

Prion research in Dublin

A Sugary Prion Blocker

Cyclodextrins usually play the supporting role in pills and tablets, encapsulating the drug. Hilary McMahon's group in Ireland has now found cyclodextrins to be capable of inhibiting the conversion of prion protein into its harmful form. Interestingly, the inhibitory mechanism seems to be different from those of other known anti-prion compounds.

Prion diseases are still rather a mystery. Although research has discovered a lot about the causative agents of BSE, scrapie and so on in recent years, diagnosis of Transmissible Spongiform Encephalopathies (TSEs) is still in its infancy, there are no cures and basic researchers are only beginning to understand where and how the normal prion protein misfolds into its harmful variant.

Hilary McMahon was attracted to this emerging field in 1998, when prion diseases were in the news; she had just finished her PhD on amylases in *Streptomyces*. Although her previous work at the Department of Industrial Microbiology at University College Dublin (UCD) seemed to have no connection at all to prions, she appreciated the training. "It gave me the biochemical background, which I think is very important for prion biology," she says.

Also she thought, "If you get a PhD you can do almost anything and you can apply your mind to whatever you want to apply your mind to". She decided to go to France and spent eighteen months in Sylvain Lehmann's lab in Montpellier applying her mind and training to the implications of oxidative stress on prion diseases.

The prion protein can come in two types: the normal, cellular prion protein (PrPC), found predominantly in the neural cell, and its malformed twin, the scrapie isoform of the prion protein (PrPSc). PrPSc is an infectious protein as it causes every PrPC it comes into contact with to modify its conformation. This eventually leads to the neuropathologies

known as BSE, scrapie and Creutzfeldt-Jakob disease.

Prion research is full of hypotheses about the function of PrPC and why the modification of its conformation, leading

to PrPSc, has disastrous consequences in the nervous system. It has been shown that the octapeptide region at the aminoterminal of the protein binds copper, so in many of the hypotheses PrPC has a role in the oxidative state of the cell in regulating copper transport and/or through a modification of the Cu/Zn superoxide dismutase activity.

Together with her colleagues at Montpellier, McMahon added some important pieces to this puzzle. They found that a prion infection impairs the cellular response to oxidative stress in neuronal cells (*PNAS*, 2000, 97, p.13937). They also showed that the normal cellular prion protein undergoes a site-specific cleavage on exposure to a reactive oxygen species (*Journal of Biological Chemistry*, 2001, 276, p.2286).

McMahon explains, "The prion protein's ability to bind metal ions in the octapeptide region leads to a susceptibility to stress cleavage and in the presence of a reactive oxygen species the prion protein loses the aminoterminal. Another research group has extended upon that since

then and found that the cleavage leads to protective abilities for the prion protein."

After she returned to Ireland in October 2000 McMahon became a lecturer at her college, UCD, in the unit now known as Microbiology within the School of Bio-

molecular and Biomedical Science. In 2002 she was appointed to the faculty of science and continued her prion research with her own group, a group that is changing in size. "We're going through a transition at the moment. The first cohort of three PhD students graduated just last year, one of them, Marguerite Prior, was first author of the cyclodextrin-paper. She's 'postdocing' on Alzheimer's disease in the US," McMahon says.

Patents in the pipeline

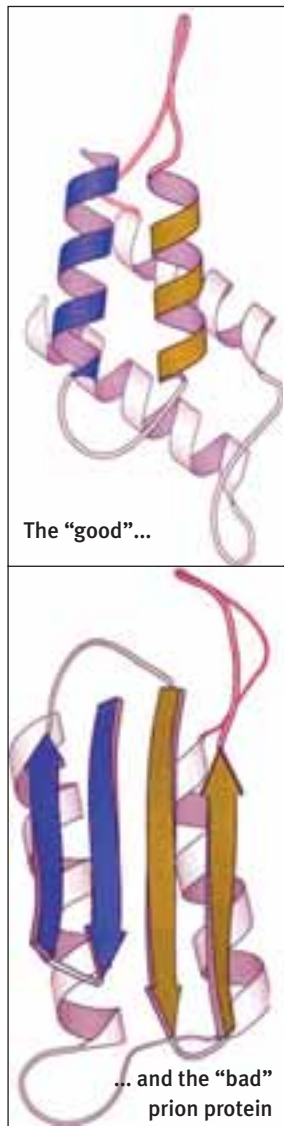
The group is still doing research into oxidative stress. However, she can't tell us much about current results, because of potential patents. She can only tell us that they are currently putting together a publication about a protein that modifies the infection process by controlling the Cu/Zn superoxide dismutase activity.

