different physiochemical properties (Basler *et al.*, 1986). The conversion of normal prion protein to abnormal form paved the way for studies involving detection of the causative agent for TSEs (Pan *et al.*, 1993). The typical characteristics of prion diseases are the accumulation of the PrP^{Sc} in the brain (Basler *et al.*, 1986). PrP^C has a high alpha helical structure of

42% and a very low beta sheet content where as in PrP^{Sc} has 21% alpha helix and 54% beta sheets (Appendix 8) (Pan *et al.*, 1993).

There were two models hypothesized so far to explain the conversion of PrP^C to PrP^{Sc}: (i) The 'refolding' model or the template assistance model and (ii) the 'seeding' or the nucleation-polymerization model (Aguzzi and Heppner, 2000). The refolding model suggests that a high activation energy barrier spontaneously prevents the conversion of PrP^C to PrP^{Sc} at detectable levels. The seeding model states that the two isoforms are in equilibrium strongly favoring the normal PrP^C (Telling *et al.*, 1995). An aggregate will be formed by the coupling of PrP^C with PrP^{Sc} and hence acts as a seed for nuclear-polymerization reaction (Jarrett and Lansbury, 1993).

2.7 Detection of prion protein

2.7.1 Introduction

Prusiner in 1982 coined the term **Prions**. He derived it from coupling proteineceous infectious as these proteins caused transmissible diseases when the normal form of prion is converted to the abnormal protein. This protein only hypothesis was widely accepted by the scientific community, even though there is no direct or pure evidence available yet (Chesebro, 2003). Several studies have revealed that both the PrP^C and the PrP^{Sc} are glycoproteins and the PrP^{Sc} is anchored to the cell membrane by a GPI. Both these isoforms are approximately 35kDa in molecular weight (Stahl *et al.*, 1993). The detection of prion proteins are of high priority in identifying the transmissible spongiform encephalopathies. The major site of infection not only in case of scrapie but in all major TSE's is the CNS (Aguzzi and Heppner, 2000).

2.7.2 Immuno-double staining

There are two techniques used to detect PrP^{Sc} proteins, using antibodies. In one technique, two different antibodies are used to stain two serial tissues segments side by side; one identifying the PrP^{Sc} and the other identifying the cellular structure (Noorden 1986). However, this can sometimes lead to the risk of misidentifying a similar cellular structure as PrP^{Sc}. In the other method, two different antibodies are used in the same single tissue. This labeling technique requires a color coding to detect and differentiate the antigen of interest (Hancock, 1984). The major disadvantage with the immune-double staining method is the cross reaction of the antibodies during detection (Noorden 1986).

2.7.3 Conformation-Dependent Immunoassay

Initially, when Prusiner's lab identified the TSE infectious agent prion, they developed a technique to inactivate the prions in the brain by using a protease enzyme, Proteinase K. It was determined that this prion protein is used for signal transduction inside the cell (Chesebro, 2003). The remaining protease resistant PrP^{Sc} after the proteolysis with Proteinase K enzyme will then react with the fluorescent coated antibody which enables its detection (Farquhar *et al.*, 1989). However the major disadvantage with this protease was that a large amount of the infectious protein is also protease sensitive and is undetectable by the antibody. Due to this reason researchers were not able to conclude the exact amount of the infectious agent present.

Farquhar and his group in 1989 developed a new technique which enables detection of the infectious agent for Scrapie and BSE at the post mortem stage. This uses a high affinity antibody that identifies the PrP^{Sc} by its structure. The infectious tissue extract is treated with the high affinity antibodies and its reactivity is measured at the

onset. The infectious agent is then treated with certain chemicals so that the protein gets unfolded and the unseen regions get exposed. This is then treated with antibodies and reactivity is measured. The degree in reactivity differs highly between the denatured protein and its native form and the ratio between the diseased native prion proteins to the denatured form gives the exact amount of PrP^{Sc} in the brain tissue (Farquhar *et al.*, 1989).

2.7.4 Western Blotting

Western blotting is one of the major techniques used by researchers worldwide to detect proteins. This method uses a specific antibody for the protein of interest. Proteins are first separated based on molecular weight by the Sodium Dodecyl Sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a nitrocellulose membrane or polyvinylidene difluoride (PVDF) membrane. The antigen or protein of interest is then detected using specific antibodies. The protein is bound to the specific antibody first and later this antibody is coupled with a secondary antibody labeled with a luminescent substrate for detection of the protein. Depending on the labeling of the secondary antibody, the detection methods can vary from chemiluminescence, staining, autoradiography and flurography. In the case of prion diseases, western blotting has been extensively used to identify both the PrP^C and PrP^{Sc} where the proteins are detected as three separate bands on the blot: nonglycosylated, monoglycosylated and diglycosylated. Each of these has different molecular weights and based on their molecular weight pattern researchers are able to identify different strains of Scrapie (Thuring *et al.*, 2002).

2.7.5 Mass Spectrometry

This is a method used for studying the chemical structure of peptides and other molecules. This technique is based on the generation of charged molecules or fragments of molecules by ionizing chemical compounds. The measurement of mass to charge ratio enables the identification of the peptide. The mass to charge ratio of the peptides are calculated by the motion of the ions when they pass through the electromagnetic field. The Mass Spectrometry method can be used both quantitatively and qualitatively: which includes identification of unknown compounds, detection of its structure and determination of isotopic composition of molecules.

CHAPTER III

EXPRESSION AND DETECTION OF RECOMBINANT SHEEP PRION PROTEIN
(RecShPrP[C]) USING HUMAN NEUROBLASTOMA CELLS AND
WESTERN BLOTTING

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ABSTRACT

The objective of this study was to over express the sheep normal prion protein

(RecShPrP[c]) in SK-N-SH cells using a mammalian expression vector with C-terminal

6XHis-tag for large scale purification by Nickel-Nitrilotriacetate column

chromatography. The full length prion open reading frame was PCR amplified and the

complete coding sequence (PrP25-234) including the signal peptide from both C and N

terminal ends of the protein was subcloned into pcDNA6.0 plasmid. The orientation and

integrity of the cloned PCR product in the mammalian expression vector was confirmed

by DNA sequencing. The prion gene was transfected using Lipofectamine2000 reagent

and successfully over expressed in SK-N-SH cells. The cells were lysed and the over

expressed prion protein was detected using Western Blot. Production of RecShPrP[C] using

this method would ensure the availability of large amounts of this protein for further

downstream applications. *In vitro* production of the full length PrP[C] and its detection is

important in further studies aimed at deciphering the biochemistry and structure of prion

proteins.

