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PERSPECTIVE

Bringing law and order to the cytoskeleton and cell junctions: An interview with Werner Franke

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PC: Where were you born?
WWF: I was born on Jan 31, 1940 during the first weeks of the World War 2, in Paderborn, the place of a thousand springs, one of the oldest cities in Germany, an important place, a King's palace or Pfalz in the days of Charlemagne. I attended the local high school, the gymnasium, as we call it, one of the oldest schools in Germany that was donated by Pope Leo the 3rd and of course had a theological focus. In 799 AD he had been thrown out of Rome and came to Charlemagne in Paderborn. They returned to Rome to reinstate him and in return Pope Leo gave Charlemagne two things: first the title: Heiliges Römisches Reich Deutscher Nation (Holy Roman Empire of the German Nation) and second, the school, which was founded in 806 AD.

PC: What was the major factor influencing you to go into science?
WWF: I and my classmate and sports club friend Theo Plesser were reading science books and doing experiments together on the side in school. He then studied physics and went on to become a Professor at the Max Planck Institute in Dortmund. Initially, I too leaned towards physics. But during my studies in Heidelberg I realized it was then still technically impossible to answer the questions that I had in mind, so I switched to biological applications of physics. As you know I am fascinated by optics. I did my University Diploma in four areas: botany, zoology, chemistry and I particularly enjoyed physics.

PC: Did your parents have any influence on your love of science?
WWF: No. My mother was supportive, but I was a loner. I entertained myself and belonged to a sports club, where I was a track athlete and Theo did pole-vault. My father was employed in the technical part of the German railway system restoring machines. My mother had a big garden that kept us self-sufficient – after the war you were very glad if you could grow your own potatoes and vegetables. Later, she took a position in a company that made priests' gowns.

PC: After growing up in Paderborn what lead you to choose Heidelberg?
WWF: In 1959 I had to join the army in the engineer troops - one of my specialties was booby-trapping. Initially I was based in the city of Minden, at the River Weser, then in Hamburg and Munich. It was for an 18 month period and this time made a big impression on my life. I became a reserve officer. I was also making my living at that time as a writer for TV and stage, in particular for German satirical cabaret. Later, once per month, I had a full one hour broadcast program involving German history in satirical terms. The best-known German stage cabaret and satirical actress at that time, Lore Lorentz, participated in one. The army gave me free time off on weekends to make these recordings. This side activity continued - though at a reduced level - up to my days as a professor.

PC: How did you come to Heidelberg and then to Freiburg University?
WWF: Initially I was attracted to Heidelberg by the University Sports Club, which wanted to have me for their relay team. Then I found Peter Sitte, with whom I did my Diploma. He was the first person in Germany to do cell biology-oriented polarization microscopy and electron microscopy and various other optical techniques. I also helped with practical courses for other students, but again I was a loner, doing things by myself. My first publication in 1966 "Isolated nuclear membranes" was completed as an undergraduate and was published as a single author paper in the J. Cell Biology. It was very unusual in those days for a student to publish in that journal. I did it by myself and my Professor generously said, "It's your work - you publish it yourself", which for a German professor in those days was unheard of.

PC: Very enlightened! So your mentor really encouraged your independence. What else can you tell me about the topic?
WWF: This was the decade of cell fractionation, the kind of approach that was taken by George Palade for example. I noticed from reading the literature that no one had thought about what separated the nucleus from the cytoplasm in biochemical terms. So I decided to isolate the nuclear envelope, originally from onion root tips as I was in the Botany department, and do biochemistry and electron microscopy on it. There were hundreds of watering cans in the laboratory and I grew the onion roots as the source for my biochemical isolations. I discovered the 8-fold symmetry of the "annulus granules" of the nuclear pore complex. Then I turned to tetrahymena ciliates and mammalian livers and it took only a few months to gather the same kind of data from these sources - much easier than working with plants!

PC: So your mentor gave you free rein - did he give you advice?
WWF: He could not. There were four great figures that influenced me early on: Joe Gall, George Palade, Don Fawcett, and of course Keith Porter. I met them as a young fellow of 35 or 36 – I will never forget being at the first International Congress of Cell Biology in 1976 and sitting on the stage with them. A discussion was held as to where the next congress should take place and Keith Porter said "If Werner says he can do this in Berlin, he can do it, I know he can" – Keith Porter was always supportive.

PC: How did you first meet these people - did you travel to the USA in those days?
WWF: No, originally I was influenced by these scientists through reading their papers. I would take their papers with me when I went to restaurants – I still like reading in cafes and restaurants. So when I eventually met them, there was some sort of mutual recognition - we spoke the same scientific language, enjoyed the same micrograph data. Then I met everyone at the World Congress in Berlin and from 1980 onwards most cell biologists knew of me because I had organized the congress.

