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Cellular and Molecular Life Sciences

Memories of a Senior Scientist

From cancer cells to trypanosomes and back again

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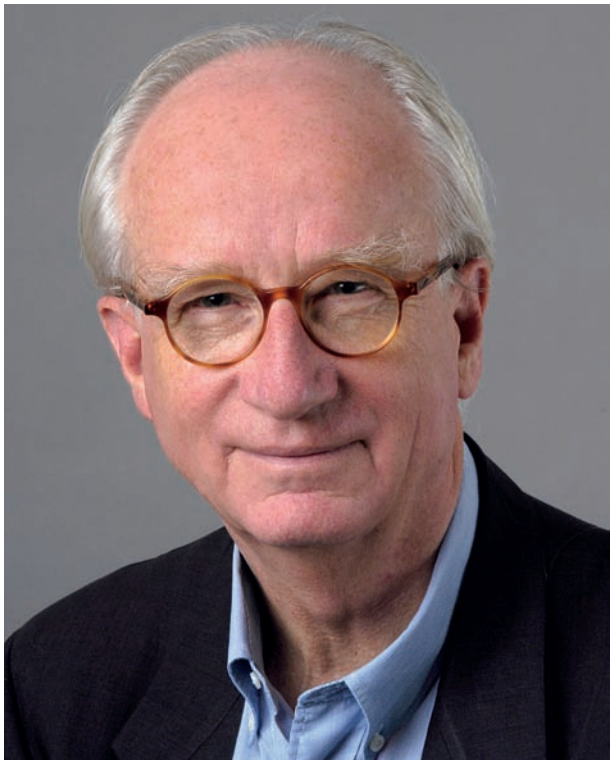

I was lucky to get a flying start in my scientific career. My father was a professor of internal medicine in the University of Amsterdam in The Netherlands, a famous and charismatic doctor, and an excellent clinical scientist. Becoming a doctor, preferably a clinical scientist, was the obvious career in our family. By accident I ended up in biochemistry, however, as I have described elsewhere [1]. While I was doing my clinical internships, E. C. (Bill) Slater, then professor of biochemistry in the University of Amsterdam, induced me to take out 3 years for a PhD. I produced a series of solid papers on tumor mitochondria (which I found to be depressingly normal) and at the end one real discovery, the malate-aspartate shuttle (briefly known as the Borst cycle) for the reoxidation of glycolytic NADH by mitochondria [1–3]. Notwithstanding this good start in biochemistry, I still wanted to become a clinician and managed to get myself accepted in endocrinology, but not immediately. This gave me the opportunity to do two post-doc years with Nobel laureate Severo Ochoa in New York University, 1963–'64. There I met Charles Weissmann, who had arrived 6 months before me, to start a new Ochoa project on RNA bacteriophage MS2 of *E. coli*. We were to isolate the replicase involved in phage RNA synthesis, which turned out to be a tough problem to crack. With a partially purified RNA replicase preparation, we managed to make duplex RNA and get some idea of the intermediates involved in phage RNA replication [4–6]. My most unexpected finding, however, was that the duplex phage RNA, thought to be an intermediate in RNA replication, was actually a side product. The replicase enzyme copying plus-strand RNA into minus-strand is able to keep the two strands apart. As soon as the enzyme is removed, however, the plus- and minus-strands, which are in register, zipper up to form the (artefactual) duplex RNA [7]. This conclusion was so unlikely that it took years to get accepted. I consider it one of my more interesting findings.

While I was doing experiments in New York, university education was rapidly expanding in The Netherlands. A new position for an associate professor, teaching Biochemistry to dental students, was created in Amsterdam and Slater offered the job to me. I was enjoying experiments, my interest in endocrinology had waned, and I accepted the offer without much hesitation. Back in the Netherlands in 1965, I decided to combine my old experience with mitochondria with my new knowledge of nucleic acids and set up a project on nucleic acids in mitochondria. With Van Bruggen and Ruttenberg I soon found that animal mitochondrial DNAs consist of 5-micron circles [8, 9], and with quantitative DNA renaturation experiments we showed unambiguously that there was only a single type of circle in the mitochondrial DNA. This proved that the genetic content of mitochondrial DNA is very limited and completely insufficient to encode the proteins known to reside in mitochondria [8].
A more detailed account of these highlights of my early years in biochemistry can be found in ref. 1.

