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## Interview with Benny Shilo

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Ben-Zion (Benny) Shilo received his Ph.D. in genetics at the Hebrew University of Jerusalem under the supervision of Prof. Giora Simchen on the kinetics of cell cycle initiation in yeast in 1978. From 1979 to 1981 he was a post-doctoral fellow with Prof. Robert Weinberg at MIT, where he worked on the characterization and isolation of mouse and human oncogenes. Since 1981 he has held various positions at the Weizmann Institute of Science, where in 1994 he became full professor in the Department of Molecular Genetics.

His research focuses, among other things, on the cell-cell interactions that bring about development in Drosophila. He is working on the problem of how the conserved signalling pathways that mediate these interactions succeed in generating reproducible patterns despite fluctuations in the doses of the signalling components due to genetic and environmental alterations. He aims at identifying and characterizing the mechanisms of this developmental robustness by combining experimental and theoretical computational approaches. In 2013 he was awarded the EMET prize in Genetics as a lifetime achievement award.

*From yeast genetics to oncogenes*

UD: Let's start with your scientific biography. You studied biology at the Hebrew University and did a PhD in genetics. Then you had a post-doctoral fellowship with Professor Robert Weinberg where you isolated and characterized some oncogenes in the mouse. What made you go to his lab and why did you go into cancer research?

BS: First and foremost, what influenced my attraction to biology and in many respects my taste for questions in biology was my father, who was a professor of microbiology at the Hebrew University. Growing up next to someone who is a passionate scientist makes you realize what kind of life this is and what were the upsides and downsides (though not so many) to it. The 1960s, when I was in high school, was the time of great discoveries in molecular genetics, the genetic code, etc. so I was really fortunate to be exposed to these findings through my father and to get to know some of the key people in the field who were his friends.

So I knew that I was attracted to biology, but I wasn't sure if this attraction was because this was what I knew or what I really liked best. So before going to the army in 1970 I had three months' extra time. I took one semester of chemistry and this convinced me that what I like is biology and not chemistry! I like life, and chemistry was too dead even though it was very interesting.

After I finished the army I went to study biology and my eventual encounter with the PhD thesis advisor was actually an indirect result of the Yom Kippur War because during my second year at university, the time of the Yom Kippur War, we were essentially drafted for half a year. But the university wisely decided not to halt the academic year but to start it normally and give special support to the people who were drafted. So every four people got a tutor after coming back from the army and my tutor was Giora Simchen, who was a yeast geneticist. As a result of this acquaintance, my wife who was also studying biology, did her master's thesis with him and I joined later sort of indirectly. I was interested in the mammalian cell cycle and its kinetics and then together with my wife we had the idea of actually examining the kinetics of cell cycle initiation in yeast where everything can be done much easier. Unlike in any subsequent experience within my scientific life within two months we had a major paper on the kinetics of cell cycle initiation and this was really nice. It used yeast genetics to examine the factors which influence how cells

sense the environment, how it impinges on their growth rates, and so on.

So I did my PhD in the lab of Giora Simchen. When, in the last year of the PhD, he was on sabbatical at Harvard, I was able to get a temporary position at the lab or Arthur Pardee at Harvard Medical school and continued my work on the yeast cell cycle there and during that year I looked for a place for a post-doc. I looked for places at the M.I.T. Cancer Center that was established and headed by Salvador Luria, who was also a friend of my father. He introduced me to what were considered some of the stars of the MIT Cancer Center, but in the end there wasn't a place for me. But at that time Bob Weinberg was just starting out at the Cancer Center and I had a friend on that floor who told him that I was looking for a place for a post-doc. So he contacted me, I came to see him, and within five minutes I fell in love with him and decided to come to his lab. What attracted me to him was the direct personal interaction and his unconventional view or approach to cancer and cancer genetics.