Key Words: Expression, Fusion protein, His tag, Prions

INTRODUCTION

The unique nature of the prion protein triggered the interest of researchers worldwide to study the molecular mechanisms underlying the cause of transmissible spongiform encephalopathies (Prusiner, 1994). Prions are infectious proteins that cause the neurodegenerative diseases in humans and animals. According to the 'Protein only' hypothesis (Prusiner, 1982) the conversion of normal PrP^C to abnormal PrP^{Sc} is by post translational modification in its secondary structure (Pan *et al.*, 1993, Weissmann, 1995). Although similar in its chemical properties, sensitivity to proteases, altered solubilities and different cellular distribution differentiate the normal PrP^C from abnormal PrP^{Sc} (Prusiner, 1982, Stahl *et al.*, 1993). To understand the biochemical principles and molecular mechanisms differentiating the PrP^C from PrP^{Sc}, it is important to study the structural properties of both isoforms. Even though scientists attempted to extract and purify PrP^C from tissues of various hosts, low expression and yield of the protein was always a limiting factor (Scott *et al.*, 1988). We expressed the entire open reading frame of sheep normal PrP^C in a mammalian cell system fused with a hexa histidine tag at the C terminal end. The expressed protein was confirmed using western blotting and the purification of the protein was performed using nickel nitriloacetate purification system. Sheep prion protein was used in our study as they are safer to work with and is one of the more highly studied prion proteins.

MATERIALS AND METHODS

Cloning the full length open reading frame of normal Sheep prion protein gene (ShPrP^c) into Mammalian expression vector pcDNA 6.0 with His-tag

Polymerase chain reaction (PCR) amplification of full length ovine prion gene: The ovine prion gene in the Zeroblunt vector was amplified by the PCR using the following oligonucloetide primers: forward 5'- TCGGATCCATGTGGAGTGACGTG-3' and reverse 5'-CAGAATTCTCCTACTATGAGAAA -3' obtained from Integrated DNA Technology (IDT). The PCR reaction was performed with 100ng of the plasmid DNA using Roche's Fast start high fidelity PCR system (Cat # 3553426001; Indianapolis, IN) and the PCR conditions as follows: (1) Initial denaturation at 95^0 C for 2 min, (2) denaturation at 95^0 C for 30 seconds, (3) Annealing at 55^0 C for 30 seconds, (4) extension at 72^0 C for 1 min, (5) final extension at 72^0 C for 30 seconds, and steps (2) through (5) were repeated 30 times. The 790 bp PCR product was analyzed by 1.2% agarose gel electrophoresis for the confirmation of the quality of PCR. The gel was analyzed by an HR 6100 system, (UVP Gel Doc-IT Imaging system, Upland, CA).

Expression construct: The mammalian expression vector pcDNA™6/*myc*-His A, B, & C was obtained from Invitrogen Corporation (Cat # V221-20; Carlsbad, CA), which has a 6X histidine repeat at the C terminal which would help later in the purification of the protein. The plasmid was amplified using PCR and transformed following the manufacturer's instructions and stored at -20^0 C.

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Restriction digestion, Ligation and Transformation of the pcDNA 6/myc-*His*

Mammalian expression vector into One shot TOP'10 competent cells. About 20ng

each of the PCR amplified plasmid DNA of the full length ovine prion gene and the

pcDNA 6/myc-*His* mammalian expression vector were digested using the EcoR1 and

BamH1 restrictions enzymes (1/10 of total digest volume) obtained from New England

Biolabs (Cat #s R0101 and R0136; Ipswich, MA). The treated plasmids were analyzed by

1% agarose gel electrophoresis and gel purified using the Zymoclean™ Gel DNA

Recovery Kit (Cat #D4001; Orange, CA) according to the manufacturer's instructions.

The extracted vector and the full length ovine prion gene were ligated and transformed

following the manufacturer's instructions using the Rapid Ligation and transformation kit

from Fermentas (Cat #K1431; Glen Burnie, MD). Positive clones with the complete

plasmid were identified by plasmid extraction and PCR following the above said

conditions. The sequence of the isolated PrP^C-6X *His* gene was confirmed by DNA

sequence analysis using Applied Biosystems (Foster city, CA) Model ABI 3700 DNA

analyzer at the DNA-Protein Core Facility at Oklahoma State University, Stillwater.

Cell culture

  The mammalian cell line used for transfecting the full length mature ovine prion

gene is the Human neuronal cells, also termed as SK-N-SH cells and was obtained from

ATCC (Cat # HTB-11™; Manassas, VA). The cells were grown in RPMI 1640 culture

medium supplemented with 10% fetal bovine serum, Penicillin and streptomycin (Cat #

SV30010, Hyclone, Logan, UT) and Amphotericin B (Cat# SV 30078, Hyclone, Logan,

UT) in a humidified atmosphere with 5% CO_2 at 37^0 C as described in the ATCC's

protocol with a few modifications. The cells were maintained by passaging them at regular intervals of 7 days or when attained 90% confluency (Masters and Stacey, 2007).

Transfection of pcDNA 6/myc-His-full length ovine prion gene into SK-N-SH cells

Before transfection, the cells were divided into aliquots of 2×10^5 cells per mL (counted using a haemocytometer) in a 100mm culture dish. After 18-24 hour, the cells were transfected with pcDNA 6/myc-*His*-ovine prion gene by using Lipofectamine 2000 reagent (Cat # 11668-027; Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. After 24 hour, cells were collected and analyzed for gene expression.

