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VIDEO INTERVIEW TRANSCRIPT

Bakker, Bert: transcript of a video interview (27-Oct-2015)

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Bakker, Bert: transcript of a video interview (27-Oct-2015)*

Biography: Professor Egbert (Bert) Bakker PhD (b. 1951) studied chemistry in Delft (BSc), and continued his studies at Leiden University (1975-1976) where he was also a technician (1977-1989). During this period he worked closely with Professor Peter L Pearson and pioneered molecular genetic techniques, which led to the first prenatal diagnosis of Duchenne muscular dystrophy in 1985. In 1989 he completed his doctoral research on Duchenne muscular dystrophy, and the same year he was awarded the Lustrum Prize by the Dutch Human Genetics Society. In 1990 he became Head of the DNA diagnostic section in Leiden University's Clinical Genetic Centre and Associate Professor at the Department of Human Genetics. In conjunction with these roles, he was Head of the Forensic DNA Laboratory at Leiden (1994-2000). In 2000 he was appointed Professor of Molecular Genetic Diagnosis at Leiden University Medical Center, where he continued as Head of the Laboratory for Diagnostic Genome Analysis, till April 2015, when he stepped down as Head of the laboratory and ceased his management duties.

[1]. BECOMING A MEDICAL GENETICIST; LEIDEN & PETER PEARSON

I was trained as a biochemical technician, and I had to do my internship in a place in Rotterdam where they were working with heart perfusion of rats. I didn't like that so much, so I went out and looked for another place to do an internship, and it was in 1974. And I found a place in Leiden at the Human Genetics Department - at that time it was called "Anthropogenetics" - in Leiden, and there Peter Pearson offered me an internship and I started to work in genetics. And it really fascinated me a lot because there was not so much known at that time. It was looking at the microscope at chromosomes, finding out how these replication worked by adding BrdU [bromodeoxyuridine] in cell lines and getting sister chromatid exchange. And it really fascinated me how this whole process worked. After my internship I had to go in the Army. Actually, I knew I had to go in the Army, but there was some time left so I started first [the] study of chemistry, but after [the] first year, I couldn't finish the first year; I had to go in the Army. And when I came back from the Army I phoned Peter Pearson and said, 'Can I give you as a reference for my new position?' I wanted to get another job somewhere. And he said, 'Yeah, it's fine, but why don't you come here. I have an opening for you.' So I could come and work in his lab as a technician, and I started; that was 1977 at that time.

[2]. LEIDEN & PETER PEARSON: HARLEQUIN CHROMOSOMES & CELL LINES

Thinking of the start of my career with Peter Pearson, at that time he was very enthusiastic and trying to stimulate in all kinds of ways the work that we did. When I came in there as a trainee, he gave me three articles, and I read three articles. There was one in German, French and an English one. And he said, 'Okay, here,' so I just started there and I got three articles, and it was about BrdU incorporation in cells and making these harlequin chromosomes on… And there was another article, so it was from the French group of Dutrillaux and they had made these harlequin, so adding BrdU in culturing cells, and doing that for 24 hours. You would see that the first replication round everything was doubled; the second replication, one chromatid of chromosome was completely incorporated in the other half. So it was a very nice article, and you could see changes between the two sister chromatids. And so he came back after a few days and said,

* Interview conducted by Ms Emma M. Jones, for the History of Modern Biomedicine Research Group, 27 October 2015, in the Wellcome Trust, London. Transcribed by Mrs Debra Gee, and edited by Professor Tilli Tansey and Ms Emma M. Jones.

'Okay, have you read these articles?' I said, 'Yes, it's interesting.' 'Okay, well we're going to do.' 'Going to do?' 'We could try to reproduce it.' 'Yeah,' I said. 'That's what I want. Just order the stuff and start doing it.' So I had to order all kinds of chemicals, and getting cells to grow, and try to do that.

In the same time we also used that BrdU incorporation for late replication X chromosomes; so if you just add seven hours BrdU, you harvest the cells, you can see that the one that replicates late is one of the X chromosomes, and you could very nicely see the X chromosome on these spreads. And with assisted chromatid exchange - so 24 hours' incorporation - you could see very nicely all these exchanges. And with that, people used that kind of technique to look at Bloom syndrome, and look at all kinds of chromosomal breakage syndromes, to diagnose them. But at that time it was more interesting to see that we could replicate it and reproduce it in the lab. After that we started to work on cell lines. There was a tumour cell line that was called "HEK-273". It was a human embryo kidney cell, HEK, and that cell was originally made from a human embryo kidney cell on the floor above us in the building with the group of van der Eb. And Graham and van der Eb together had done the first transformation of a human cell with SV40 [Simian virus 40] DNA, and [with] this transformation, this cell line became stable.