PC: What was your position in Freiburg?
WWF: I was a University Assistant, which is not quite the same as an Assistant Professor in the USA because generally you have to do what the Full Professor wants you to do. But in my case it was good because with Peter Sitte I had freedom. I was in Freiburg for six years, from 1967–1973. In Germany there is this thing called habilitation, a formal qualification for professorship.

PC: You continued working on the nuclear membrane with your trainees?
WWF: And on other membranes. During this stage Ulli Scheer biochemically isolated and did electron microscopy (EM) on the nuclear membrane of amphibian oocytes and Hans-Walter Zentgraf worked on the nuclear envelopes and plasma membranes of avian erythroblasts. Juergen Kartenbeck worked on the endoplasmic reticulum (ER) and the Golgi apparatus membranes. These people were my students in Freiburg and I could bring them along with me to Heidelberg where I had much more space and money and could expand. Then we were joined by Michael Trendelenburg and Herbert Spring and later by Juergen Kleinschmidt who extended the work on *Xenopus* oocyte nuclear contents. I took the position in Heidelberg primarily because it was a cancer center that was federally funded - like the NIH, but at the same time it was linked to a state position at the University of Heidelberg. I knew I would have to teach and give courses to the students but I would also have the opportunity to have them in my lab. Throughout my entire research life I held this dual position. This was good because it facilitated daily communication with students.

PC: How were you recruited to the DKFZ?
WWF: Until that time, The DKFZ was spread over various "barracks" within the Neuenheimerfeld (Science Park). Then a big federally-financed cancer center was built at the end of 1972. I saw this would be a good position. I knew Heidelberg, I was lucky, I made it. At the time the German system had not been very attractive for young people, in particular in cell and molecular biology. That changed quickly: Walter Keller and Gunther Schutz came to our institute a couple of years later, then Ingrid Grummt. There were still some older professors around in classic areas of cancer research but from then on our center was based mainly on contemporary molecular and later developmental biologists.

PC: When I joined your lab in 1983 you used to travel regularly to the USA to attend the membrane and cytoskeletal meetings.

WWF: I began to focus on cytoskeletal structures for several reasons – first, there was obvious architectural and functional interactions with membranes; second, not much was known about some of them, notably the intermediate filaments; third, one day after giving a talk in Brussels I met Klaus Weber on the train back to Cologne. He had just come back from Cold Spring Harbor and taken a position in the Max Planck Institute in Gottingen. During that railway trip we exchanged slides from our talks and thoughts and afterwards I kept in constant contact with Klaus. Originally, I had learnt of Klaus Weber and Mary Osborn from Gunter Blobel. When I visited New York he told me – "I was recently at a CSH meeting and there is this guy, Klaus Weber, you will know him with his student, Elias Lazarides - they have wonderful results that fit very nicely with what you see in your structures and they do this by immunolocalization." So when Klaus and I travelled back together from Brussels we noted that we and our methods fit together and from there on we collaborated like crazy, making frequent exchange visits between Gottingen and Heidelberg. We brought the techniques together and we made antibodies that mutually benefitted one other. It was an ideal collaboration over decades. As most people know, Klaus was originally a protein chemist, so, if he saw something, he always had to do the protein sequence and think about the possible functions and the evolution of this protein. This is where the first intermediate filament (IF) protein sequence came from. I felt the additional obligation when discovering a new protein, antibody or method, to ask "can it be useful for tumor diagnosis of cancer?" I always found it important that the basic research results are translated into diagnostics and to take the initiative to apply what you have found to the real world of medicine.

PC: While at the DKFZ you were doing a lot of other things besides formal science – you had editorial positions at Cell, The Journal of Cell Biology, and your own journal, Differentiation.

WWF: Yes I held editorial positions on many journals. For almost a decade I was the only non-American editor of the J. Cell Biology. I took on Differentiation because Springer Verlag is in Heidelberg and asked if I could manage it.

PC: So you had a rather unusual emergence into science – most people have a mentor here or there who guides them, and, in fact it's often said that you need good mentors and that lineage is very important in getting on. But you seem to have emerged "out of the egg" by yourself, you met these influential Americans by reading their work and then later in your career you teamed up with Klaus.

WWF: We were also influenced by and interacted with, for example, John Gurdon and Ron Laskey in our work using amphibian oocytes.

PC: And you were quite closely connected to several Israeli scientists who passed through the lab Benny Geiger, Jose Schlessinger and Avri Ben-Ze'ev, for example.