So I moved back to Israel and wrote my thesis and then a few months later we were back in Boston and joined his lab. It became clear that he introduced a revolutionary method of transfecting DNA from cancer cells into mouse cells. The main challenge was to actually clone and isolate these genes. We first started by transfecting mouse cancer genes into mouse cells and then human cancer genes into mouse cells. Even though in the case of the mouse into mouse transfection we had some nice strategy to tag these genes and isolate them, there were technical difficulties. Then when we did human to mouse transfection, the idea was that we could use the repetitive sequences of humans which are different from the mouse sequences in order to tag the relevant oncogenes and isolate them in this way. Conceptually it worked very nicely. Again, there were technical difficulties, but in the end it did work.

What we found out – and additional people in the field subsequently found out - was actually that the genes that were identified from a

variety of human tumours - represented just three types of genes. And these genes are already known; they were the Ras genes, the Harvey, Kirsten and N-Ras genes that were previously identified by retroviral oncogenes. So the whole exercise, in retrospect proved a little bit like a mute exercise because the genes were already known but still it demonstrated that these were the cardinal genes that played a role in human tumours.

UD: Weinberg – what his scientific biography?

BS: He spent most of his career at MIT. He was an undergraduate of MIT, did his graduate work with Sheldon Penman at MIT on RNA modification, a short post-doc at the Weizmann institute, a short post-doc in San Diego with Renato Dulbeco and then back to MIT.

UD: Did he have connections to Germany?

BS: His family comes from Germany and he's very much into the genealogy of his family. His father came to the States and Bob was born in the States.

UD: He was born in 1942 I think.

BS: Yes, so his father came in the mid-1930s or even earlier; his father was a dentist. Bob was very German in his attitude even though he was born in the States. There were many German aspects to him. Now, already before we knew that the genes that were being transferred by this method were already known, it still became apparent that the repertoire of cancer genes one could pick up in this method was limited, it was just from the fingerprint of the repetitive sequences that we could identify three fingerprints and that's it regardless of the origin of the tumour. Tumours from different origins, and different tissues would give just one of three of these patterns. So it was clear that this, mathematically speaking, was a small repertoire of cancer genes. And this was towards the end of my post-doc and as a post-doc it makes you worried because it means that if

all the world is going work on these three genes your specific contribution will be limited.

So this was how things were at the back of my mind. I saw a paper in *Nature* on cloning a Drosophila metabolic gene for adenine biosynthesis in yeast, using functional complementation. So they had this cDNA library, and they used that to express it in yeast cells. And they took a yeast mutant that was defective in Adenine metabolism and complemented it with a Drosophila gene. So the idea of functional complementation between organisms indicating evolutionary conservation of function somehow stuck in the back of my mind and at that time – this was 1981 - there were no sequences around. I mean there were only some sequences of actin, tubulin, and cytochrome C. So the question of structural and functional conservation was totally open and there was really no idea of how much conservation exists. And this issue is especially pertinent to the cancer-causing genes that were known at the time, that were isolated by being hijacked from cells by oncogenic retroviruses. So whether these are genes that are specifically dedicated to mouse or to growth control in vertebrate species, or whether they have broader functions – was totally open.

I thought that if we could find homologues of oncogenes in yeast cells, then we could actually begin to do genetics to unravel their function. And then I remember in December 1980, at a Christmas party, I told Bob Weinberg: "Today I started the approach for genetic functional identification of oncogene functions." The idea was to find conservation. I went around Boston and collected DNAs of yeast of course, nematodes, sea urchin, and I went to a friend of mine at Harvard Rich Kessin who worked on slime moulds to also get slime mould DNA. I was sitting with him, explaining to him why I thought this could be an interesting approach, and he gave me slime mould DNA. Mike Hoffman, who was a post-doc with Bill Gelbart at the time working on Drosophila, had a bench in the same room. So he overheard the conversation and as I was leaving the room he came to me with a little test tube of Drosophila DNA and said, "why don't

you try that as well?" I wasn't thinking about Drosophila because at that time no-one had done reverse genetics on Drosophila and I was thinking more about single-celled organisms or at most C. elegans.

UD: So that's how Drosophila came into your life and stayed?