On to yeast and trypanosomes

Once I had a foothold in mitochondrial biogenesis, my group rapidly expanded. We tackled mitochondrial DNA (mtDNA) replication and transcription and branched out into yeast and *Tetrahymena* [10]. While studying mtDNA replication, we found displacement-loop DNA (D-loop DNA) at the same time as Vinograd's lab [11]. In search of a convenient way to check the intactness of our animal mtDNA circles, Cees Aaij and I developed ethidium-agarose electrophoresis [12], a technique rapidly adopted by other labs for the analysis of restriction digests of DNA [13]. Soon uni-cellular eukaryotes came to dominate the lab, however. Yeast entered first. We found that yeast mtDNA circles were 5 time the size of animal mtDNA [14, 15]; we made the first restriction map of this DNA [16] (at the time considered a huge DNA); we discovered the first intron in an organelle DNA in the yeast large rRNA gene [17] and we found introns in yeast mtDNA that were dispensable, because they were absent in the mtDNA of some yeast strains [18–20].
My most lasting relation with uni-cellular eukaryotes I developed, however, with trypanosomes. Since I have described my 35-year love affair with tryps in detail on the Parasitic Protozoa web site (http://164.67.39.27/parasite_course-old/personal%20stories/personal_histories_of_parasitolo.htm), I'll only give a summary here. The affair started by accident: as a mitochondrial expert I was asked to attend a workshop on kinetoplast DNA (kDNA), the unusual mtDNA of trypanosomatids, and I became fascinated by the unusual structure of kDNA, which consists of large catenated mini-circle networks. I started working on the structure of these networks and we soon found that the mini-circles were heterogeneous in sequence [21, 22] and that there were larger circles hidden in the network, which we called maxi-circles and which contain the protein-coding mitochondrial genes [23]. We also made the first EM pictures of segregating kDNA networks, impressive structures. The most important discovery on kDNA in Amsterdam, however, came much later when I had handed over the project on mitochondrial biogenesis in trypanosomatids to Rob Benne, a new associate professor: he discovered RNA editing [24].
In the meantime I became increasingly occupied with two other projects, the glycosome and antigenic variation of trypanosomes. The discovery of the glycosome arose from my long-standing interest in oxidation of glycolytic NADH by mitochondria [25], which had already led to the malate-aspartate shuttle [2, 3]. In their mammalian host the African trypanosomes, such as *Trypanosoma brucei*, suppress the synthesis of a mitochondrial respiratory chain and they live off glycolytically generated ATP. The excreted end product of glycolysis is pyruvate, not lactate, and the trypanosomes reoxidize their glycolytic NADH via a glycerolphosphate cycle. We located the glycerolphosphate oxidase in the mitochondria, but on the side discovered that the entire glycolytic pathway from hexokinase down to phosphoglycerate kinase is located in a modified peroxisome, for which I coined the name glycosome [26]. I remained interested in glycosome/peroxisome biosynthesis [27, 28] and I even helped to set up the first prenatal test for a severe human inborn error of peroxisome biosynthesis, the Zellweger Syndrome [29, 30]. However, in the end my trypanosome work would become focused on the mechanism of antigenic variation of *T.brucei*, one of the most successful scientific projects in my life.
African trypanosomes freely multiply in the bloodstream of the mammalian host, allowing efficient pick-up by the insect vector, the tsetse fly. The mammalian host makes antibodies against the trypanosome coat, but the trypanosome population manages to avoid total destruction by repeatedly changing the coat in a small subfraction of the population. George Cross (Cambridge, England; now Rockefeller University) had shown that this coat consists of a single protein, the Variant Surface Glycoprotein (VSG) and with George we started in 1979 to study the molecular genetics of VSG switching. We soon found that *T.brucei* has an enormous repertoire of VSG genes (10^3) and that it can activate a silent VSG gene by transposing a duplicate to an expression site (ES), where it displaces the resident gene by gene conversion [31, 32]. This explained in essence how antigenic variation works [33], although we and other labs would need decades to answer many remaining questions.
The ES was adjacent to a telomere [34] and this allowed us to clone trypanosome telomeres and show that they end with $(GGGTTA)_n$ repeats [35] and that these repeats grow and contract [36]. Other labs found only later that

the same hexamer sequences are present at the ends of mammalian chromosomes and that growth and contraction is a feature of eukaryotic chromosomes in general. Our experiments on antigenic variation spun off several other interesting findings:

– We showed that the ES is a 60-kb transcription unit [37, 38] and that the primary transcript is processed by transsplicing [39–43] (also found by the labs of Agabian and of Cross). Transcription of the ES proved insensitive to α-amanitin [44] and we concluded that the ES is transcribed by RNA polymerase I, rather than Pol II. The availability of transsplicing allows the synthesis of pre-mRNA by any polymerase [45], and we reasoned that Pol I would allow more efficient transcription of the ES than Pol II would be able to provide. As only a single VSG gene in the ES yields about 5% of the total trypanosomal mRNA population, transcription has to be efficient. This reasoning has survived until today.
– In an attempt to find out whether VSG genes can be transposed to another chromosome, we hooked up with David Schwartz in New York. David was developing Pulsed Field Gradient (PFG) gel electrophoresis in the lab of Charles Cantor and he had succeeded in size-fractionating the chromosome-sized DNA molecules of yeast. Alerted to this unpublished technique by David Baltimore, I managed to convince Schwartz and Cantor to allow us to try their tricks on tryps. Like yeast, pathogenic protozoa do not condense their chromosomes during cytokinesis and there are no metaphase spreads suitable for *in situ* hybridization. The collaboration with David Schwartz was a resounding success [46, 47]. We found inter-chromosomal VSG gene transposition and a unique set of about 100 mini-chromosomes (50–150 kb) in T. brucei. We showed that PFG gel electrophoresis can be used to size-fractionate the chromosome-sized DNA molecules of other kinetoplastid flagellates [48] and the technique would become indispensable for studies on protozoa in general.
– The 60-kb ES transcription unit contains several genes, besides the VSG gene, as shown by the Cross lab. The Overath lab then found that two of these Expression Site Associated Genes (ESAGs) encode a heterodimeric transferrin receptor (Tf-R), which is essential for taking up transferrin (Tf) from the mammalian host to cover the trypanosomal iron requirements. We and others had already found that trypanosomes do not have a single VSG gene ES, but about 20, only one of which is active at the time [49, 50]. The regulation of the activity of these 20 ESs adds another layer of complexity to the mechanism of antigenic variation and it was long unclear why this was useful to the trypanosome [51]. We found that the Tf-Rs encoded by the 20 ESs are similar, but not identical, and suggested that this provided an explanation for multiplicity of ESs.
  At first, I thought that the ability to produce 20 slightly different Tf-Rs allowed the trypanosome to escape from host antibodies against one Tf-R by switching to production of another Tf-R encoded in another ES [52]. This purely immunological explanation proved to be an oversimplification. The diversity of Tf-Rs appears to be required to allow *T.brucei* to tightly bind the diverse range of Tfs from all the mammals it can infect [53, 54]. Antibodies can only interfere with Tf uptake, if the affinity of the receptor for Tf is low. I think that we have presented overwhelming evidence for this interpretation, but not everybody in the field is overwhelmed yet. I probably need to go to Africa to verify the predictions of our model in lions, wildebeests and pigs, but I have not found time for this extended scientific safari.
– When an ES is turned off, the VSG gene in it acquires DNA modification [55]. It took us 10 years to show that the modified base is a new one in DNA, β-glucosylhydroxymethylU, or J for short [56]. J is present in all kinetoplastid [57] flagellates and in *Euglena* [58] and it replaces a small fraction of T in repetitive DNA, predominantly in telomeric repeats [59]. Our indirect evidence indicates that J is made in two steps, conversion of T into hydroxymethylU, followed by transfer of the glucose moiety [60]. We have found a specific protein that binds to J in duplex DNA [61] and this protein is essential in *Leishmania* [62]. We are still trying to find out what J is doing.