WWF: I first met Benny in CSH. He had been in California with John Singer and discovered vinculin. We walked over the meadow in CSH - this CSH meadow was the most important place for scientists to get together and was critical for the development of molecular biology. This was also where I met Richard Hynes. These leisure things facilitate talking to one another. When I met Benny I knew he was interested in cytoskeletal membrane interactions so I went to the Weizmann Institute. Leo Sachs was also there. I had a few mini-sabbaticals over the years and our families got to know each other well as we have spent vacations in Israel.

PC: You also held international offices, correct?

WWF: I was also connected to the EMBL in statu nascendi. In the mid-seventies Kai Simons and Ari Helenius were in the Cancer Center for a few years and on saturdays, we would meet John Kendrew and Sydney Brenner. I remember Sydney sometimes came to these meetings with assemblies of gigantic EM pictures glued together of sections through stages of C. elegans development and asked questions about EM – these were the days when he started a novel rigorous way of developmental biology research. When finally Heidelberg was lucky and was selected as the EMBL place, then I became engaged in EMBL's development and for many years was the German representative on its Council when its laws and programs were established. In the early 1990's - after the East-West reunification - I was then the Secretary General, responsible for the whole of European molecular biology. I did this work together with John Tooze, who was the Secretary General of EMBL.

PC: You were also well connected within the eastern bloc prior to the fall of the wall.

WWF: I followed Max Birnstiel as the President of European Cell Biology Organization. I was elected in Paris in 1982 and served until 1990. It was very challenging and difficult at the time because Europe was divided into East and West. We had to alternate meetings, for example holding one in Budapest and the next in Florence. We also had to figure out how to finance them in the specific political system and how to resist certain political pressures. So in that difficult era I was one of the few persons who knew and co-operated with colleagues on both sides.

PC: If you had to sum up in one sentence your most important contribution - do you think it was your paper cataloguing the IF?

WWF: No. The catalogue by the way was originally rejected by Nature then became one of the most cited papers in Cell. I told Roland Moll, the first author, that

this could be his life's work. He later became Full Professor of Pathology at the University of Marburg.

PC: So what do you think was your most important contribution or insight?
WWF: The most important insight was when I noticed there were filamentous structures and bands in the SDS-PAGE that were very similar but not identical – one turned out to be keratin X, the other a different keratin Y, another was vimentin, and another from a muscle cell was desmin. By raising antibodies we found them to be related but different and importantly they were cell-type specific. So it was the recognition of their complexity as a family and their cell-type specific expression patterns.

PC: I remember from editing your papers, all those years ago, that I had to find many English words for "different": distinct, various, variety, etc. I have heard you be described, and I think its fair but I wonder what you think, as a biochemical Linnaeus of the intermediate filament world – would you like that title?
WWF: (laughter) Linnaeus – of course - formally I liked it at the beginning, Linnaeus was an important scientist but of course this has the additional connotation of just seeing and giving names to things. I always wanted to go one step further and describe their cell-type specific gene expression and their architectural structures formed. I am also happy that in my original paper in 1981 on liver cells, I mentioned that these cell-type specific expression patterns may prove to be useful in diagnosis of carcinoma metastasis. In the case of intermediate filaments they have turned out now to have world-wide application.

PC: But I think you do like to classify things – that's what I mean by the Linnaeus analogy - I mean this in a very positive sense (laughter).
WWF: Yes - as a German I like law and order (laughter).

PC: So now we have our title! You like to bring order into the world. But I know you also get very excited by the exceptions to the rules.
WWF: Yes because they are the challenges – first you have the general rules and order and then you have the exceptions. One of the most important things I work on now are the exceptions that happen accidentally. I had a paper in 1989 together with Anita Knapp, in Cell, that described cloned cells that should be positive for vimentin but here and there was a single cell that was also positive for keratin and thus violated the rule. We selected and cloned them and found these spontaneous and at random expression changes were clonally stable. More recently, we could select and accumulate diverse clonally stable changes resulting in new additional molecules, which may result in the formation of novel structures and novel types of cells and tissue. For example, we can show that single cells of hematopoietic origin and their derived clones can make desmosomes or other junctions and spontaneously transform into multicellular "epithelioid" tissue structures. This is evolution in the test tube - it is rare - but the new cell-types may survive if they are not out-competed. This is what I try to systematically study in an *in vitro* system. Previously, if you followed up on such strange anti-dogmatic exceptions you would have been burned on the market place. But now I can follow up on this. The exception is what is important for evolution.