That was a kind of stable growing cell, so indefinitely growing cell, and with that you could actually study these cells. What Peter was interested in was where in this human DNA, is that SV40 DNA, this viral DNA, where is that incorporated and can you see that? In the lab there was a PhD-student that worked on this topic, and she was working with hybrid cell lines, fusing these lymphocytes (actually, the HEK-273 cells) with hamster cell lines, so the lymphocytes from the human and the ovarian hamster cells from the hamsters, fuse them, grow them and you lose chromosomes. And at a certain point you have a set of chromosomes and you can see (via immunoprecipitation) if T-antigen, which is expressed by this SV40 gene, if that's in there or not, and try to locate it on one of the (human) chromosomes. That turned out to be very difficult because the cell lines were very dysregulated. When you looked at the chromosomes of these HEK-273 cells, we made karyotypes of that, and if you look at the chromosome, 104/105 chromosomes, and from each (chromosome), three (to) five copies, and each cell had almost a different composition. But because there's so much DNA in there the cell was growing, growing further.

So from these cells he wanted to know: where is this located? And because it (localization by immunotyping) didn't succeed with the hybrid cells, okay, what we can try is to do that at the DNA level. If the T-antigen is not working for some of the cell lines that we have, we can maybe look at DNA, isolate DNA from the hamster hybrid cell lines and hybridize the probe for SV40 (T-gene) to that. So it was another difficult job that we had to do and we couldn't localize it, also not with Southern blotting, so it was very difficult to get it to work. But on the other hand, from there we learned to do Southern blots, hybridization, and try to get this to work. And one of the first hybridizations that I did at that time was with these hybrid cell lines, so that's how it came into DNA and to learn Southern blots; that was something that was not in our Department there yet. I had to go to Amsterdam to the lab of Piet Borst, and Dick Flavell was working at that time in Amsterdam, Jan Swammerdam Institute, and there I learned to do the first Southern blots and see how that worked. And from that time on we had connection with the people from the Jan Swammerdam Institute and also Dick Flavell, who later moved to Mill Hill in London, and in Mill Hill in London I went there to make the first (DNA) probes by cloning these pieces of human DNA. So it's all connected.

But the first Southern blots on these hybrid cell lines were really difficult. So, and Peter Pearson always tried to stimulate and to get the best out of you, and [he was] very enthusiastic if there was something new he wanted to know immediately. 'Oh, what's new? What did you find in this? Let's see…' A disadvantage from that same way of thinking of Peter Pearson that he was always going for … that the people who were steadily going and working on other diseases in the lab; Huntington's, that was already there, it was localized we knew where, how it segregated in the families and it was not so interesting. And we had a lot of reports on that also and there was also a PhD-student working on Huntington's disease and trying to replicate all kinds of negative studies on effects on the cells and he got less attention. And that's what happened then. So he was always trying to get new things first. I had a very good time working in Leiden with Peter Pearson and when he left in 1989, he left to Baltimore, at that time I did my PhD myself and became Head of the DNA Diagnostic Laboratory, and have run that ever since.

[3]. CLONING DNA, MILL HILL IN LONDON

Yes; so if you go back to 1979 when we first started to do, trying to clone pieces of human DNA, it [was] possible to have plasmid and to digest the plasmid and to ligate a piece of DNA in there, but it was not allowed in the Netherlands to clone pieces of human DNA in bacteria, or to get a small piece of human DNA into a plasmid and put it in a bacteria to grow and to multiply this. There was a special committee that was working on genetic modified organisms, and this committee was saying, 'Okay, it's not allowed, we have to clear first the bacteria in which it has to be done, and plasmids that have to be used.' And there were a few plasmids known that could be used, for instance to produce insulin, and so they were already working on that. But they were very strict on which cells to use and which bacteria and which plasmids. So at that moment it was not possible to do this experiment in Leiden, to take these pieces of DNA, because we had no probes, so I had to make the probes. I collected the placenta from hospital, isolated DNA from the human placenta. This DNA was then digested in small pieces, in fragments, in fragments of around 1 kb, which was separated on the sucrose gradient and from these fragments I made these plasmids by ligating this fraction of 1 kb, 2 kb, and 3 kb into plasmids, transforming them in bacteria.

But it all had to be done in Mill Hill in MRC [Medical Research Council] in London, in the lab of Dick Flavell, which we already knew from the time that I first learned the Southern blots. So these difficulties were only a few years because after, I think 1982, at that time we got permission to do it in our own lab in Leiden and we got a VMT-safe microbiology technology lab to do these transformations. And from then, from that time on, we also could clone bigger pieces and make cosmids, and it was Gert-Jan van Ommen actually that came in our laboratory in 1983. He had already experience of making cosmid libraries, which he also learned in Mill Hill, but he was at that time working in the lab of Jan de Vijlder in Amsterdam, in the same Institute also where Piet Borst was so he worked there and together they, Gert-Jan made these cosmids. And because, for [the] Duchenne gene we wanted to clone the whole region, so at that time Peter Pearson attracted Gert-Jan van Ommen to come to Leiden to work on Duchenne muscular dystrophy. It was in 1983.