SDS-PAGE and Western Blotting

The whole cell lysate was prepared using the Mammalian Protein Prep Kit (Cat #37901; Qiagen, Valencia, CA) following the manufacturer's protocol. The lysed cells were centrifuged at 5000 x g for 10 minutes and the supernatant was collected for SDS/PAGE and Western blot analysis. The samples were analyzed according to Laemmli (1970). The whole cell lysates were denatured in 2x SDS-laemmli buffer by boiling it for 5 minutes at 95^0 C and were loaded into a 10% precast Tris-glycine gel obtained from Biorad (Cat #161-1101EDU; Hercules, CA) to do the electrophoresis using Biorad's Mini Protean vertical electrophoresis system. Protein staining was performed by Coomassie Brilliant Blue staining (Laemmli, 1970).

Western blotting was performed from the unstained SDS gel by transferring to a PVDF membrane using a Mini Trans Blot cell (Biorad Laboratories, Hercules, CA) for 1 hour at 100 volts. The membrane was blocked using 5% Bovine serum albumin in Tris Buffer Saline (TBS) (20mM Tris and 500mM NaCl) for 1 hour and was incubated with the primary antibodies (1:3000) F89/160.1.5 (Cat # P6488; Sigma, St. Louis, MO) and

L42 (Santacruz Biotechnology, CA) at 4^0 C for 1 hour. The membranes were washed

three times, 5 minutes each in Tris Buffer Saline with 0.1% Tween-20 (TTBS) to remove

any unbound primary antibody and incubated with the secondary antibody(1:5000), Goat

anti-mouse IgG HRP conjugate (Cat# 170-6516 ,Biorad Laboratories, Hercules, CA).

After thorough washing with TTBS, the labeled fusion protein was detected using the

chemiluminesce in a Versadoc^{TM} Imaging Systems, (Biorad, Hercules, CA). The primary

antibodies used in the study were monoclonal mouse anti prion IgG1 F89/160.1.5 which

recognizes the IHFG amino acids of PrP^C and PrP^{Sc} from a variety of species including

sheep and L42 which recognizes the amino acids 145 to 163 in the prion protein gene.

Purification of the RecShPrP^C Using Ni-NTA Purification System

Purification of the overexpressed protein samples from 2 x 10^5 cells per 1mL

from a 100mm culture dish was performed under the denaturing conditions as described

in the manufacturer's instructions in Invitrogen's Probond purification kit. The

Guanidinium lysis buffer, pH 7.8 was equilibrated to 37^0 C and 3mM, 50mM and

100mM Imidazole was added to it in respective tubes. The whole cell lysate was

resuspended in 8 mL of the lysis buffer with protease inhibitor cocktail (Cat #37901;

Qiagen, Valencia, CA) and the cells were mixed thoroughly at room temperature for 15-

20 minutes. The lysed cells were centrifuged at 7000 x g at 4^0 C. The supernatant was

collected into a fresh tube and was purified on a Ni-NTA purification column (supplied

with the kit). The protein was allowed to bind with 2mL of the Ni^{2+}- nitriloacetate resin at

room temperature for 30 minutes. The tip of the column was snapped off and the samples

were collected as 1 mL fractions and saved at 4^0 C for SDS-PAGE analysis. The columns

were washed with Denaturing binding buffer with 3mM, 50mM and 100mM Imidazole,

by resuspending the resin and incubating for 2 minutes. This step was repeated and the eluate was collected and stored at 4^0 C. The column was then washed with Denaturing Wash buffer with 3mM, 50mM and 100mM Imidazole, pH 6.0 and was repeated twice. The eluate was collected and stored at 4^0 C. The protein was eluted by adding Denaturing elution buffer, pH 4.0 and 100mM Tris-EDTA buffer in 1 mL fractions. The concentration was measured using a Nanodrop ND-1000 Spectrophotometer. All the fractions were concentrated by passing through a membrane filter with a cut-off molecular weight of 30kDa (Cat# UFC9 030 24, Millipore, Bedford, MA) and stored at -80^0 C. All the purification steps were performed in both room temperature and refrigeration temperature.

RESULTS AND DISCUSSION

Meat and meat products contaminated with prions are a major concern to the food industries with regard to food safety (Kay, 2005). Multiple investigations have shown that BSE infection can occur in cattle due to exposure to prions present in the meat bone meal (MBM; (Taylor *et al.*, 1997). Therefore, it is important to develop newer detection methods to identify the presence of both normal and abnormal isoforms of the prions in the animal and human food systems. As a first step, it is important to over express different isoforms of prion protein in various *in vitro* and *in vivo* systems and quantify and characterize their expression trends. This, in turn, will also enable researchers to study the structure of the protein expressed in each biological system and develop newer detection methods suited to each system. Researchers have successfully constructed molecular models to study the protein structure of the PrP^C and PrP^{Sc} using NMR techniques (Huang *et al.*, 1996). However, due to the highly unstable nature of the PrP^C

and difficulties in obtaining large amount of the protein from mammalian tissues, expression systems were developed (Baron *et al.*, 1999).

Initial attempts to overexpress the full prion protein using non denaturing conditions led to very low expression (Mehlhorn *et al.*, 1996). Researchers attempted to over express only the functional part of prion gene and these experiments were successful in producing higher rate of gene expression and detection (Gasset *et al.*, 1992, Zhang *et al.*, 1995, Mehlhorn *et al.*, 1996). A study by Scott and others stated that apart from bacterial expression system, cultured cell lines also lead to poor or low expression of PrP^C (Scott *et al.*, 1988). Other studies also reported the poor efficiency of mammalian cells in expressing the native and abnormal prions (Kocisko *et al.*, 1994). However, later research led to the development of *in vivo* and *in vitro* tissue culture or cell culture models to produce larger amount of prion protein which can be used for biochemical and structural studies. It is also important to develop an expression system that can produce recombinant PrP^C and PrP^{Sc} as it will help in the investigation of the role of prions in scrapie and other TSEs using molecular genetic techniques (Scott *et al.*, 1992). Non neuronal cell lines capable of expressing the abnormal infectious form of prions have been reported previously where rabbit kidney epithelial cells were used to over express ovine prnp gene (Vilette *et al.*, 2001).