PC: So that brings us to junctions which was your third big area. Maybe you would like to give me your thoughts on that phase of your career.
WWF: This was the most natural development I could take after studying IF to study what they are attached to. If you remember already in 1981 and 1982 I had identified a new junctional protein, the major desmosomal plaque protein, which I called desmoplakin. This work came again from the German desire for law and order. We studied the composition of desmosomes of various cell-types in a systematic fashion. One major surprise was that desmosomal junctions from different tissues contained similar but not identical molecules and secondly, that major internal epithelial desmosomal proteins were also found in the adherens junctions of the intercalated discs (ICD) of the heart.

PC: But why was that so surprising Werner? We saw the same thing and realized Don Fawcett had written reviews showing EM pictures of desmosome-like structures in the ICD.
WWF: But the surprise came when we found that everything that was in a simple epithelial desmosome was also present in the entire adherens junction system of the heart. When I did immuno-EM I could not distinguish desmosomes: their constitutive molecules were admixed and amalgamated with other kinds of adhering junction components, such as N-cadherin, the catenins and so forth. So I had to conclude that the molecular structure of the heart junction was not comprised of *fascia adherentes* and discrete desmosomal structures as Fawcett and McNutt had described, but the entire ICD junctional region was positive for classic desmosomal molecules. So I had to conclude that this was one amalgamated junction because all of the molecules that were known to belong to adherens junctions also colocalized in these junctions. This is why I dared to propose a special term the area composita junction, which forms peri- and post-natally in mammalian hearts and has now been confirmed by several other groups like the Belgian group of Frans van Roy and that of Glen Radice in Philadelphia etc. This "Composite Junction" is not a desmosome or an adherens junction, it is a mixture of both plus a number of special additional ICD molecules. So this is why one little mutation may bring about severe effects in the entire heart, up to "sudden death" with no effect in skin and other organs, which may comprise the

same mutated molecule but just not in this special joint structure - the composite junction of the heart.

PC: I guess what also influenced you here was your work together with Walter Birchmeier's group on the plakoglobin (PG) knock-out (KO) and the plakophilin (PKP2) KO mouse that "died of a broken heart" - that was the first desmosomal gene to be shown functionally to have a specific involvement in the heart.
WWF: Correct, we studied the first PG KO with Walter Birchmeier. We did the colocalization and saw the whole heart was broken open. In those days Walter went for lunch at the Max Delbrueck Center, Berlin and met Ludwig Thierfelder, a cardiologist and epidemiologist. Thierfelder asked "what is this strange PKP2 you are working on with Werner Franke?" Walter told him that in embryology it produced a very severe heart phenotype and so they published together right away in Nature Genetics 2004 that about half of the "sudden death" cases are due to mutations in PKP2. That was the breakthrough that brought things together. So all of a sudden there was this correlation of epithelial and heart diseases and of the molecular biology of desmosomal genes and with that of other junctions. More recently, for example, T-alpha-catenin has been added to the list and has been found in complexes with PKP2 and mutations in other non-desmosomal proteins in the ICD result in certain diseases in genetic animal models. One sees now that when the desmosomal molecules and non-desmosomal molecules are brought together in this new structure they are in new neighborhoods and now we want to elucidate, by chemical cross-linking and immunoprecipitation, the molecular organization of these new complexes.

PC: Tell me about the meeting you and Walter organize on this topic.
WWF: It is called "Heidelberg Heart - When Molecular Biology Meets Cardiology". The first meeting was in 2007. It turned out to be something new and everyone was excited. The second one was in 2011– we would play the famous song "Memories of Heidelberg" by Peggy March during the meeting breaks and take a boat trip up the River Neckar with readings from Mark Twain's stories about the surroundings. The next one will be in 2015. It has fostered many interactions on this topic across Europe and across the world, recently for example with special emphasis on Canada.

PC: Why Canada?
WWF: In Newfoundland and Labrador there occur surprisingly many special life-threatening gene clusters, often resulting in "sudden death", which have been molecularly identified recently, and may result in improved diagnostics and treatment.

PC: What are your plans for the future?
WWF: I would like to clarify the protein interactions in the composite junctions of the ICD and in other cell-cell junctions, that are so consistently ignored in the textbooks, such as the "*complexus adhaerens*" of the lymphatic endothelial sytem, the "*cortex adhaerens*" of the eye lens and the "*tesselate junctions*" of the stratified epithelia.

PC: How long do you think you will continue to do research?
WWF: Germany has strict age retirement rules. I have been given an honorary Helmholz professorship extension. In addition, I have created a little company, rented a room here on the campus and have two private EM's installed. I will continue to do research because I can only do research.

PC: So now you are free to "follow your heart" (laughter).
WWF: And my brain too: its one junction-connected organ system. I hope (laughter).

PC: What is your advice for young scientists?
WWF: Go for the unknown. Don't jump on the bandwagon. Go for the provocative its more interesting and often its more important: for the world and for your career.