BS: Just because Mike Hoffman was in the same room at that moment! So I took all these DNAs and did what we called a "zoo blot", where you just take DNAs from different organisms and cut them and run them in a Southern blot with probes that you can look for and see where you find homology.

Now lucky for us, first of all, we were able to find low stringency hybridization conditions that could work in spite of sequence mismatches. And the other lucky break was that because the complexity of the genome of Drosophila is 20 fold lower than that of mouse, and that of yeast is even 5 lower than that, it means that you can load many more genomic equivalents on a lane than with mouse-to-mouse. This increases the sensitivity of hybridization because you just have many more copies of every gene on every lane.

So I had this blot of different organisms and hybridized it with probes for oncogenes that were available. These were the oncogenes that were isolated by virtue of their association with retroviruses – the Ras genes src and abl – mostly these three.

UD: I read that Weinberg later also founded a company together with Eric Lander and developed drugs against cancer. Were you ever involved in that?

BS: No. So this was in May 1981, and I have these zoo blots and probes from oncogene homologues. I hybridized them and exposed the filters to film. We were on the fifth floor of the cancer center and on the sixth floor was the darkroom where we had the tanks for developing the films. When I took them out I saw multiple bands on different lanes. I didn't know which organism they corresponded to, but there were multiple bands and they are fairly dark. When I went

back to the notebook they all turned out to correspond to the Drosophila DNA lanes. The results of this hybridization procedure were amazing because they indicated that there was significant conservation of DNA sequences in organisms that have separated 600 or 800 million years ago in the course of evolution. They indicated that these genes are not important just for vertebrate growth control, but they have a much broader function. And that provided an opportunity to begin to study them in these organisms.

Now yeast didn't work at that stage because the probe that we had contained only part of the genes; it didn't contain the most conserved part. In a way this was lucky for me because had I found it in yeast, I would have gone after yeast and I think that eventually the stuff in Drosophila was much more interesting.

So I came back home with this blot and told my wife that every one of these bands is a lifetime of work for a whole lab, which turned out to be true. And then the question is where do you go from there? So once it was published –in 1981 in PNAS – this not only changed people's view on the role of these sequences, and this was even before they were sequenced, just from the mere homology. But obviously also other people became interested in it, most notably Michael Bishop who now said that he was converting his lab to study Drosophila, quite a frightening prospect. It was May and I returned to Israel in August, starting a lab knowing actually nothing about Drosophila!

*Oncogenes in Drosophila*

UD: Did you return to the Department of Virology because of the retroviruses?

BS: No, at that time there was genetics and virology. The distinction between them was more semantic. But they hired me and felt I would work on oncogenes and they were pretty disappointed when I told

them that I want to work on Drosophila, but that was my choice. Eventually a year or two years later Michael Wigler found the Ras genes also in yeast. But we focused on Drosophila. The initial work was more structural. At that time is was good enough to get every part of the work into *Cell*, but it was not that interesting. It was more cloning and finding out how much conservation there is and so on, pretty structural work. The more interesting work was the functional work. But when you go to do reverse genetics, and this was what we had to do - you start with a gene and you isolate mutations and go after the function – it's really a matter of intuition or luck which genes you actually decide to go after.

UD: There is not a 1 to 1 relation between gene and phenotype.

BS: Well, it's a matter of luck for two reasons. One is that if you go after a gene which has a strong maternal RNA contribution where the females contribute a lot of normal maternal RNA to the embryo, then even if you knock out the zygotic function of the gene in the embryo you're actually going to get a very muddled and variable phenotype because the normal maternal transcript that the female deposits is enough to get the embryo through all the various stages. So this is in fact what happens for the Ras genes. Ras is a critical component, but if you knock it out zygotically there is a strong maternal contribution so they only die as larva or pupa and you really don't learn much about the primary function of the gene.