She can, however, talk in detail about the other field of research she started after her return to Dublin. To find compounds with possible therapeutic value in prion diseases, McMahon and her group have screened numerous substances for their effects on prion diseases in cell culture. "We have looked at proteases, amylases, dextrans and other glucose-containing moieties, that I came across in my PhD," she says.

From starch to prions

They have identified cyclodextrins as a new anti-prion drug that has the ability to reduce the pathogenic isoform of the prion protein, PrPSc to undetectable levels in scrapie-infected neuroblastoma cells (*Journal of Virology*, 2007, 81, p.11195). "It seems bizarre, that in some way my research in prions has a direct link to my PhD as some of the enzymes I studied back then have the capacity to produce cyclodextrins when degrading starch," McMahon says.

Cyclodextrins (CD's) are cyclical oligosaccharides made of glucose monomers linked alpha-1-4 to one another. Some typical, naturally occurring cyclodextrins are alpha-CD (six glucose-units), beta-CD (seven glucose-units) and gamma-CD (eight). "The ring of sugar molecules creates a cone



shape, so the compound forms a cavity that can encapsulate moieties in it," she says.

Cyclodextrins have found a wide range of applications in the food, pharmaceutical and chemical industries, as well as agriculture and environmental engineering over the last few years because of their ability to lodge hydrophobic compounds, while being water-soluble themselves. They are used to encapsulate the active pharmaceutical ingredients in drugs as well as to remove cholesterol in food, producing "low-fat" products.

McMahon states, "The first way that I believed the CD's work is through the sequestering of cholesterol. Cyclodextrins are well-known for their ability to do this and compounds that interfere with cholesterol have been shown to have a potential therapeutic value. However, if they work purely by sequestering cholesterol, then if

ferent raft domains to the PrPSc. Therefore it appears that cyclodextrins move the different proteins away from each other. This observation is consistent with the assumption that lipid rafts are a potential conversion area where PrPC unfolds and refolds abnormally leading to PrPSc and that separating the two should lead to a hindrance of the conversion.

More than one skill

However, as there is still some overlap in their location, separation cannot be the sole mechanism of action. That's why they presumed the CD's to have some effect beyond that. They also know that cyclodextrins also have the capacity to bind proteins. "In the group, where I did my PhD, we used cyclodextrins as a one-step-purification method for amylases," McMahon says. Affinity-chromatography confirmed that cyclodextrins

have the capacity to bind the normal prion protein.

"The consequence of this binding is that it interferes with the potential conversion as we showed in an *in vitro* conversion assay. It's possible that cyclodextrins cause some hindrance so the PrPSc can't bind the PrPC or that it can't cause the PrPC to unfold and convert."

Besides looking at

the new drug's mechanisms of action and the interaction of oxidative stress with prion diseases, McMahon's group opened up a new field recently, which will provide another link to her training in industrial biotechnology. "We have so many resources available within microbiology, that we are screening numerous *Streptomyces*, fungi and bacilli for new proteases with the capacities to degrade the abnormal prion protein. This is important, because one of the characteristics of PrPSc is to be protease resistant with the usual enzymes. We've identified a new strain of *Streptomyces* that has the capacity to degrade PrPSc." Another surprise: it's a thermophile again, as was the one in her PhD.

It will be interesting to see how many connections she can make between the two fields of *Streptomyces* enzyme technology and prion biology, fields that did not seem to have much in common at first glance.

BRYNJA ADAM-RADMANIC



Young prion experts in Dublin (from left to right): Mary Murphy, Karl McEvoy, Hilary McMahon, Brendan Molloy

you reintroduce cholesterol you should be able to actually reverse the effect of cyclodextrins. When we did that, it didn't work, so it must be by some other effect that the CD's work."

The group also found that the administration of cyclodextrins modifies the location of PrPC and PrPSc within the cell, which is something unusual. Both PrPC and PrPSc are found in specialised membrane domains, cholesterol- and glycosphingolipid-rich lipid rafts also called Detergent Resistant Microdomains (DRMs). There the prion proteins associate with saturated raft lipids with their glycosyl-phosphatidylinositol (GPI) anchor.

Shifting rafts

Prior to treatment with cyclodextrins the two isoforms are located in similar raft domains, but when the cells are treated with the cyclodextrins they shift their location, so that the PrPC is floating in dif-