Back to cancer

When I moved my lab to The Netherlands Cancer Institute in 1983, the institute pressured me to stop my research on yeast and trypanosomes, but I resisted the pressure. Dropping yeast and organelle biosynthesis was OK, because my involvement in those topics had petered out anyhow. The research on antigenic variation in trypanosomes was going fine, however. I had a very strong group of students willing to make the move with me to the Cancer Institute. We produced a string of high-profile papers in Cell and Nature and I was convinced that a scientific institute needed a director with scientific authority, which mainly came from my ongoing trypanosome research. The strongest argument, however, was that I liked to work on trypanosomes. I had grown fond of these elegant organisms with their original biochemical solutions. Dropping trypanosomes was out of the question.

In the end my trypanosome research proved highly beneficial to the Cancer Institute. Publicly funded institutes were increasingly judged on the impact of the publications produced by their staff and my trypanosome papers produced a substantial fraction of the impact generated

by the Cancer Institute in the years between 1983 and 1986. After that period, the new staff members that I managed to recruit increasingly helped to generate a scientific output.

Nevertheless, it was not unreasonable for the director of a cancer institute to work on cancer in his own lab and in 1984 I started working on the mechanism of multidrug resistance (MDR) in cancer cells. It was one of the few moments in my scientific career that I did not start a new line of research by accident, but made a real decision. I chose MDR for 4 reasons:

- It looked like a biochemically interesting topic, for which I was reasonably qualified. It was known already that MDR was associated with DNA amplification and I had gained considerable experience with DNA rearrangements in the trypanosome work.
- Drug resistance is a key problem in cancer therapy. Our patients do not die of loco-regional disease, but usually because their metastases have become insensitive to all available chemotherapy.
- The field did not seem overly competitive making it difficult for a newcomer to get a foothold.
- Finally, there was nobody working on mechanisms of drug resistance in the Cancer Institute and I would therefore not find my self in the awkward position of directly competing with one of my staff members.

In 1985 I started looking for genes involved in MDR. Initially, I had difficulty in getting well-characterized MDR cell lines from the labs that had generated them. I was a nobody in the drug resistance field and my letters were usually not even answered. This all changed when I went to the Gordon Conference on clinical and experimental chemotherapy of cancer. Sports acts as an important socializer in Gordon Conferences and I therefore brought my tennis racket, because I am a reasonably good tennis player, having started early. I soon found out that one of the key social events in the Gordon Conference was a doubles match pitching New York University against Yale. Yale had won in recent years, but when the team of New York University found out that I had been a post-doc at NYU, I was recruited to their noble cause. Fortunately we won. American professors are fanatic tennis players, but they never learn to play well as they start only when they go to college at the age of 18. Our victory was celebrated and discussed even during the scientific sessions and from that moment I was accepted as a member of the chemotherapy research community.

We started a collaboration with Victor Ling, who had discovered P-glycoprotein as a constant element in MDR cells, and found DNA amplicons in his cells that had a limited set of three genes in common [63]. The genes encoded large proteins and looked like good candidates for the putative drug transporter responsible for MDR. As Ling decided to sequence the hamster versions of these genes, we switched to the human ones. By the time we had sequenced half of the human MDR1 gene the roof came down: In papers in Cell and Nature other groups described the sequence of the human MDR1 P-glycoprotein gene (cDNA), one of the murine genes and an incomplete version of a hamster gene. I had been rather naïve to tackle a major problem in cancer with a single graduate student and expecting to get away with it.

Fortunately, my student Alexander van der Bliek had found evidence for a second expressed P-glycoprotein gene in the cDNA clones that he was sequencing [64]. The sequence of this gene most resembled the third of the three hamster P-glycoprotein genes and we therefore called it the MDR3 gene at the suggestion of Victor Ling. We now had our own P-glycoprotein gene to work on, but unfortunately, we were unable to find a plausible function for this gene initially. The MDR3 P-glycoprotein looks exactly like its MDR1 counterpart. The 2 proteins are 76% identical in their amino-acid sequence, but cells transfected with a MDR3 construct did not become MDR and we were initially unable to find any drug transported by this protein. (Much later another student in the lab, Alexander Smith, did find transport of a limited set of drugs and apparently the MDR3 P-glycoprotein has retained some of the drug transporting capacity of its MDR1 counterpart, which is a dedicated transporter of xenotoxins [65]).