So one aspect is the maternal contribution; the other aspect is that if you work on a gene which either doesn't have a very pronounced function or, like Ras is a cytoplasmic component, which gets inputs from different receptors and pathways and so on, then the phenotype that you are going to get will be confusing and muddled because it reflects a combination of several pathways. It's not a distinct 1 to 1 relationship with a given pathway. So luckily Mike Bishop chose the wrong genes – src, fps, and so on – it took them years and they got phenotypes that were very non-descript and not very clear. We waited for about a year or so and in 1983, Yossi Yarden who was a

PhD student with Yossi Schlessinger, who was still here at the Institute, isolated peptide fragments of the EGF [epidermal growth factor] receptor and sent them to Michael Waterfield who was doing peptide sequencing, and he found out that the fragments correspond to an oncogene called ErbB [avian erythroblastosis oncogene B] which is a tyrosine kinase – just like src.

So when Yossi Schlessinger found that out, he was very excited and came to my lab and told me that ErbB is the tyrosine kinase receptor – we already knew that ErbB is conserved because we've done the zoo blots with the ErbB probe – so realizing that ErbB is a transmembrane receptor, I thought that this was a fantastic nodal point to go for mutants because at the receptor level you're really looking at the distinct pathway and you're really likely to get phenotypes that correspond one-to-one with the actual function of this pathway. So literally, when Yossi left the room after telling me that, I went to my freezer and took out the ErbB probe that we had and used it to isolate clones from Drosophila.

So we isolated in 1984 and '85 the first clones of the Drosophila ErbB (EGF) receptor, sequenced and characterized it and so on and began to go after its function. Now the way to go after a function in these days was to map or identify the chromosomal region where the gene maps and then obtain a chromosomal deficiency that uncovers that region, saturate that region for mutants and define and cluster them into complementation groups and then figure out which complementation group corresponds to your gene. So we went through that elaborate procedure and identified the complementation groups that corresponded to the receptor and in parallel work from the group of Trudi Schupbach at Princeton had identified mutations in the same gene– so the two groups came to the conclusion at the same time that we'll identify mutations in the receptor that give rise to a severe phenotype which was previously – or this mutation was previously identified as a severe phenotype mutation – and we could now begin to characterize the effects of this mutation.

This was at the time when the reverse genetic efforts come together with findings from the direct genetic approach. The work of Janni Nuesslein and Eric Wieschaus, for which they got a Nobel Prize, looked for mutants that disrupt the anterior posterior polarity, they find the mutants, they group them into functional groups, and then try to go after the genes and figure out what they do. So in a way the two efforts converged because it turned out that through the reverse genetic approach we were finding the genes that were actually identified genetically by the direct genetics approach. We essentially were finding out key signalling pathways that play a role in embryonic development, and when they go awry, participate in human cancer.

*Molecular embryology in Drosophila*

UD: When did you go into molecular embryology?

BS: It took us a few years to actually learn the biology properly. We started knowing very little about Drosophila and focusing initially on the more molecular characterizations of these oncogenes. As we were doing the screen for mutants we got more into the biology but were still pretty naïve about it and we could have done things more efficiently and better had we known more about the biology. But towards the late '80s we gradually got more deeply into the actual biology and having isolated the mutants in the EGF receptor we realized that we would have to know the biology better in order to really understand the full function of this gene. And this was partially aided by the fact that I did a sabbatical with Spyro Artavanis-Tsakonas at Yale in '89 and students went to courses and we gradually looked at it in a much more biological way.

The EGF receptor plays a central role in development where it has multiple functions, in contrast to other receptors that were isolated at the time like Sevenless or Torso, which have a single function in development and therefore are more easy to dissect. Therefore, the challenge was to actually be able to isolate each one of these

functions and look at it in detail rather than just getting a final end product which is a composite of all the roles that the receptor plays throughout embryonic and post-embryonic development. And it really turned out to be one of the five major pathways which shape the embryo and post-embryonic development and plays a role in dozens and dozens of roles in development.

UD: It's a receptor of what?

BS: It's a receptor which is expressed on the cells and responds to ligands – to hormones – which are provided by neighbouring cells.

UD: I am interested to know what you are doing now. Developmental signals that are transmitted to and received from the embryonic cells and the mechanisms they provoke - how is signalling genomically controlled?