[4]. GENETIC PROBES; DUCHENNE MUSCULAR DYSTROPHY; ALZHEIMER'S DISEASE

Proudest achievement: so I have been working now for over 40 years in genetics. One of the first things that really was new and what was making the probes (to detect RFLPs or restriction fragment length polymorphisms), the small pieces of human DNA cloned in plasmids, and to make these probes to work on Southern blots and see differences between individuals and see segregation in families. And at that time it was really new - that was 1980, 1981, 1982 - and I know that the Head of Department, Peter Pearson, came in and almost when I had developed the X-rays, he came in and started to say, 'What's new? What's…?' So it was very exciting at that time with these restriction fragment length polymorphisms; we could start genetic linkage in families, and for Duchenne muscular dystrophy we started to do carrier detection and prenatal diagnosis. And the first prenatal diagnosis for Duchenne was, of course, very impressive, that we could do that at that time. That was 1980, end of 1984, and it was published in *The Lancet* (March 23, 1985). That was one of the major things in my career.

One other thing is, a few years later when we had this family where we couldn't work out carrier status of a woman. She had a son with Duchenne muscular dystrophy carrying a deletion, but this deletion was not present in the mother (in her DNA). So she was not a carrier. But on dosage, it was difficult, and we said (to be on the safe side, we did offer her a prenatal test for the next child), 'No, you never are sure if there might be a somatic mosaic or there (she) might be (a carrier), which we missed, so she could transmit it a second time.' And we proved that the second child, the second foetus that we tested, was also affected. So there was a germline mosaicism, which was already known from 1926 or so that that could happen, and it was published in hamsters, guinea pigs. And this, actually we had the proof that it really occurred (in humans: published in *Nature*, 1987), and how frequent it was came a few years later that we could see it was more often. So that there was really a recurrent risk for these women. So these two things are very new things to be proud of.

And there was another thing: a few years later we had a neurologist in our Department in Leiden University Medical Centre that had a family that had brain haemorrhages, and these brain haemorrhages looked, if you look at the small vessels and you stain that (they) looked very much like the same accumulation that you have in Alzheimer's disease. So it was the staining with Congo red, and it was amyloid that was seen, not in plaques as you see in Alzheimer's, but now in the small vessels. And this huge family was from the area of Leiden; they asked us to look at segregation, and mainly because there was a geneticist from Antwerpen [who] came to our lab to learn the tricks or to work with RFLPs and to work with Southern blots. And she (Christine Van Broeckhoven) was interested in Alzheimer's disease. She was from the Born-Bunge Institute and she was interested in these [families]. And she had, together with people from London, John Hardy, they had the probes for the Alzheimer gene, for the amyloid precursor gene (*APP*), which they thought would be involved in Alzheimer's disease. Earlier they had already proven with these probes that it didn't segregate with the Alzheimer's in these families, but we were bound to see how that worked in this family. And here we could prove that it (the genetic defect) segregated fully with the amyloid locus. So the brain haemorrhage, and this [is] called a very long name, "hereditary cerebral haemorrhage with amyloidosis of the Dutch type" (HCHWA-D).

And this small, this family was then linked to this amyloid locus, and with that we could say that amyloid, the gene is involved. Later it turned out that the Belgian family with the Alzheimer's, early onset Alzheimer's, that in that family it (the disease) also segregated, but it had one case of Alzheimer's that didn't fit in this family so now they could show that this was an outlier, and was actually having Alzheimer's disease due to another problem. So it was a "phenocopy", as we call it. So this was, this linkage for the amyloid gene was published in *Science* in 1989.