It is known that neuronal cells serve as good media for infectious prion over expression but non infectious full length sheep prion protein has not been over expressed in such a system. This study is one of the first efforts in this direction. In this study, a Histidine tagged fusion system was used to overexpress the full length (residues 1-256) of $ShPrP^C$ with Human neuroblastoma cells (SK-N-SH). We subsequently conducted

experiments to detect the overexpressed RecShPrP^C using Western blotting and DNA fingerprintng. Sheep prion protein (ShPrP^C) was chosen in this study as it was one of the most studied and well defined among the prion proteins. It was also safer to work with from a safety standpoint as it highly differ in the sequences from humans and reduce the incidence of transmission of sheep prions to humans (Prusiner, 1989).

Expression of the recombinant fusion protein *His*-PrP^C into pcDNA 6/*myc-His* B Mammalian expression Vector

The entire ORF of the sheep prnp gene was previously cloned in our lab using Invitrogen's Zero Blunt Vector (Figure 1). The *His*-PrP^C was constructed by sub-cloning the sheep normal prion gene (790 bp) into the pcDNA 6/*myc-His* B mammalian expression vector (Figure 2). This vector codes for 5126 nucleotides, i.e. 1708 amino acids. The sheep PrP^C sequence was inserted between the EcoR1 and BamH1 restriction sites with a downstream hexahistidine tag. This enables the purification of the fused recombinant protein by immobilized Ni-NTA chromatography column.

The length and orientation of the subsequent clone (full length scrapie ORF in the mammalian expression vector) was confirmed by DNA Sequencing. The translation product of the cloned product was further confirmed to sheep prion protein using the SWISPROT, Geneva software. Also the molecular weight (Mw) of the resultant prion protein with the (*His* tag) was predicted to be 27 kDa (27887.4 Daltons) and the isoelectric point (pI) was theoretically projected to be 9.47.

The histidine tagged full length scrapie prion gene was overexpressed in Human neuroblastoma cells (SK-N-SH) as described in the materials and methods. The resultant protein was solubilized and extracted using the mammalian lysis buffer using appropriate

protease inhibitors and nucleases. The cell lysate with the expressed proteins were separated from the debris by centrifuging the lysate for 15 minutes at 7000 x g. The whole cell lysate was analyzed for prion protein expression by SDS-PAGE and Western Blotting.

SDS-PAGE and Western Blot

The expressed gene from the SK-N-SH cells contained the sheep normal prion protein with two mobilities of 27kDa, 33kDa which are the unglycosylated and diglycosylated forms respectively as shown in the Coomassie blue staining of SDS-PAGE (Figure 3). The proteins were identified after Western blotting by two monoclonal antibodies, mouse IgG F89/160.1.5 (Figure 4) and mouse IgG L42 against the prion protein (Figure 5). The expressed recombinant fusion prion protein had the 27kDa recombinant ShPrP^C (1-256) as visualized in the Western blots with both the anti-prion antibodies.

Purification of the Recombinant fusion protein (RecShPrP^C) using Ni-NTA Purification system

The full length PrP^C was expressed in SK-N-SH cells and purification was attempted using the Nickel-Nitriloacetate purification system. The SK-N-SH cells were used to overexpress normal sheep prion protein and were solubilized using 6M guanidinium chloride along with protease inhibitors initially. The solubilized cell lysate was centrifuged at 7000 x g and the filtrate was loaded directly into a Ni-NTA purification column. The major fraction eluted should be His-PrP^C when the pH was changed from 7.8 to 4.0. The eluted protein concentration was measured by Nanodrop ND1000 Spectrophotometer (Delaware, USA) and was determined to be 0.03mg/mL. As

this concentration was too low to be detected by western blotting, we tried several variations in the purification protocol. Changes were made in the pH of the elution buffer. The new pH tried was 3.5 instead of 4.0. Imidazole was used at varying concentrations (50 and 100mM) as a reducing agent based on previous studies in denaturing purification buffers. Some of these purifications yielded specific bands but of different molecular weight which were later identified as non- significant proteins by DNA fingerprinting performed at the DNA-Protein Core facility, Oklahoma State University. Repeated attempts using the native i.e. the non-denaturing conditions to purify the PrP^C and subsequently using the denaturing conditions also yielded no positive results. Earlier studies by others have also shown that even under strong denaturing conditions the efficiency of purification was very low (Caughey *et al.*, 1988, Scott *et al.*, 1988, Turk *et al.*, 1988, Pergami *et al.*, 1996).

Our original plan was to identify a mammalian expression system for the production of the native PrP^C, and to develop a purification method to make the protein available in the pure form. We were successful in fulfilling the first objective. However, we were unsuccessful in purifying significant amounts of this over expressed protein, a problem faced by earlier researchers. The successful development of a protocol for the overexpression of sheep PrP^C ($ShPrP^C$) using the Human neuroblastoma cells was developed during the course of this study. This is the first human neuronal cell system used for expressing the sheep prion protein. Also literature and previous studies in our lab have shown that the Nickel affinity column chromatography is not a good method for the purification for the prion protein. This can be due to a multitude of reasons, singly or in combination, for example, incompatibility of the tag and low efficiency of the