BS: Let me just - before I answer that question – explain in general, that developmental biology is now at a turning point in the sense that the last 30 years have really been remarkable and it was a privilege to work in this field during that period, knowing very little about the way an embryo develops. We now know the major pathways, the major principles; the conservation of these pathways is really a central guiding theme not only for development but for biological processes in general. And in a way, some of the research in developmental biology today is more dotting the I's and crossing the T's in the sense that it's the same pathways again and again, we know the components, we know the principles, so it's important for every tissue to find out the subtleties, but in a way it's more of the same. It's becoming less interesting.

The next challenges, I think, are going to come from two directions. One is to integrate the developmental signalling with cell biology because, in a way, when these pathways work in the context of the cell they have to integrate into the context of the cell. The question is "how do you use general cell biological features in order to modulate and tweak these global signalling pathways to adjust them to the

specific features of different cells and different tissues during development?" So that's one challenge – to integrate cell biology and developmental biology.

And the other challenge concerns computations. When we initially looked at the signalling pathways, we did a crude manipulation of knocking out the genes completely and looking at the consequences and you get dramatic phenotypes because when you knock out these pathways you really affect central signalling processes. But during normal development the challenge that organisms face is not a complete knockout of these pathways, but quantitative perturbations in the levels of signalling because embryos are faced with continuous fluctuations in the level of these signalling elements. These fluctuations can come from stochastic variations, from temperature, from environment. The embryo is not like a mold that you pour stuff into and that's why it comes out the same again and again. Every embryo is a new creation which is a result of cross-talk and communication between cells and getting reproducible patterns. it is really, in a way, a miracle because it's a consequence of many signalling and communication events which build on top of each other so every inaccuracy will essentially be amplified in subsequent stages. So getting a precise outcome every time is really a miracle. And the fact that embryos can compensate for huge differences in signalling is perhaps exemplified most dramatically by the fact that you can genotypically make embryos that have half the level of a morphogen just by knocking out one copy and despite the fact that you have half the level of this critical signalling molecule you get normal-looking embryos. So how do they know? I mean, how do they sense, how do they correct these inaccuracies?

So clearly the major challenge is to look at quantitative aspects of signalling and ask how embryos sense variabilities or fluctuations in the level of the signalling components and how they correct for it so that the end result becomes very accurate.

And to do that you need, on the one hand, accurate ways of measuring signalling – not just on and off, but actual measuring the fluctuation – and imaginative computational approaches to begin to get the quantitative aspects of these systems and this is part of what we are trying to do. So coming back to the original question-

UD: To my question - I am influenced by the work of Eric Davidson. He said that the fact that the outcome of development is always the same – more or less the same – is due to the fact that the early stages of development are genomically hard-wired. He related to regulatory genes and their hierarchical organization in regulatory networks and their connections by transcription factors. I wanted to know how these two things relate. So how do transcription factors relate to the signalling cells? Do they influence them or -

BS: Yes, it's an interplay. Davidson ignored, or didn't like, the role of extra-cellular signalling molecules because he was into transcription factors - that's fine. He was a bit too dogmatic, but the hardwiring of transcription factors is there. But it is generated or triggered by extra-cellular signalling components. You start with an extra-cellular component, a hormone, which is distributed asymmetrically in space, and functions as a morphogen, and this single molecule can give rise to multiple cell fates because the cells can sense not only the presence or absence of this molecule, but also its level, and respond differently to different levels of the morphogen. This is the classical French Flag model of Lewis Wolpert.

UD: But again, how come the outcome is the same? And which agent controls the cells which produce the hormones?

BS: So with every stage, you need things that will – let me say this a different way – the distribution of the morphogen is very critical because since the morphogen affects different cell fates and the cells sense the absolute level of the morphogen, you need a way to first of all sense the level of morphogen that you have at a given time and then to correct the distribution in a global way. It's not trivial how you

would actually do that. On top of that you have a situation where you have embryos of different sizes – now obviously if the morphogen is distributed you will want to adjust the morphogen to get the size of that particular embryo because you want to scale things. So how do you do that, where do you sense, and how do you correct it globally?

