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Aaron Klug and the study of Alzheimer's disease

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Aaron Klug, who died on November 20, 2018, was a giant of molecular biology (1–3). He was also a major figure in the study of dementia, especially Alzheimer's disease. In 1982, Aaron was the sole recipient of the Nobel Prize in Chemistry “for his development of crystallographic electron microscopy and his structural elucidation of biologically important nucleic acid-protein complexes” (4). In 1983, Martin Roth, Professor of Psychiatry at the University of Cambridge, brought the tangle problem of Alzheimer's disease to Aaron at the MRC Laboratory of Molecular Biology (LMB). Roth is perhaps best known for showing that presenile and senile dementias are similar, both clinically and neuropathologically (5,6). The work that developed in Cambridge over the next five years was a collaboration between the LMB and the Department of Psychiatry with its associated Brain Bank.

Neurofibrillary tangles consist of a majority of paired helical filaments (PHFs) and a minority of straight filaments (SFs). Before being part of the multidisciplinary effort set up by Aaron, which was aimed at identifying what PHFs are made of, Claude Wischik and Tony Crowther used tangle preparations from Alzheimer's disease brain and 3D image reconstruction to show that the PHF consists of a double-helical stack of transversely oriented subunits (7,8). Crowther later demonstrated that PHFs and SFs share a common structural unit (9). Both types of filament have a cross- β structure, the defining feature of amyloids (10). Although Michael Kidd described the PHF as the major structural component of the neurofibrillary tangle of Alzheimer's disease in 1963 (11), its molecular nature was only elucidated in the 1980s.

The PHF, which is devoid of known biological activity, is defined by its appearance. Purification entails loss of morphology, implying that electron microscopy alone cannot be used. Aaron reasoned that one required a label that identifies both intact filaments in microscopy and the protein bands from purified tangle preparations in gel electrophoresis. Thus, Michal Novak and Cesar Milstein produced monoclonal antibodies against purified tangle fragments. Ross Jakes, Crowther and Wischik showed that antibody 6-423, which recognises tau protein truncated at E391, decorated PHFs isolated from tangle fragments in electron microscopy and labelled a 12 kDa band in gel electrophoresis. John Walker obtained the partial amino acid sequence of this band, which I used to clone and sequence cDNAs from a human brain library. The predicted amino acid sequence of 352 amino acids was unlike that of any known protein, but RNA blotting suggested that it might correspond to an isoform of tau protein. This was confirmed with the publication of the predicted amino acid sequence of a mouse tau isoform by Gloria Lee, Nicholas Cowan and Marc Kirschner

in January 1988 (12), part of which Kirschner had read to Aaron over the telephone in late 1987.

Although there had been several studies reporting the presence of tau-like immunoreactivity in neurofibrillary tangles or in PHFs, the first by Jean-Pierre Brion and colleagues in 1985 (13), the Cambridge work proved that tau protein is an integral PHF component. Tau filaments consist of two parts, which were called ‘core’ and ‘fuzzy coat’. The core region corresponds to the tau sequence required for a filament to look like a filament. It was shown to contain repeats (six tau isoforms are produced in human brain through alternative mRNA splicing from a single gene. Three isoforms have three repeats each and the other three have four repeats each; their ratio is approximately 1:1. In non-assembled tau, the repeats, which are 31 or 32 amino acids long, function in microtubule binding). The fuzzy coat consists of the rest of tau and can be removed by protease treatment, leaving the core region intact.

We presented these findings at the Banbury conference on “The Molecular Biology of Alzheimer’s Disease” organised by Caleb Finch and Peter Davies in April 1988 (14,15). It was my first meeting on Alzheimer’s disease and it was memorable, not least because of some of Aaron’s comments during the discussion. Our papers demonstrating that tau protein is an integral component of tangle filaments were published in June and July 1988 (16–18).

From the beginning, Aaron saw the work on tau as a means towards understanding the pathogenesis of Alzheimer’s disease, not as an end in itself. Two papers with Maria Grazia Spillantini and others described the morphological features and spatial characteristics of tau and beta-amyloid deposits in Alzheimer’s disease brain (19,20). Over time, it became clear that tau is also central to several other neurodegenerative diseases, where it assembles in the absence of beta-amyloid deposits. Nonetheless, the relevance of tau pathology for the neurodegenerative process was repeatedly questioned.

