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At autopsy, the brains of Alzheimer's patients (right) are filled with amyloid plaques, reminiscent of the plaques seen in the brains of animals with scrapie (left).

AMYLOID

Little proteins, big clues

After a quarter of a century, the amyloid hypothesis for Alzheimer's disease is reconnecting to its roots in prion research.

BY JIM SCHNABEL

In September 1984, a group of prominent researchers from around the world met in Scotland to discuss a disease that afflicted sheep and goats.

Scrapie, as they called it, was important for more than agricultural reasons — it was also the most easily studied example of an emerging class of diseases that destroyed the brain. The illnesses jumped infectiously from animal to animal, yet yielded no trace of a virus or other microorganism. One big clue was that these diseases left behind insoluble clumps, or plaques, made from millions of tiny fibrils, each of which comprised hundreds or thousands of proteins. A striking new hypothesis proposed that these fibrils and their plaques marked the toxic passage of infectious proteinaceous particles, or prions.

On the first night of the conference, several researchers gathered for dinner. Among them were Colin Masters, a neuropathologist from the University of Western Australia, and Konrad Beyreuther, a protein-sequencing expert from the University of Cologne in Germany. Masters began telling Beyreuther about a human disease that featured plaques like those seen in scrapie and seemed to be very common. It was called Alzheimer's disease.

"Until then I had never heard of Alzheimer's disease," Beyreuther recalls.

It is easy to forget how recently Alzheimer's disease entered the public consciousness. For many decades after it first appeared in the medical literature, the term referred only to an obscure, early onset form of dementia. What we now know as common, late-onset Alzheimer's was then called 'senile dementia' — and it was so prevalent among the elderly that it hardly seemed worth classifying as a disease (see 'A problem for our age', page S2).

THE MYSTERY PROTEIN

It is also easy to forget that at the dawn of Alzheimer's research, the disease was suspected of being a prion disease — we tend to think of the connection between prions and Alzheimer's as being much more recent. In late 2010, for example, a team led by Mathias Jucker at the University of Tübingen, Germany, reported that they could, in essence, transmit Alzheimer's-type brain pathology in a prion-like manner by injecting Alzheimer's brain matter into the bodies of mice (Eisele, Y. S. *et al. Science* 330, 980–982, 2010). Such findings have contributed to a major rethink of the cause of Alzheimer's disease. But this rethink is partly a renaissance because, as the story of Masters and Beyreuther's early interest in Alzheimer's reminds us, the prion connection is not new. "It was there at the beginning," says Masters.

As Masters knew in 1984, autopsies of Alzheimer's patients revealed brain plaques

resembling those seen in scrapie, often surrounded by dying neurons and their twisted axons and dendrites. When doused with Congo red, a standard pathology stain, and illuminated with polarized light, the Alzheimer's plaques — just like scrapie plaques — displayed an apple-green shimmer, a prismatic sign of the hydrogen bonds that held their fibrils tightly together. Protein aggregates that had this peculiar property were called amyloids.

Earlier that year George Glenner, an amyloid researcher at the University of California, San Diego, reported isolating a small protein from amyloid deposits in brain blood vessels in people with Alzheimer's disease. Was the protein embedded in Alzheimer's brain plaques the same as the one in Glenner's vascular deposits? Or was it more like the scrapie protein?

Masters and Beyreuther, at their dinner in Scotland, agreed to collaborate to find out, and their partnership probably did more than any other to launch modern Alzheimer's research and its central idea: the amyloid hypothesis.

Masters had already painstakingly purified a quantity of Alzheimer's amyloid, in a process akin to bomb-grade uranium enrichment. When a sample arrived in Germany, Beyreuther and his colleagues broke it down with formic acid and sifted through

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the debris to find the smallest stable protein. This turned out to be a tiny peptide of roughly 40 amino acids, and Masters and Beyreuther called it A4. Sequencing A4 showed that it was not the scrapie protein, or indeed anything like it, but was essentially the protein Glenner had isolated from blood vessels.

Beyreuther's team quickly determined that A4 is a fragment of a much larger neuronal protein, amyloid precursor protein (APP). They found the gene that encodes APP on chromosome 21. This was a big clue, as people with Down's syndrome, who have an extra copy of chromosome 21, were known to develop Alzheimer's-like brain plaques by 40 years of age. The overproduction of APP and A4 was now revealed as the likely reason for the plaques in Down's syndrome — and probably in Alzheimer's disease too.

TOO MUCH AGGREGATION

Other Alzheimer's investigators readily pursued the APP lead. But three other important clues from this initial burst of research by Beyreuther and Masters would be almost entirely overlooked for most of the next decade.