We have a theory which we have shown already in several cases to operate – this is in cooperation with Naama Barkai in our department. The idea is: you sense at the edge of the gradient – this is a very powerful place to sense because it gives you an input or indication about the entire profile. Through a mechanism that we have postulated, you produce a molecule which is diffusible, extra-cellular, and impinges on the entire signalling profile. The general idea is that through this molecule you bring the level of signalling at the edge of the gradient to a fixed level which is invariable and you do this by impinging on the entire profile - you adjust the entire profile so you get it to a fixed level by sensing at one point and impinging on the whole gradient. It's a challenge – where to sense and how to impinge on the whole distribution profile. It's a challenge and an open question.

UD: How does the morphogen gradient get to the embryo?

BS: It gets to the embryo because the idea is that you produce the morphogen in a restricted domain -

UD: It is embryonic, not a maternal effect?

BS: It's embryonic but in a way like the Spemann organizer is embryonic; it's zygotic, but it's a consequence of maternal effect. You somehow generate an asymmetry which affects gene expression in a restricted position in the embryo and this generates diffusible protein which is produced in one place but is then distributed to other places in the embryo. It can also be maternal in origin, like the Bicoid along the AP axis.

UD: So that the cells know their position?

BS: Yes. The protein that you produce has a certain half-life time. if you know it's very stable then eventually you would have equal levels everywhere because it just accumulates. But it has a limited half-life time because it's degraded, because it's taken up by the cells, and so on, so you end up with a distribution where cells that are closer to the source would see more, and cells that a further from the source would see less.

Now, the idea is that initially this somewhat unstable protein a fairly coarse source of information; it's not very precise, but it gives a global input to the coordinates of the embryo. And, after some buffering and adjustment, the cells of the embryo will use this information to sub-divide themselves into two or three distinct domains of gene expression. And then the process refines; you can actually shift from gradient to borders and so on, each time on a smaller and smaller scale. You start with a gradient that can encompass the whole embryo - you use that to sub-divide the embryo, let's say, into three domains of distinct gene expression like the French flag with clear borders and so on, and now, for each of these domains you can generate, let's say, a gradient that will emanate from one domain to the next domain on a smaller scale. And you sub-divide that into regions and so this shift between a gradient to borders to a gradient continues but each time on a smaller and smaller scale, subdividing the pattern further.

The pattern is hard-wired in the sense that the order of events is sort of anticipated, the complexity is increased over time, and the pattern is refined to smaller and smaller regions as a consequence. But it is not hard-wired in the sense that at every stage where you employ a morphogen gradient, the cells are actually sensing the levels and you can shift the borders depending on the distribution of your morphogen gradient. So the cells are sensing the communication between them. It's not a situation where every cell knows from the outset what could actually be, but at every stage, depending upon the signal from the environment, every cell decides which course it will pursue. Now this course is affected, on the one hand, by its history – I mean, it's

already a cell in a particular position with a particular combination of transcription factors so that's the hard-wired aspect, but it's now still listening to the environment and deciding which choices to make based on the environment.

UD: But this environment is obviously the same in all embryos of a species.

BS: The environment is the same in what sense?

UD: It's not an accidental kind of gradient. It is the same in every embryo of the species.

BS: Right, right. For example, the position of Spemann Organizer is dictated by the position of sperm entering into the egg. So even though the egg is radially symmetrical, this random symmetry breaking event will define the Spemann Organizer exactly. And that would be the same of embryos, it's true. But the actual monitoring of the gradient and the level of the gradient, and so on, will differ from one embryo to the next. This is where variability comes in, and where you want to adjust things and to-

UD: -right, within, let's say, the species characteristics.

BS: So every embryo is listening to the same molecule; some are listening stronger and some are listening weaker, and you want to adjust that listening in order to get the right pattern.

*Molecular interpretation of the Spemann-Mangold experiment of the induction of a second head in 1924*

UD: Can you tell me what the organizing molecules in the Spemann-Mangold experiment are? I know that a lot of molecules were found that could function as inducer, but they were not specific. What is the view today?