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components of the column can interfere with purification. Excessive amounts (2mL) of resin used can result in the overbinding of the nickel ions to the proteins. This can have an effect on the subsequent steps in the purification process. Use of lesser volume (100-200 µL) of the resin or use of a two tag purification column where, the protein is denatured in the first system and then renatured to its original form using the second system might unravel the difficulties in purification. However, this study failed to identify the specific cause for this.
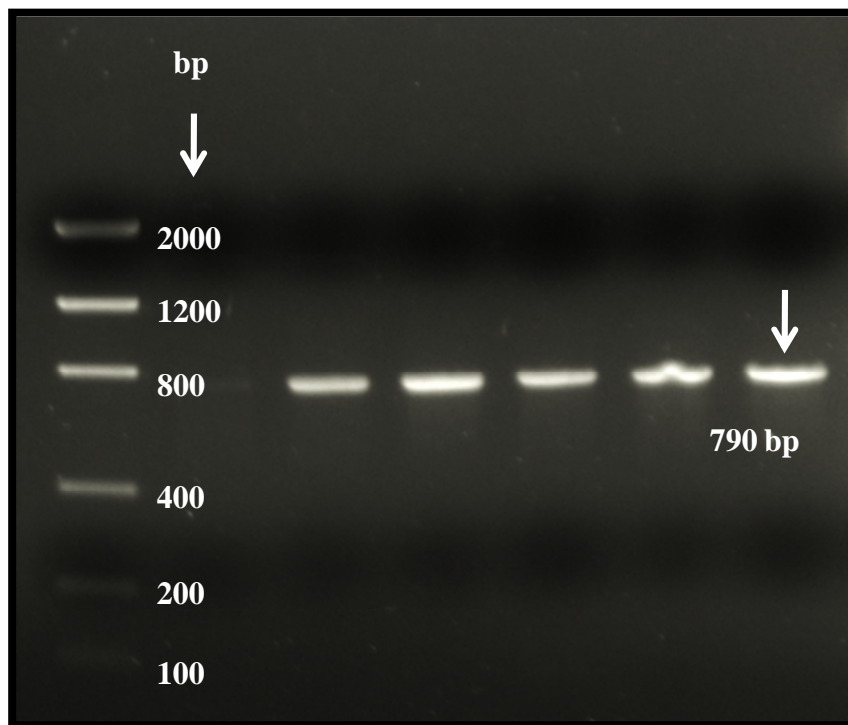
Figure 1
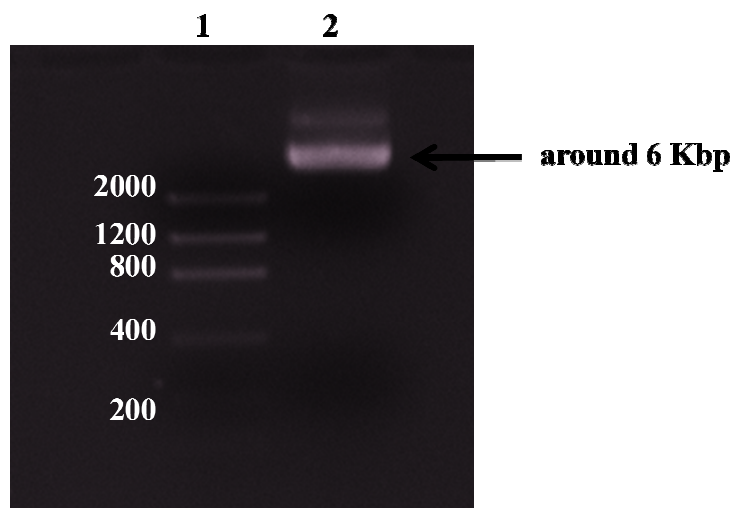
Figure 2

Figure 3

Figure 4

33 kDa
27 kDa
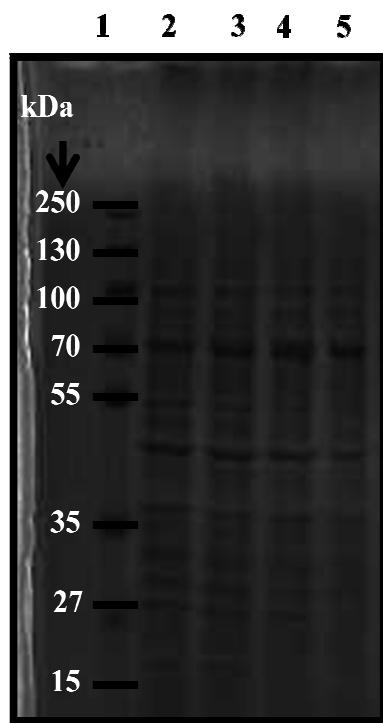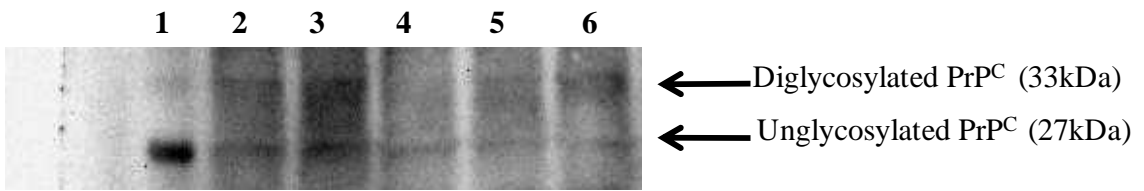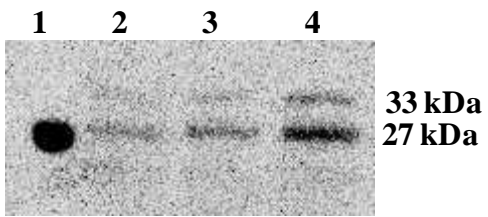
Figure 5

Figure Legends

Figure 1: PCR product of the entire open reading frame of sheep PrP^C in Zero Blunt vector ran on a 1% agarose gel. The PCR product was visualized by ethidium bromide staining.

Figure 2: The PCR amplified product of the *His*-PrP^C in pcDNA 6/*myc-His* B vector ran on a 1% agarose gel. The DNA bands were visualized by ethidium bromide staining. Lane 1 is the molecular weight marker ranging from 100 bp to 2000bp and lane 2 is the PCR product of the transformed *His*-PrP^C in mammalian expression vector.

Figure 3: SDS-PAGE analysis of the expressed $RecShPrP^C$. Prestained molecular marker (lane 1), 40 µg each of the cell lysate with varying concentration of Lipofectamine 5 µL, 10 µL and 20 µL were loaded onto lanes 2, 3 and 4. Lane 5 was the no target gene control.