Confronted with a long series of negative experiments, my student Jaap Smit and post-doc Alfred Schinkel decided to take a major leap: they started generating a knockout mouse, unable to make the mouse homologue of the MDR3 P-glycoprotein, called Mdr2. The approach made sense: the MDR3 P-glycoprotein gene is mainly expressed in the liver and the protein is localized in the canalicular membrane, which separates the hepatocyte from small bile ducts. Hence, we were convinced that the KO mouse would reveal the function of the MDR3/2 P-glycoprotein. The protein was obviously secreting something from the liver into the bile. In the KO mouse, this something should either accumulate in the liver or be missing in the bile, or both. With hindsight the approach was pretty obvious, but when we started, around 1990, it was a risky adventure. Generation of KO mice was in its infancy and still a major undertaking. We were fortunate, however, that Hein te Riele and Anton Berns in the Cancer Institute had just introduced a major technical improvement in the gene technology. They had discovered that the very low efficiency of homologous recombination between targeting construct and targeted gene in the murine ES cells was due to the use of DNA constructs from other mouse strains than the one from which the ES cells were derived. The polymorphisms between mouse strains result in imperfect homology between their DNAs. This is recognized by the DNA mismatch repair system,

which removes most of the targeting construct trying to insert into the target gene by homologous recombination. By using DNA constructs of the mouse strain from which the ES cells were derived, it was possible to get a much higher frequency of targeting and avoid the use of complex selection strategies. This shows that having the right strategy is not enough; it really helps to work in a top institute where you have optimal access to new technology, new ideas and new biochemical results.

We were also lucky in that our first KO mouse had a clear phenotype: it developed a mild hepatitis, which looked interesting enough to get the attention of hepatologists. We teamed up with 2 biochemists with a reputation in hepatology: Ronald Oude Elferink and Bert Groen at the Academic Medical Center in Amsterdam. Together we soon found out that our KO mice were lacking a major component in their bile, phosphatidylcholine (PC). Even more significant was the finding that mice, heterozygote for the disrupted allele, had half of the amount of Mdr2 P-glycoprotein in their liver and half the amount of PC in their bile, but no other defect whatsoever. This proved that the murine Mdr2 P-glycoprotein and its human counterpart, the MDR3 P-glycoprotein, are PC translocators [66].

The mild hepatitis in our KO mice made sense: PC in bile is essential to form mixed micelles with the high concentration of bile salts present in bile. When PC is absent, the bile salts start eluting phospholipids from the membranes they contact, resulting in a sterile inflammation, exactly the pathology observed in our mice. Humans have even more hydrophobic, and therefore more aggressive bile salts than mice. Total absence of the MDR3 P-glycoprotein in humans leads to a very severe form of liver disease that requires liver transplantation. This is progressive familial intrahepatic cholestasis type II, which was much later found to be caused by the MDR3 P-glycoprotein deficiency by a French group in collaboration with Ronald Oude Elferink.

It was great to find a function for the MDR3 P-glycoprotein, especially since it was a function with direct clinical relevance. Although every discovery is obvious with hindsight, I remember that the professor of gastro-enterology in Amsterdam would not believe it at first. Transfer of phospholipids into bile was considered a passive process, mediated by bile salts, and since he had taught generations of medical students this fact, it could not be wrong.

Writing up our results was an interesting, but challenging experience. Biochemistry and physiology of bile formation is a specialized field and for months I was combing through the literature to see what was fact and what was fiction. It did not help that I was writing up the elucidation of the structure of our new base J in trypanosome DNA at the same time, as that work involved a lot of complex analytical and biophysical chemistry. I do not think 2 papers have ever been published by Cell in the same year from the same lab, which have so little in common as the 2 papers that we produced [66, 67]. It was obvious, however, that continued research on bile was a bridge too far and we left the further analysis of our Mdr2 KO mouse to the real experts, Oude Elferink and his friends.

