Dr. Ken Korach Interview

Office of NIH History
Oral History Program

Interviewee: Dr. Ken Korach
Institute of Environmental Health Sciences

Interview Date: April 21, 2004
Transcript Date: April 23, 2004

Prepared by:
National Captioning Corporation, LLC

820 South Lincoln Street
Arlington, VA 22204
703-920-2400

Sara Shostak: Today is Tuesday April 13th and I am interviewing Dr. Kenneth Korach of the National Institute of Environmental Health Sciences.

Interviewer: The tape recorder is on now. And you had started to talk about…

Dr. Ken Korach: Oh, I was just saying that part of our role or activities, job duties here is to keep in mind the development of research tools and reagents and models for evaluating toxicological studies as well and the fact that there are a number of environmental estrogens. Part of our role and part of our interest is understanding the estrogen signaling system so that we can understand how environmental chemicals or endocrine disruptors will disrupt that system which changes the physiology and then elicits the toxicology that – from exposures and all of that. So, that's one aspect – so it's not in – it's not significantly different than probably what some of Ray's goals were in developing transgenic models and we had actually – the first mouse we made –

Interviewee: Other over-expression of the estrogen receptor which is a key molecule in eliciting or mediating estrogen's actions throughout the body would provide or synergize or sensitize tissues to hormonal carcinogenesis, and it was suspected because two of the tissues that have the highest expression of estrogen receptor in which estrogen produces a growth response are the ones that are most susceptible to hormonal carcinogenesis of the reproductive tract in the endometirum as well as the breast.

So, we made this – Vicki Davis at the time and John Kost [spelled phonetically] made this mouse but we found very early on that animal models do not like to express – over-express estrogen receptor the way you see other cancer models. And we've known this and once we published the papers on this people told us – they said, "You know we've tried to do this" and I had numerous conversations with people at meetings and all that trying to do exactly what we were doing and we had limited success, but we were able to basically make the proof of principle that some over-expression of the estrogen receptor did produce a sensitivity to, in this case, the hormonal agent was DES or diethylstilbestrol and we never really understood why animals won't over-express it, but we – subsequent studies have now indicated that there is apparently a critical role in development that causes the blastocyst not to actually implant and this – there's a requirement there for, you know, apparently a particular balance of estrogen receptor activity. If you have too much it produces an effect and if don't have – if you have too little it produces a similar effect. So nature has devised some kind of a balance for this signaling mechanism it produces.

So, the knockouts that we made were – our strong interest in trying to understand exactly what the role is of this protein in estrogen signaling, because there have been, through the years, people who have claimed that estrogen works through a variety of other mechanisms and sometimes these, depending on the cells people used in vitro, they were able to demonstrate this, but the question has always been is: what really goes on physiologically, on biologically? So, that was what our goal was in making the ERKOs. And at the time –

Interviewer: The ERKOs are the estrogen receptor knockouts.

Interviewee: Receptor knockouts. Right. So, we collaborated. At the time we had no knockout facility here and so we collaborated with Oliver Smithies who is the world-renowned geneticist that was involved in the original development of the knockouts. He and Mario Capecchi I hope one of these days will actually – people will finally realize the importance of their discovery and give them a trip to Stockholm, but…

Interviewer: Where are they institutionally?

Interviewee: So, Smithies is at the University of North Carolina.

Interviewer: Okay.

Interviewee: A colleague - a guy that I had – a student who was at Duke who had worked on estrogen receptor, and I was on his committee, went to Oliver's lab and he and I were talking one day and he was learning how to do knockout technology and he had an interest in estrogen receptor and so I said – we were talking and I said, "What about making a knockout?" Well, if you looked in the literature and what people were taught in graduate school and medical school was that mutations in the estrogen receptor were lethal. And so it was very – it was virtually impossible to get anybody to fund – like through a study section – I mean I had people call me and they said, "I'm reviewing this grant making a transgenic for ER, knocking it out – " people just felt that was not going to be a worthwhile endeavor.

Anyway, so I – we are also interested in development of the role of estrogen in early development, because certain chemicals, environmental agents that you are exposed to early in development, can effect your subsequent adult life and effects. So, I said, "Well, look even if we do it and we don't get living animals, viable animals, we'll at least know where estrogen receptor was critical in development and to see if it links, for instance, with DES carcinogenesis and so forth." So we, Dennis and I, worked on this thing and he did the fair amount of on-hands work in Oliver's lab because they had the technology there.