Figure 4: Western blot analysis using the Monoclonal antibody F89/160.1.5 from the whole lysate after tranfection of the fusion protein. 8 µg of the Prestained protein marker was loaded onto lane 1 and 40 µg each of the cell lysate with varying concentration of Lipofectamine 5 µL, 10 µL and 20 µL were loaded onto lanes 2, 3 and 4. Control (No target gene) was loaded onto lanes 5 and 6 which show low expression.

Figure 5: Western blot analysis using the Monoclonal antibody L42 from the whole lysate after tranfection of the fusion protein. 8 µg of the prestained protein marker was loaded onto lane 1. 40 µg, 60 µg and 80 µg of the cell lysate were loaded onto lanes 2, 3 and 4.

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APPENDIX 1

Preparation of 1.2% agarose gel

1. Weigh 1.2 grams of agarose (Cat # A5304, Sigma, St.Louis, MO) and add it to 100mL TBE buffer in an Erlenmeyer flask.

2. Heat this mixture in a microwave approximately for 2 minutes, so that the agarose melts. Take caution that this doen not get boiled and spills over.

3. Working under the hood, add 5µL of ethidium bromide (final concentration of 0.5 µg/mL) (Cat # 7627, Sigma, St. Louis, MO) to the melted agar and mix gently by swirling it. (Note: Ethidium bromide is a carcinogen).

4. Prepare the gel cast ready by taping both the ends to hold the melted agarose and pour the agarose to it. Place the comb and allow to set.

10X TBE Buffer

1. Tris Base (Cat # T4661, Sigma, St.Louis, MO)   : 108 g

2. Boric Acid   : 55 g

3. EDTA (Cat # E7889 Sigma, St. Louis, MO)   : 9.3 g

4. ddH$_2$O make up to   : 1 L

   Total volume   : 1 L

APPENDIX 2

Polymerase Chain Reaction (PCR)

1. Make the following recipe for the PCR mix

   ddH$_2$O : 14.5 μL

   PCR buffer (10x) with MgCl$_2$: 2.8 μL

   (Cat # E00007, Genscript, Piscataway, NJ)

   Forward primer (10mM) : 0.4 μL

   Reverse primer (10mM) : 0.4 μL

   dNTPs (10mM) : 0.4 μL

   (Cat # D0056, Genscript, Piscataway, NJ)

   Taq polymerase (2.25mM) : 0.25 μL

   (Cat # E00007, Genscript, Piscataway, NJ)

   Plasmid DNA (100ng) : 1.0 μL

   ....

   Total : 20 μL

2. Touch spin the PCR mix on table top centrifuge and place onto a thermocycler
   (MJ Research PTC-200 Thermal cycler, DNA engine, Ramsey, MN) with the
   following PCR conditions:

   i. Initial denaturation at 95^0 C for 2 minutes,

   ii. Denaturation at 95^0 C for 30 seconds,

   iii. Annealing at 55^0 C for 30 seconds,

   iv. Extension at 72^0 C for 1 minute.

v. Final Extension at 72^0 C for 30 seconds and repeat steps (ii to v) 30

times.

3. Upon completion of the PCR reaction, add 3 µL of 6X sample buffer (Cat# 161-
0767-EDU, Biorad Laboratories, Hercules, CA) to the PCR product and run on a
1.2% agarose gel with ethidium bromide (final concentration 0.5µg/mL).

4. Visualize the gel using an imaging system HR 6100 system, (UVP Gel Doc-IT
Imaging system, Upland, CA) to analyze the PCR results.

APPENDIX 3

Diagram showing cloning the full length ovine prion gene onto pcDNA6/*myc*/His

mammalian expression Vector (Invitrogen, Carlsbad, CA)
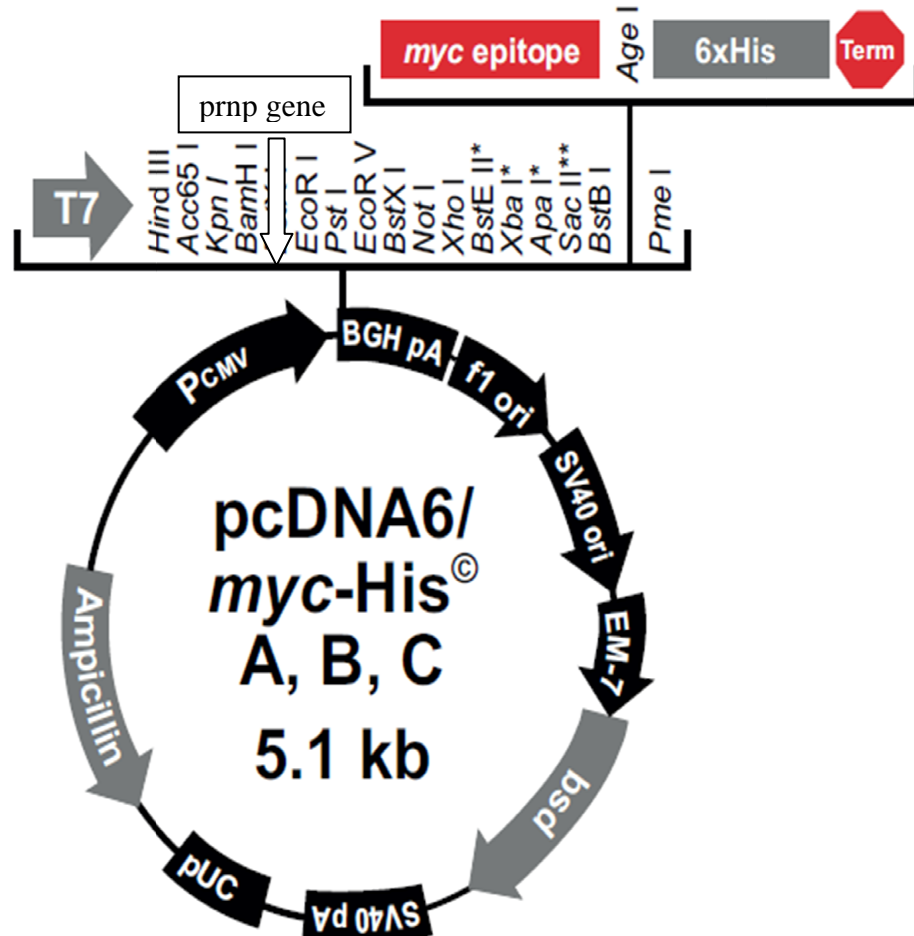
APPENDIX 4