Although the analysis of the MDR3 P-glycoprotein was fun, our real interest was in the mouse homologues of the drug transporting P-glycoprotein, involved in MDR. There were good reasons to test what would happen in mice without drug transporting P-glycoproteins: industry was busily developing inhibitors of P-glycoprotein to be used in patients. No doubt these inhibitors would also block P-glycoprotein in normal tissues and this made it important to get more information about the physiological role of drug transporting P-glycoproteins. The location of these transporters in the body made it likely that they would affect disposition of substrate drugs. The ability of P-glycoprotein to transport some steroids had also led to the suggestion that P-glycoprotein would be essential for the excretion of corticosteroids from the adrenal cortex. If true, this would compromise long-term treatment of cancer patients with P-glycoprotein blockers. So post-doc Alfred Schinkel started to generate a mouse with no drug transporting P-glycoproteins. This was complicated, because mice have 2 P-glycoprotein genes, usually now called Mdr1a and b to carry out the job done single-handedly by the human MDR1 P-glycoprotein. Alfred Schinkel knocked out the 1a gene, then the 1b gene and then made the double KO. All mice were fine, showing that drug transporting P-glycoproteins have no essential role in normal metabolism, at least in mice living in cages in the Cancer Institute [68, 69].

The excellent health of our KO mice was good news for clinicians, planning to use P-glycoprotein blockers in patients, but a little meager for a paper in a top journal. We expected substantial effects on disposition of drugs, however, and these should provide the positive arm of the KO mice analysis. While Alfred Schinkel was brooding on suitable protocols, he was rudely disturbed by a message from our animal house: 'your KO mice are dying.' Of course 'nothing' had been done to the mice, but after some probing Alfred found out that the mice had been treated with ivermectin for a parasitic infection. More controlled experiments showed that ivermectin is 100 fold more toxic to the Mdr1a KO mice than to their wilde-type littermates, that ivermectin is an excellent P-glycoprotein substrate, and that P-glycoprotein is present in the apical membrane of the brain capillaries, where it prevents ivermectin from entering the brain [68]. This is why ivermectin, which acts as a nerve transmitter agonist analogue, can be used to treat parasitic infections in mammals: P-glycoprotein prevents it from reaching its targets in the brain. Removal of this 'guardian' of the brain removes the barrier and results in ivermectin-induced epileptic insults that kill the mice.

Medical students are usually taught that entry of drugs and nutrients into the brain is a matter of hydrophobicity. The more hydrophobic the compound, the better it gets into the brain. Hydrophilic compounds, like glucose, require a dedicated transporter to pass the blood-brain barrier. What medical students do not learn is that there are many exceptions to this rule, only mentioned in the small lettering. To our great satisfaction many of these exceptions proved to be P-glycoprotein substrates and most of the other ones have been shown to be substrates since.

Although this may seem pretty obvious now, it was not in 1994. I remember giving a talk to the European Society of Brain Research Meeting in Amsterdam. The preceding talk was by an eminent pharmaceutical scientist, who explained with ingenious chemical arguments why many hydrophobic compounds do not go to the brain and why no compound with a mass over 800 enters the brain at all. When I showed our ivermectin results and explained that we can get quite large molecules, such as cyclosporin A, into the brain by removing the P-glycoprotein from the blood-brain barrier, I was met with complete incredulity. How could I possibly have shown the presence of P-glycoprotein in every single capillary in the brain? We had not, of course, but I explained that the ivermectin-treated mice were stone dead, whereas their heterozygous littermates were fine, showing that every part of the blood-brain barrier must contain P-glycoprotein.

A second pharmacologically important location of P-glycoprotein proved to be the apical membrane of the gut mucosa, where P-glycoprotein hinders the entry of substrate drugs. The importance of this emerged from experiments with digoxin by Alfred Schinkel and Ullrich Mayer, a German doctor who spent 2 years in our lab. Digoxin is an excellent P-glycoprotein substrate and we used it to study whether the available P-glycoprotein blockers were as effective as the complete removal of P-glycoprotein in the KO mice in allowing access of digoxin to mouse brain. On the side Ullrich found that the KO mice excreted nearly all digoxin via the urine, whereas in wild-type mice half of the digoxin leaves the body with the feces. A possible explanation was that digoxin is excreted from the liver into bile by P-glycoprotein and that this route to the feces was not available in the KO mice. This explanation proved incorrect, however. Intravenously administered digoxin in bile duct ligated mice appeared in the feces in wild-type mice, but not in the KO's. Apparently, digoxin was able to diffuse from the blood into the gut mucosa from which it was excreted by P-glycoprotein into the gut contents in the wild-type mice [70].