[5]. MAJOR OBSTACLES; AGAROSE GELS IN SOUTHERN BLOTS; SINGLE STRAND CONFORMATION POLYMORPHISM

Thinking about obstacles in the technologies that we used, then there was in 1982 when we had had this international course for restriction fragment length polymorphisms; that was an international course that Peter Pearson and I and other people from the States that - Ray White, Web Cavanee and Mireille Schäfer - organized this course to use RFLPs in research and later diagnostics. And so we had some 30, 23 people, coming to our lab to learn this new technique. And it was all running very smooth; we had nice Southern blots, we had nice results, and it worked fine. But then, when the course was finished, we were also running out of all, lots of chemicals. The membranes ran out, the agarose was almost finished, the buffers were gone, so, restriction enzymes were all out. So we had to buy them all new and start again and show, and then for half a year we couldn't get it to work. So then we also had contact with Ed Southern and ask[ed] what happened there and he said, 'Oh, I've had that also.' He had also had some problems during the time that it (Southern blotting) didn't work. So sometimes the technique worked and sometimes it didn't work, so it was very frustrating to have half a year to get no proper results. Sometimes it worked and then it didn't work, and actually it turned out that the batch of agarose we had was almost run out, but we had a new one, and if you use new one it (the result or signal on the X-rays) was gone, and if you use the old one it was working fine. So actually the type of agarose, if you make Southern blotting you have the gel, within the gel your DNA, the gel was pressed so flat that DNA couldn't come out so you got no results in your Southern blot. And it took a long time before we found that out, because we first thought of the buffers or the enzymes or the denaturation agent, and all this stuff, and the radioactivity, but it worked. At the end we found out what it was, and also later with other techniques where we had these SSCPs, single strand conformation polymorphisms, so by melting out the DNA and hybridizing you get secondary structures if you run it in agarose, in PAGE, or polyacrylamide gels. If you run DNA there you get different conformation; if there's a mutation in there. And that was also a very tricky technique to use but we got it to work, and, but we only used it for half a year or two years, and then it was gone. It was not a technique to use for a longer time.

And so we have seen during all these years, the 'wave' that was a technique also using this hybridization and melting out and DGGE (denaturing gradient gel electrophoresis) was a very successful technique, we used

it for many years in the lab. And, but sometimes you have a hurdle that you have to take to get it to work, and that's why, at a certain point, if you are going to use it in diagnostics, from 1985 when the first prenatal diagnosis from Duchenne was possible, we were going to move towards diagnostics, and were getting more diagnostic samples. And, at a certain point, we really chose that I became Head of the Diagnostic Laboratory and Gert-Jan van Ommen was Head of the Research part, and at that time we said, 'Okay, we have to really be sure that we have proper validation of the techniques, and quality system, and getting all the things in place to do real diagnostics.'

[6]. MAJOR CHANGES; PRENATAL DIAGNOSIS; GENETIC TESTING AND COUNSELLING

Ja, one of the major developments in genetics has been, of course, the new technologies that came along all the time, but on the other hand also the prenatal diagnosis that become possible from 1985 onwards. And then, looking at all the other diseases like haemophilia, OTC (ornithine transcarbamylase) deficiency, brain haemorrhage (HCHWA-D), where we had this large family, we could do segregation analysis. So to offer genetic tests that could be used in genetic counselling, we had a Counselling Unit quite close to us; to do the carrier detection, prenatal diagnosis, helping families to cope with what's in the family, the disease, and… Of course, you would like to come towards therapies, and that's the next step. We have seen over the years that there is the antisense oligos (oligonucleotides) that people start using. Especially in Duchenne muscular dystrophy there has been very successful research in mice and in all kinds of studies now; that it's now in the first trials with patients. And that will take some time before that really will be implemented, but that will be one of the major steps as well. So getting therapy involved. Of course, with all the diseases where you do have a substitution therapy like, for instance, Hunter's disease where you miss a gene called "*IDS*", the iduronate-2-sulphatase gene, if you have that deleted or mutated, then you end up with Hunter's disease, and patients die very early or they get lots of problems. But if you could supplement that early on, then they might live longer. But also these therapies are very expensive, so having the enzyme made is not really working yet. So I think there will be some time needed to get all the therapies in place.

[7]. HOPES AND PREDICTIONS FOR THE FUTURE. THERAPIES; A CURE FOR DUCHENNE MUSCULAR DYSTROPHY?

Ja, if we're going to look at therapies and look into the future and what's going to be the major challenges and the major developments, maybe is that with the use of IPS [induced pluripotent stem] cells, and with the use of changing skin cells towards neurons or towards other types of cells, that you can help to make a change. For instance, now for diabetes patients they can make these Islets of Langerhans and produce the insulin in small packages, and they can do that separately so they can make a kind of membrane part with cells in there that produce the insulin. So there's lots of developments in that area. For the replacement therapy, it will be also possible, for instance, the genes like Factor VIII, Factor IX, it has been there already made by recombinant technologies, and it can be used and can be given to patients. So these replacement therapies are not so difficult, but if you want to change a cell producing a gene, then you come into these antisense, and antisense oligos are fine and you can use them and they work in cell culture, and they work in mice and we can maybe get them to work in patients, but every patient is different. Their genetic background is different, the reaction on these (oligos) is different. The outcomes until now are not that great yet so it will take some time before that (antisense oligo therapies) will work. If you make a change here, then other changes follow, and that will help, not so easy to get the problem better. So I don't think we can cure Duchenne patients in the next 20 years. That will take some time.

[END OF TRANSCRIPT]

Further related resources:

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