BS: Unfortunately, this screen for the molecule failed at the time because the assay was so complicated. I mean the duplication of the axis is really a very, very complicated assay, so the molecules could not be isolated chemically. They were identified in the '90s when people like Richard Harland at Berkeley started to look molecularly for genes that are enriched in the Spemann Organizer. And they found secreted molecules that were enriched in the Spemann Organizer – noggin, follistatin, and chordin - and then they were able to look molecularly at the function of these molecules. Again, once these molecules were characterized in more detail, they turned out to be elements in one of the five cardinal signalling pathways that regulate all aspects of development.

UD: Protein molecules?

BS: Yes, of course. But they were initially identified as genes and RNAs and so on. So the cardinal molecule in the Spemann Organizer is chordin (the Drosophila homologue). It intuitively was very surprising because this molecule dictates the position of the head and the central nervous system of the frog. And you would think that you need positive things in order to induce such elaborate structures as a head structure. It turns out to be an inhibitor. So you actually have to inhibit a signalling pathway in order to make a head. If you inhibit the pathway, the default is to make a head. Conceptually it doesn't matter; it's just that psychologically it was a little bit of a shock!

So how does it do it? As I said the egg is initially symmetrical. And interestingly, while the signalling pathways that guide development are conserved, there are events that we call "symmetry-breaking events" which break the symmetry and these are actually not conserved; they are very different from one organism to the next and they depend more on the biology of that organism. So in frogs, where the fertilization is done in the water and the egg's radius is symmetrical, you have to break up that symmetry. And the way that you break up that symmetry is that the sperm enters at a random point, but the point of sperm entry alters the cytoskeletal organization

of the egg such that you start to get movements, and something begins to happen in the point exactly opposite sperm entry. And you recruit maternal transcription factors at that particular point, you begin to express particular genes, and eventually this induces the expression of zygotic genes like this chordin protein. This chordin molecule generates, essentially, a gradient of the activating ligand – the activating ligand comes from the opposite side. In the end, you end up with a gradient of activation where you have the least activation in the head and more and more activation as it gets further and this essentially induces the different body parts of the embryo.

In the Spemann experiment they transplanted chordin into the wrong region, so you essentially duplicate the head and duplicate the structures and everything will be half the size, but you are essentially generating two embryos in place of one embryo.

UD: So the solution of the problem of the Spemann organizer had to wait for molecular biology.

*Molecular biology of growth factors; translational research*

UD: I have two more questions: One, which role do growth factors play in your research? Are they morphogens?

BS: Yes, growth factors are morphogens.

UD: And when did it become known that growth factors were morphogens?

BS: Essentially in the late '80s, early '90s from two opposite directions. First, when people did reverse genetics and took different members of different oncogenes like Ras or EGF receptor, they found homologues and found that these homologues participate in patterning. And in the opposite direction, once the pattern mutations were identified and the genes were cloned, they turned out to be synonymous with these oncogene signalling pathways. So when the

two elements came together – signalling and oncogenes on the one hand, and embryo patterning on the other – it was clear that these elements which were known as growth factors in mammalians are actually patterning elements and morphogens in the course of development.

In some cases, they function more as on/off switches and in other cases they have more long-range functions and they actually function as morphogens. The distinction is pretty clear, I mean, when you have just one cell fate that you can induce, you function as an on/off switch. But when you have multiple cell fates that you can induce depending on the level, then you function as a morphogen.

UD: Great. Another question. Do you know of research in which growth factors are used for the development of cancer drugs?

BS: Yes. Since signalling pathways are intimately involved in cancer, the Notch pathway, the Wnt pathway, the EGF receptor pathway, there are many anti-cancer drugs that are based on these pathways. Some of the best-known ones are antibodies against the human EGF receptor, Herceptin, and other antibodies. The elevation in the level of EGF receptor is one of the most telling hallmarks for the severity of breast cancer, so treatment with antibodies against the EGF receptor combined with chemotherapy, are amongst the more prevalent treatments today. So many of these pathways are also involved in anti-cancer drugs. Usually because these drugs are given to a mature organism, the risks of compromising development are minimal, but still even for tissues that self-renew, like stem cells, it's a concern to be able to affect these pathways but maintain the self-renewing capacity of these tissues.