The first was an observation by Beyreuther about the forms of A4 in different solvent mixes. He noted the presence of stable clusters, or oligomers, made of two, four or more copies of A4. So strong was the peptide's tendency to form these oligomers that in certain solutions, dimers made of two copies of A4 were more prevalent than monomers.

The second clue was that full-length A4 is extremely prone to aggregate. After obtaining the full A4 sequence, Beyreuther began to synthesize various lengths of it in his lab, including a series that started at the 42nd (and terminal) amino acid of its longest variant and worked towards the opposite end. "When we came close to the end of the peptide and took it off the resin, we saw it getting aggregated," he remembers. "I thought 'Mein Gott, it's snowing!' It was aggregating so quickly. It was horrible."

The third clue came after Beyreuther and Masters raised the first antibodies to A4 and used them to detect amyloid deposits with unprecedented sensitivity in autopsied brains. The deposits were much more extensive than anyone had realized and were almost always present in people older than 80 years of age. In younger brains the plaques tended to be sparser and more diffuse, but they were still detectable in about 20% of cognitively normal people who had died in their fifties. The implication was that Alzheimer's disease is almost inevitable, with plaques beginning to form in the brain three decades before symptoms develop. "I thought that was amazing," says Beyreuther.

THE AMYLOID HYPOTHESIS

By the end of the 1980s, Beyreuther and Masters had largely completed their discovery work on A4. Other scientists, mostly from the United States, took the lead on Alzheimer's research,

Like prions (above), amyloid- β might spread in an infectious manner within tissues.

and one of their first acts was to rename the A4 protein amyloid- β , where the β referred to the classic β -sheet molecular structure of amyloids. They also put much less emphasis on the original prion connection. "Some of these young guys who came after us didn't seem to know what a prion was," says Masters.

Even so, they seemed to move swiftly towards an understanding of how amyloid- β causes Alzheimer's disease. In the early and mid-1990s, *in-vitro* studies indicated that amyloid- β becomes toxic to neurons when it begins to aggregate. Genetic studies of families with early onset Alzheimer's disease detected mutations within the gene that encodes APP, and analysis of one of these mutant APP genes found that it causes a sevenfold overproduction of amyloid- β (see 'Finding risk factors', page S20). Transgenic mice that overproduced human APP and amyloid- β developed plaques resembling those seen in Alzheimer's disease, and their behaviour in standard tests suggested some cognitive deficits. The amyloid hypothesis seemed straightforward: when the amyloid- β concentration in the brain becomes too high, the protein aggregates into fibrils and plaques, and begins killing neurons.

It eventually became clear that the situation was not quite that simple. Further genetic studies showed that familial, early onset Alzheimer's is usually caused not by the overproduction of total amyloid- β , but by the relative overproduction of a less common variant of amyloid- β known as A β 42, the full-length, 42-amino-acid variant whose extreme proneness to aggregation had so alarmed Beyreuther.

The A β 42 findings were still consistent with the plaque hypothesis, particularly once researchers recognized in the mid-1990s that the variant in most plaques is A β 42. The problem was that mouse models with an over-dose of A β 42 — like the first Alzheimer's mouse

models that overexpressed APP — lacked the heavy neuronal losses and cognitive decay associated with the human disease. "These models have some cognitive decline, but it's not as much as a person with full-blown Alzheimer's disease, by any stretch," says Harvard neurologist Bruce Yankner, a long-time Alzheimer's researcher.

Some researchers suspected that mice, with their small brains and short lives, cannot accurately model such a slow-burning, big-brain disease. But another possibility, which gained currency in the late 1990s, is that amyloid- β plaques are not the real drivers of dementia. Autopsy studies showed, for example, that the progress of Alzheimer's dementia does not correlate well with the development of plaques. As Beyreuther and Masters had initially observed, the plaques become dense in the brain long before any signs of cognitive decline.

Unfortunately, the major pharmaceutical companies had already placed their bets on the amyloid- β plaque hypothesis, and numerous drug-development programs would go on to fail in clinical trials. But in the meantime, a small group of researchers had begun to develop a new hypothesis that encompassed Alzheimer's and a variety of other amyloid-forming diseases.

OLIGOMERS REVISITED

The genetic evidence made it almost certain that the aggregation of amyloid- β somehow leads to Alzheimer's disease. The fibrils in plaques were the most obvious type of aggregate, and therefore the most obvious suspect. Only after the plaque hypothesis began to fail did researchers return to the other aggregates: the amyloid- β oligomers first seen by Beyreuther and his colleagues in Cologne.