This immediately suggested that variations in MDR1 expression in the gut could affect uptake of digoxin in patients and this was later shown to be the case by others. Digoxin is a heart drug and we were more interested in anti-cancer drugs. An obvious drug to test was paclitaxel (Taxol), an excellent P-glycoprotein substrate that has to be administered intravenously, because it is hardly taken up from the gut. In a collaboration with our pharmacological colleague in the Institute, Jos Beijnen, who has played an essential role in our pharmacological studies, we found that the oral availability of paclitaxel is dramatically increased in the KO mice [71]. Since I work in an integrated cancer center, combining research and patient care, this finding was rapidly translated into clinical trials in patients by the medical oncologist Jan Schellens with help of Jos Beijnen.

Although we set out to generate KO mice in the context of MDR, the most important insights that came from these mice were in pharmacology and these insights have found their way into the clinic. Most biochemists that I know, whether originally medically or chemically trained, like to see their findings be translated into practical applications in medicine. There is no need to push them. The current emphasis on 'translational research' by funding agencies is therefore misdirected and it results in the funding of a lot of trivial clinical research. Once there is a useful new concept or fact emerging from basic science, clinical application follows fast. I have worked for over 20 years on mechanisms of drug resistance in cancer cells, and it has struck me how few investigators are looking at mechanisms and how many are available to test expression of any new resistance gene on patient samples.

When Alfred Schinkel came back from a postdoctoral period in the USA and set up his own lab in The Netherlands Cancer Institute, – on another floor, a strict rule that I had instituted as a director to avoid nepotism/servitude –, he continued the P-glycoprotein work and I concentrated on a second class of drug transporters, the multidrug resistance-associated proteins (MRPs), now part of the ABCC subfamily of ABC transporters. I first met the MRPs through our work on *Leishmania*. I was frustrated that we did not have a suitable plasmid system in trypanosomes for transformation experiments, whereas in another kinetoplastid flagellate, *Leishmania*, plasmids abound. I therefore asked post-doc Ted White to look at the plasmids in an innocuous lizard *Leishmania* species, *Leishmania tarentolae*, to see whether he could find a plasmid that would also work in *T. brucei*. With hindsight, this was a stupid suggestion, because the evolutionary distance between *Leishmania* and trypanosoma is probably more than a hundred million years and it is not very likely that these organisms can still replicate and express each others plasmids. Undeterred by these considerations Ted started to study the H-circles of *Leishmania*, plasmids that had already been described by Steve Beverley. We, like Steve, obtained evidence that these plasmids may be involved in drug resistance [72] and since half of my lab was working with P-glycoprotein and drug resistance in animal cells, Marc Ouellette, a Canadian post-doc who had taken over from Ted White, decided to look for a P-

glycoprotein gene on the H-circles of *Leishmania*, using hybridization probes derived of mammalian P-glycoprotein genes. This was a very long shot and I was not in favor of these experiments, but Marc pushed on and found the Pgp A gene [73]. The Pgp A gene has very low homology with human P-glycoprotein genes, – it was a miracle that Marc had picked it up at all –, and it can be considered the founding father of the MRP (ABCC) subfamily. Its involvement in resistance to arsenite and antimonite [74] was further worked out by Marc after he had returned to Canada.

Our work on MRPs yielded a lot of useful results (and highly quoted papers), but no major discoveries as with P-glycoproteins. We showed that drug transporters like MRP1, that are located in the basolateral membrane of surface epithelia can nevertheless be vital for the protection of tissue stem cells and internal spaces, like the testicular tubuli and the CSF [75–77]. We found several additional MRPs and we helped to characterize the function and substrates specificity of some of these, by vesicular transport studies and in KO mice. One of our most unexpected findings is that MRP4 transports the prostaglandins E1 and E2 [78]. The physiological significance of this finding is still unclear, however.

Scientific side activities

Some scientists get their best ideas in solitude, I like talking to people about experiments and I often get my best ideas in discussions or during seminars. Anybody who talks tends to express opinions and people with opinions in academia get involved in administrative chores. I sat on lots of university councils, I was the director of 2 institutes, and I was a member of more scientific advisory boards and prize juries than I care to admit here. All these side activities depended on up-to-date scientific knowledge, however, and without running my lab I would not have been able to be useful. Let me give a few examples.