DNA sequence analysis of the entire prion gene ORF with C and N terminal

signal peptides in Mammalian expression vector

C03_PrPinMEV-T7PRO.ab1

KB 1.2 KB.bcp

PrPinMEV-T7PRO

KB_3730_POP7_BDTv1.mob
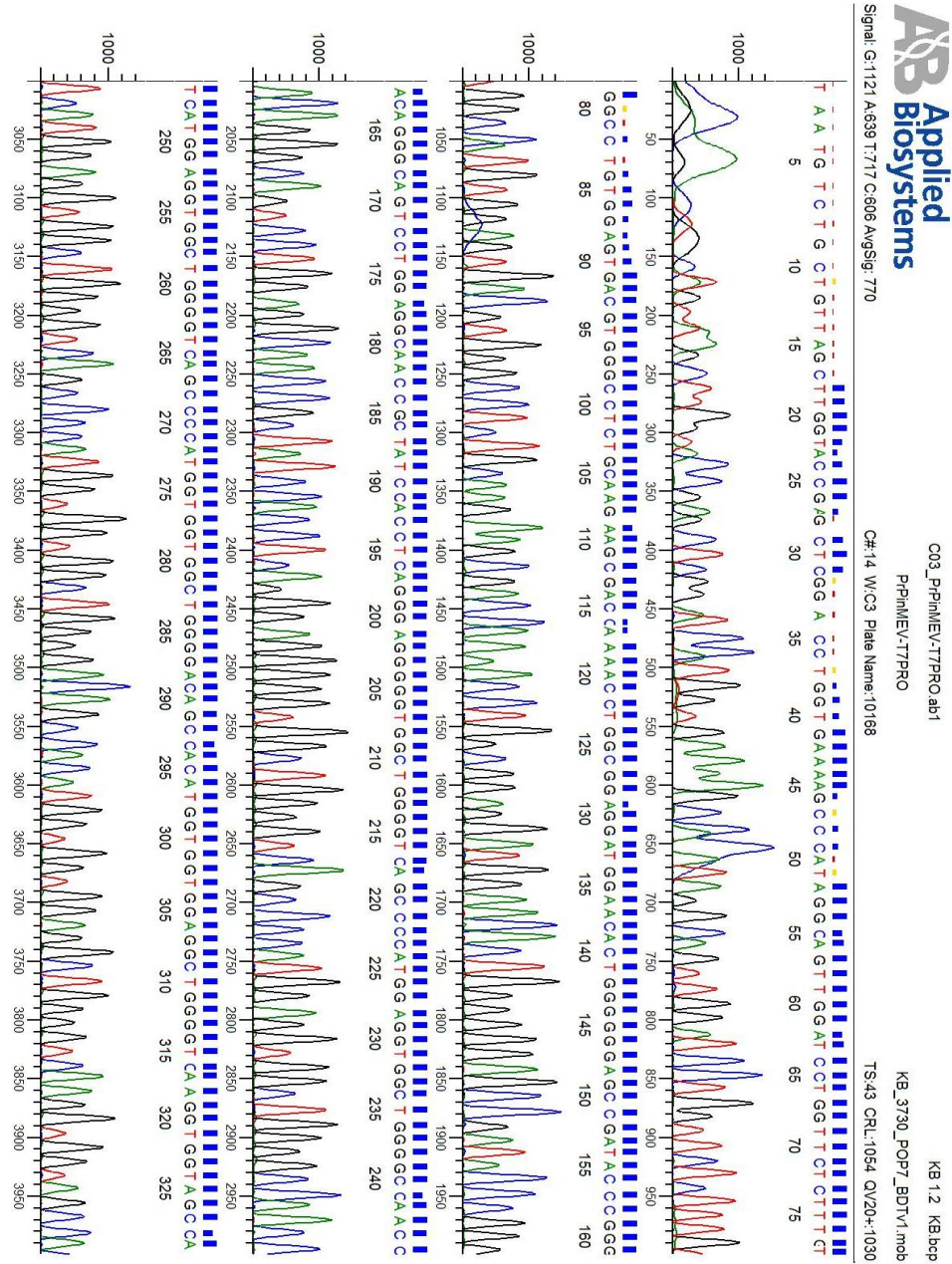
Signal: G:1121 A:639 T:717 C:606 AvgSig: 770

C#:14 W:C3 Plate Name:10168

TS:43 CRL:1054 QV20+:1030

Diagram showing cloning the full length ovine prnp gene onto pCR- BLUNT II -

Topo Vector (Invitrogen, Carlsbad, CA)
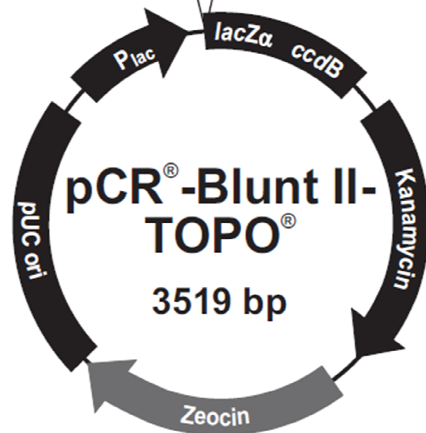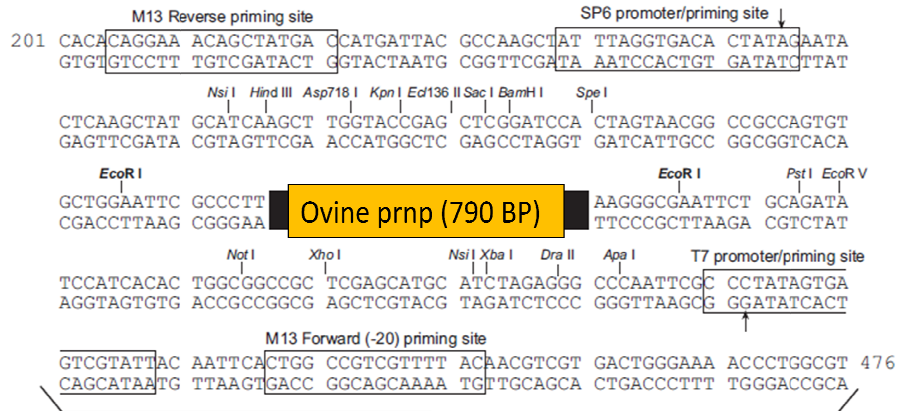
DNA sequence analysis of the entire prion gene ORF in Zero blunt vector