In the early and mid-1990s, Charles Glabe at the University of California, Irvine, and Dennis Selkoe at Harvard University reported finding oligomers in experiments with amyloid- β . They saw them as briefly existing intermediates on the way to disease-causing fibrils, rather than fully fledged drivers of disease. But in 1998, William Klein's lab at Northwestern University in Evanston, Illinois, reported that oligomers could be the true culprits in Alzheimer's disease. When Klein's team added a chemical to a solution of amyloid- β to stop it forming fibrils, the amyloid- β instead formed oligomers, which then began to kill nearby neurons. At least some of this toxicity seemed to be the result of the oligomers weakening the synapses — the junctions between neurons — and impairing their ability to contribute to learning and memory (see 'Two pathways of aggregation'). Similar results soon followed from the Selkoe and Glabe labs, and in time mouse models also demonstrated oligomer toxicity.

In the 2000s, a new consensus began to emerge: that amyloid- β fibrils are weakly toxic on their own, that they seem to provoke harmful inflammation, and that they are prone, especially

TWO PATHWAYS OF AGGREGATION

Individual amyloid- β peptides, which are produced normally by neurons, can assemble in at least two ways. One pathway leads to insoluble, plaque-forming fibrils. The other pathway leads to soluble oligomers, which are small enough to enter synapses. These oligomers are suspected to be the main toxic species in Alzheimer's disease.

when their plaques become especially dense, to slough off soluble amyloid- β that can then reform into oligomers. But in this model, amyloid- β oligomers are the more worrisome neurotoxins. Indeed, the amyloid- β fibrils are probably protective to the extent that they trap aggregating amyloid- β in a less harmful form.

Amyloid- β oligomers are now thought to exert their harmful effects by binding directly to the membranes of neurons, or to specific receptors — the insulin and NMDA glutamate receptors are suspects — needed for neuronal signalling. But if amyloid- β oligomers were merely toxic to neurons, they might never overwhelm the clearance mechanisms of the brain and cause disease. To do that, they seem to need another deleterious property that is associated with prions: infectiousness.

PRIONS REVISITED

The idea that Alzheimer's might be a prion disease was first suggested in 1984 by the future Nobel laureate Stanley Prusiner of the University of California at San Francisco. His idea was widely dismissed after amyloid- β was found to be different from scrapie protein. But by the mid 2000s, it was clear that Prusiner had been essentially correct. Both amyloid- β and prion-disease proteins could fall into a state that was both toxic and self-replicating.

Prusiner, who was also at the dinner in Scotland with Masters and Beyreuther, was apparently wrong about the replication mechanism. He had initially proposed that an infectious prion is a protein monomer with

a misfolded shape that can induce the same misfolding in normal versions of the protein.

But as the chemist Peter Lansbury, then at Massachusetts Institute of Technology, showed in a series of *in-vitro* experiments in the mid-1990s, the key self-replicator in prion diseases and Alzheimer's disease appears to be an oligomer, not a monomer. Once formed, the oligomer becomes a template, or 'seed', that attracts new monomers, and aggregation around that nucleus proceeds rapidly. "This is one of those nonlinear phenomena in which small changes can have big effects," says Lansbury, now chief scientist at Link Medicine, a biotechnology company in Cambridge, Massachusetts.

One type of nucleus would serve as a template for new oligomers. Another would seed ever-lengthening fibrils. Lansbury showed that this initial nucleation event happens faster with a particularly sticky stretch of amino acids found on both prion proteins and A β 42. Adding this stretch from A β 42, or even adding full-length A β 42, can trigger the runaway aggregation of all the amyloid- β in the vicinity. Beyreuther's snow metaphor

Alzheimer's disease is almost inevitable, with plaques beginning to form in the three decades before symptoms develop.

was apt: a similar nucleation phenomenon lies at the heart of ice and snow crystallization.

More recently, Jucker and others have shown

that brain matter containing amyloid- β from Alzheimer's patients can nucleate plaques in mice. Amyloid- β is less hardy than prion proteins and so is much less likely to jump from one person to another, but it does seem to spread in an infection-like manner within tissues. "I was away from amyloid- β research for years, but I've become interested again since Jucker showed that the stuff is infectious," says Beyreuther, who is now at the University of Heidelberg.

Similar infectious properties have been observed for aggregates of tau protein, which appear in Alzheimer's-affected cells late in the disease, as well as for α -synuclein protein in Parkinson's disease. Researchers suspect that numerous other amyloid-linked diseases feature the same toxic, oligomeric mechanisms and involve a slow spread of pathology starting in the regions of the brain most vulnerable to the disorder. "We know, for example, that people who present with Parkinson's motor signs are almost always going to have Parkinson's dementia 20 years later," says Lansbury. In contrast, Alzheimer's disease affects memory and cognition quite early on.

In principle, according to Beyreuther, there could be protein structures in our food, air and water that get into the brain and promote disease-causing spirals of protein aggregation "like the little bit of dust that seeds the ice crystals in the windows," he says. "If that's true, then we are in trouble." ■

Jim Schnabel is a science writer based in Miami, Florida.