UD: Are you doing research on this application?

BS: No.

UD: Not at all? Who is doing it here in Israel?

BS: There are many groups, I mean the notion of combining antibodies against the EGF receptor with chemotherapy came from the joint work of Michael Sela and Yossi Yarden.

Some of the antibodies against the EGF receptor are developed in the Yarden lab. Different small molecules which interfere with receptor tyrosine kinase signalling were developed in the lab of Alex Levitzki at the Hebrew University.

UD: But your work was, in a way, the basis for that, right?

BS: Was my work the basis? No, not really for the cancer stuff. I mean, the cancer-related stuff doesn't really care about the biology or the normal biological functions of these molecules.

UD: But at least they knew about the receptor and its importance.

BS: They knew, but they didn't care about the normal function. I think one place where the developmental genetic approaches really helped the field was in building up these pathways. Because for every pathway, you isolate several mutants in different genes which give rise to the same phenotype, so this indicates that these genes function in the same pathway, but it doesn't tell you what the order is in which these genes function. You can take a pipe and constrict it in different places and get the same end-result but it doesn't tell you much about the order. These five central pathways – the tyrosine kinases, BMPs, Wnts, Notch and Hedgehog – even if you had the components, it was not trivial how to figure out what is the actual order. For example, in the case of the Notch pathway, the receptor is embedded in the membrane and once it finds the ligand it gets cleaved, is released intra-cellularly, and the receptor itself goes to the nucleus and becomes a transcription factor that triggers target genes. You couldn't predict that just on the basis of the structure. So one very powerful thing that genetics offers is the ability to do what we call genetic epistasis, to place genes into a pathway and say what works, before whom, what's the order in which they work, and so on. So the genetics allows us not only to isolate all the components in the

pathway, but also to be able to order them, and from the order figure out how they work molecularly. So this was very important and it also influenced the way in which you can think about inhibitors now of different steps in the pathway.

UD: How do you perceive the interaction between basic research and applications in biology today? Is application a good thing, is it taking people away from research? Is it giving something back?

BS: I think, first of all, that there is a realization that findings in basic research have a direct implementation on applications so we see a very short interval between basic findings and implementation, which is nice and rewarding. I think that the large emphasis, especially of funding from abroad is on translational research; people tend to forget where everything came from. So someone asked a friend of mine whether Drosophila also has a Hedgehog gene, forgetting where it actually came from! So I think that for funding bodies it's important not only to fund towards where you expect to get an immediate result, but to fund basic research and directions which are unpredictable, and that's where many new findings will come from, for example CRISPR.

But people forget that, and there is less funding for basic research and less appreciation for basic research. But the connection between findings in basic research and implementation are fairly quick and impressive.

UD: Some people say that at the Weizmann Institute the largest part of research money goes into applications and others say, no the largest part goes to basic research. Do you know what is true?

BS: The success of the Institute is that it still manages to put basic research as its main theme or goal. The president said once that we are converting money into knowledge, not knowledge into money. So I think definitely that at the hiring stage and at the various stages of academic evaluation, the basic research and the basic importance of the finding is still the most important criterion, which I think is critical.

On the other hand, we are not oblivious to the implications, so when there are findings that are applicable, there are ways to take care of the industrial applications. People do worry about long-term funding and therefore consider carefully how much basic research they want to do and how much things which are more easily funded they want to include in their work. So people try to establish healthy patterns between having basic research as their primary goal and criterion, while exploiting the application stuff but not being driven by it.

So people here will say, “I want to understand cancer and use that to treat cancer”, rather than, “I want to treat cancer, is here is a protocol,” and develop it. I think this culture is deeply embedded and people are aware of it.

UD: Thank you very much for sharing your knowledge with me.