APPENDIX 7

Cell culture of Human neuroblastoma cells on Days one, three and nine
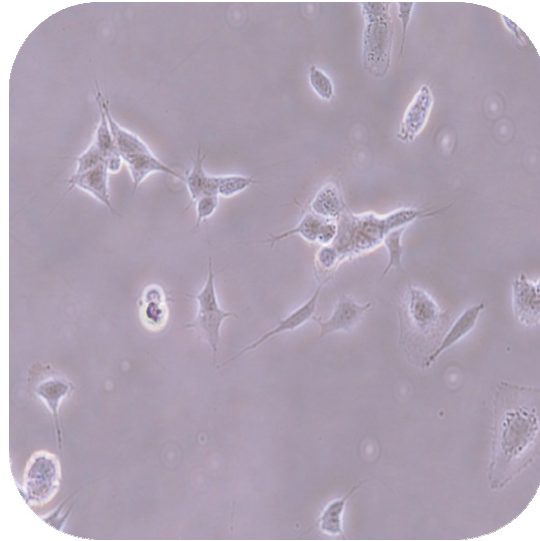
Day 1- cells attaching to the culture plate

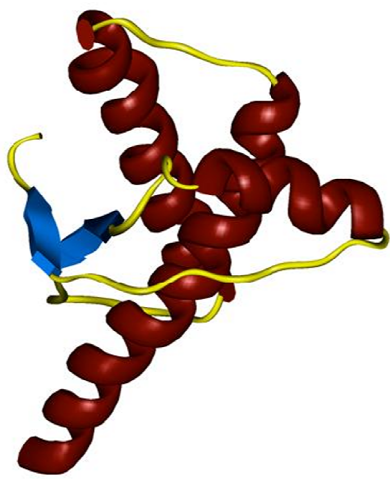

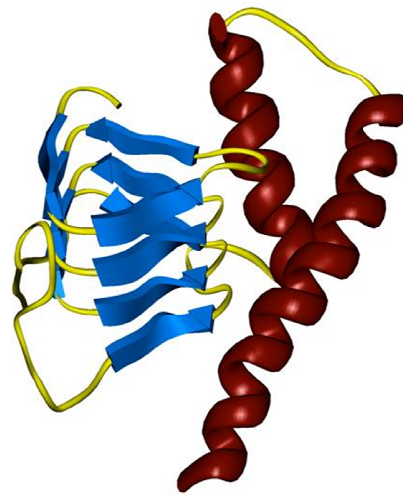Day 3- cells are 30-40% confluent

Day 9- cells are 90% confluent

Structures of normal and abnormal prion proteins

(Ory Jillian, 2006)

Normal prion protein   Abnormal infectious prion protein

APPENDIX 9

Coding region of sheep (Ovis aries) prion protein

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGWG

QPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHSQWNKPSKPKTNMKHVAGAAAA

GAVVGSLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQYSNQNNFVHDCV

NITVKXHTVTTTTKGENFTETDIKIMERVVEQMCITQYQRESQAYYQRGAS**VILFSSPPVILLISFLI**

FLIVG (NCBI accession number P23907).

VITA

Sujitha Prasad

Candidate for the Degree of Master of Science

Thesis: Expression and detection of Recombinant sheep prion protein (RecShPrP^C)

using Human neuroblastoma cells and Western blotting

Major Field: Food Science

Biographical:

Education:

Bachelor of Science in Microbiology, Bharathiar University, Coimbatore, Tamil
Nadu, India, April 2004

Master of Science in Biotechnology, Bharathiar University, Coimbatore, Tamil
Nadu, India, April 2006

Completed the requirements for the degree of Master of Science in Food
Science at Oklahoma State University, Stillwater, OK in May 2009

Experience:

Research Associate (August, 2008-May 2009)
Teaching Associate (Jan, 2007-August, 2008)

Professional Memberships:

Institute of Food Technologists

Name: Sujitha Prasad   Date of Degree: May, 2009

Institution: Oklahoma State University   Location: Stillwater, Oklahoma

Title of Study: EXPRESSION AND DETECTION OF RECOMBINANT SHEEP
                PRION PROTEIN (RecShPrP^C) USING HUMAN NEUROBLASTOMA
                CELLS AND WESTERN BLOTTING

Pages in Study: 61   Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study:
The objectives of the present study was to overexpress the sheep normal prion protein
(ShPrP) using a mammalian expression vector. The prion protein gene which was in the
zeroblunt vector was PCR amplified and was subcloned into *pcDNA6/myc/HisB* plasmid.
The quality of the PCR reaction and the orientation of the cloned gene within vector were
determined by DNA sequencing. Upon confirmation of this, the *His*-tagged fusion
protein gene was transfected into human neuroblastoma cells (SK-N-SH) for
overexpression of the protein. The proteins expressed were solubilized in guanidinium
chloride and purifications were attempted with different conditions using the Nickel
Nitriloactetate chromatographic system.

Findings and Conclusions:
We developed an expression system for overexpressing the sheep normal prion protein
with the human neuroblastoma cells. We also conclude that the prurification of this
particular protein using the nickel affinity chromatographic column did not yield
significant quantities of the same.

ADVISER'S APPROVAL: Dr. Christina Mireles DeWitt_____