In 1983 I moved my lab from the University of Amsterdam to The Netherlands Cancer Institute, where I became the Director of Research. The Cancer Institute had a long and illustrious history, but was going through a deep valley. It was in danger of losing its large government program grant, which would have meant closure. I had to make fundamental changes and running my own lab was essential in doing so. This gave me the scientific authority and helped me to simplify procedures: I just did not have the time for long meetings. Running a lab also helps a director to keep his ear to the ground. No better source of information than your own students and post-docs, complaining about the institute.

Of the Scientific Advisory Boards that I served on, two stand out: The SAC of the EMBL in Heidelberg and The Scientific Committee of the Louis Jeantet Foundation, which selects the recipients of the Jeantet Prize, the most prestigious biomedical prize in Europe. The SAC of the EMBL is one of the most powerful scientific committees in the world, because it combines top-scientists from Europe and the US. All of these scientists run big labs, most of them also run institutes or departments and each of them is used to have the last word in discussions at home. With 20 people around the table, who are used to take the final decision, it can be difficult to come to conclusions. Trying to coax those big ego's into consensus was a challenging job that I enjoyed. Key elements in success were informality and humor. Having worked in many different fields of biochemistry was an advantage, as I had some familiarity with most of the topics discussed.

I found myself in the same situation as President of the jury of the Jeantet Prize in 1998 – 2004. With a strong representation from French-speaking countries, this jury tended to operate more formally than the average scientific advisory board. French scientists also have a tendency, in my experience, to sit together and to continue whispering in French while other committee members are speaking. Regrouping this large jury in an alphabetical order and introducing first names, even for the French, helped to make the deliberations more congenial and therefore more effective. Also in this function I profited from the diversity of my research experience, as the Jeantet Prize covers all fields of medicine and medically relevant biology.

Teaching was another side activity, although it is such an integral part of an academic career that it can hardly be called a side activity. In my university period there were years that I would teach more than 100 lectures a year and to all kinds of students, ranging from second year dental students to specialized lectures to chemistry-majors. What I liked best were the clinical biochemistry lectures to medical students that I taught together with internal medicine and pediatrics. The professor of Internal Medicine would call me usually the evening before the lecture, around 21:00 hrs, describing his interesting patient and I would then spend hours in the backstreets of biochemistry, usually until deep in the night, to be able to present an interesting and understandable complement to the clinical presentation on the next morning. It was hard work in the beginning, but fortunately the number of medically relevant biochemical backstreets is limited and over the years the lectures became less stressful and more an interesting opportunity to learn about advances in clinical medicine. Even some of my research projects originated from these joint lectures, such as my work on peroxisome biosynthesis.

In addition to teaching students, I spread the biochemical gospel through reviews and commentaries. I did not contribute more reviews to Nature, Science and Cell than original papers, but they certainly get quoted more, not to mention my lengthy reviews for the Ann. Rev. Biochem. and Microbiology series.

Life after 65

When I reached the age of 65, I retired as director of The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, but I was allowed to retain my lab as long as I remained productive. This was an exceptional privilege, which is possible in The Netherlands Cancer Institute, but not yet in Dutch universities. Since I always ran an American style lab, supervising my own students and postdocs, nothing changed much in my life after 65. I just have a little more time for reseach, a little only, because I still have a lot of scientific side jobs.

My trypanosome research is now completely focused on base J, its biosynthesis and its function. We recently had a breakthrough in our studies of the enzymes involved in biosynthesis and I am confident that I shall be able to see the final solution of this problem. In the drug transporter field, I remain interested in the physiological functions of the MRPs, but I have also started a new project on studying drug resistance mechanisms in the tumors arising in the new genetically modified mouse models, developed by Anton Berns and Jos Jonkers in The Netherlands Cancer Institute. A new post-doc in the lab, Sven Rottenberg, has managed to get resistance of these (spontaneous) tumors, a feat never accomplished before in animal models, and we have high hopes that these new model systems will allow us to find out what are the most important resistance mechanisms in realistic animal models and whether these models can be useful to clinicians to work out better drug regimens. Enough to do.

Highlights in biochemistry come from teamwork and lack of space has prevented me to highlight this sufficiently here. I had the pleasure and privilege to collaborate with many excellent colleagues and with a large number of smart, motivated and really nice students and postdocs. More than 30 of these now sit on their own academic chair. Some of them have been so successful that they qualify to write a sequal to my story. They will keep the centrifuges humming.

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