The identification of mutations in *MAPT*, the tau gene, in familial forms of frontotemporal dementia in June 1998, proved that dysfunction of tau is sufficient to cause neurodegeneration and dementia, with the formation of tau filaments being the likely gain of toxic function underlying all tauopathies, including Alzheimer’s disease (21–23). Aaron was an author of the paper by Spillantini and colleagues, which identified a causative intronic mutation in *MAPT*, the tau gene, in familial multiple system tauopathy with presenile dementia, and which demonstrated that this mutation leads to the relative overproduction of soluble four-repeat tau and the assembly of four-repeat tau into filaments (23). Discussions with Aaron were essential for our understanding of the mutation’s effects and the subsequent structural work of Gabriele Varani (24). Together, we also reviewed our work on several occasions (25,26), the last time as part of the events marking the 100th anniversary of the description of the case of Auguste Deter by Alois Alzheimer (27,28). Aaron encouraged us to collaborate with Bernardino Ghetti, because he believed that access to human brain tissues was essential for our work.

I got to know Aaron in 1987. Over the next 25 years, he was an important influence. Aaron took a keen interest in our work. I vividly remember how pleased he was when he told Max Perutz that we had identified α -synuclein in Lewy bodies (29). Aaron succeeded Sydney

Brenner as Director of the LMB in 1986, a position he held for the next ten years. Between 1995 and 2000, he was also President of the Royal Society. Aaron's presidency was not a quiet time. It coincided with the bovine spongiform encephalopathy epidemic and the identification of variant Creutzfeldt-Jakob disease, the birth of Dolly the sheep, the alleged toxicity of genetically modified plants when used as food, the sequencing of the human genome and, throughout, global warming.

Aaron knew everything about almost everything (football was an exception). He said that science cannot tell one how to lead one's life, and we often talked about other topics. I so wish we could continue those conversations. The breadth and depth of Aaron's interests and knowledge were astonishing; they encompassed philosophy, religion, history, literature, politics, art and other disciplines (30–33). I recall seeing Aaron and his wife Liebe at a lecture given by Joseph Beuys in the early 1980s. There are not many scientific papers that begin with a literary epigraph. A 1987 paper by Danièle Altschuh, Arthur Lesk, Anne Bloomer and Aaron carries a quote from Stendhal's novel "Lucien Leuwen" (34). It reads (in translation): "Give us more details", he said to his son. "Give us more details, there is no novelty and truth in anything but the details". When asked who had influenced him the most, Aaron mentioned Reginald James, his mentor at the University of Cape Town, Rosalind Franklin and Francis Crick.

In science, Aaron distinguished between me-too work and the rest (he preferred the rest). For him, science was the "relentless disturber". He wrote in 1999: "There is a notion about that, since all men are equal, and all people are entitled to think what they want, all thoughts and value systems are therefore also of equal merit. This is of course not true" (35). ... "You cannot by sheer force compel scientific truths to reveal themselves. The major insights come only to the individuals who have the patience to develop an intimate understanding of a problem, who have the space and the freedom to take professional risks and explore apparently bizarre ideas, who know how to make creative use of the surprises that they encounter when they do so. These are the people who make the enduring difference" (36). In his tribute to Perutz (37), Aaron wrote: "It could be said of him, as he once wrote of Sir Lawrence Bragg, 'Fame never blunted the keen edge of his scientific curiosity, perhaps because he had no vanity or ambition to use it for personal power.'" The same was true of Aaron.

Work on Alzheimer's disease at the LMB began with the structure of the PHF and the quest for its molecular components (Aaron called it "the nub of the problem"). In 1988, we showed that tau repeats are an integral component of the PHF core. However, it was not possible to place the tau sequence in the core. In July 2017, almost thirty years later, this became reality, when we (in collaboration with Sjors Scheres) reported the high-resolution structures of PHFs and SFs from Alzheimer's disease brain (38). This work used single-particle electron cryo-microscopy (cryo-EM) and took advantage of recent advances in specimen preparation, electron detection and image processing, many of which were developed at the LMB (39,40). In cryo-EM, one needs to compute three-dimensional maps from electron micrographs, as first shown by Aaron and David DeRosier (41). On August 11, 2017 (his 91st birthday), Aaron was delighted to learn about our latest findings on

Alzheimer's disease, which marked the culmination of the work he had started 34 years earlier.

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Aaron Klug in August 1996.