Anyway, we got these mice and everybody in the endocrine field were like – they couldn't believe it, because it was supposed to be lethal. There was a textbook up there that has a paragraph that states that. And so anyway, we got these mice and then we thought there was only one estrogen receptor molecule and so we spent a fair amount of our time either using these mice to study various aspects of the biology of estrogen action or the hope is is that they could be useful in evaluating environmental agents that have hormonal activity.

So that was one of the goals and what these mice, I think – well, the mice were extremely – I mean, they were critical in identifying the one and only patient that has an ER mutation. Because this mutation was supposed to be lethal people never thought about it and there was actually a colleague who had done his pediatric endocrine residency at UNC that both Dennis and I knew. And he called me up one day and he said, "I've got this patient" and so he started describing this patient and I said, "Well, did you check this?" And so forth and so on. Anyway, we talked about treating this patient and I said, "Why don't you put him on an estrogen patch or an estrogen treatment and see – "

Interviewer: How had he presented?

Interviewee: So, he presented – he had presented originally to an orthopedist. He was 28 years old at the time and he had the bone age of a 14 year old. So, his epiphyses were not closed yet, which they should have been when he went through puberty. He had high gonadotropins. He was insulin resistant and slightly obese and, to make a long story short, it turns out that he didn't respond to estrogen treatment. His gonadotropins didn't suppress. His epiphyses did not close and so we analyzed his – Eric was sitting there trying to figure out from an endocrine – pediatric endocrine standpoint what was wrong with this patient because they see a lot of androgen insensitivity conditions and things like that, but this was – the patient wasn't responding to any known therapy and Eric said, "You know I didn't realize it, but the mice you made have no estrogen receptor and they're viable. They are not lethal. Do you think this guy could have a mutation?" And I said, "Well, you know it's possible, there's no known cases."

Anyway, he sat down with the DNA, we analyzed it and there was a mutation that essentially the gene didn't make the protein. So, he had functionally no estrogen receptor as well and, so, this was the first report of this that we made with Eric in the *New England Journal* and I like to use it as an example where basic science led to this clinical observation because unless we would have made those mice we would have never have thought that this patient had a mutation in the ER based on what we, you know, the dogma at the time. So, that I think that the patient has clearly told us clinically that estrogen closes the epiphyses both in the males as well as females; that the old hypothesis was that androgens, pubertal androgens, closed the epiphyses in the male and estrogens in the female. Now we – the patient demonstrates that it is both.

He also was osteoporotic or osteopenic. He had a bone density at 28 that was equivalent to around an 80-year old post-menopausal woman and he has cardiovascular problems as well that were identified by a group out at UCSF in San Francisco. So, I think it was an important discovery and it's still the only – he is still the only patient, and people have looked and looked and we've analyzed other patients that have turned out to be aromatase mutant patients that don't make any estrogen, but not another one with an estrogen receptor mutation. So, that was, I think, important for the endocrine community, the clinical aspects as well as the basic.

And then – so that kind of turned everything on its ear as far as that you don't need estrogen receptor for development and you don't need it for viability and people were trying to understand how this was all going on.

Interviewer: What year was this?

Interviewee: This was in – that report came out in 19 – December *PNAS* 1973.

Interviewer: Okay.

Interviewee: So, it's been a little over 10 years since that first report.

Interviewer: The report came out in ’93?

Interviewee: ’93, yeah. What did I say?

Interviewer: You said ’73.

Interviewee: Oh, no, no I’m sorry, ’93, yeah ’93.

Interviewer: You had just radically shifted my understanding of science so I had to check.

Interviewee: No, I’m glad you clarified that. So – no in ’93 and in it – this project started in ’90.

Interviewer: OK. The project of making the mice?

Interviewee: Making the mice. The technology nowadays is so much more efficient and so much better understand than compared to the early days that Oliver’s lab was involved with it –

Interviewer: The first model you made was the ERKO?

Interviewee: Right, but this was – so what we have to clarify is this was for estrogen receptor alpha because there’s two estrogen receptors and the patient had a mutation in estrogen receptor alpha. So, the other – I mean there’s phenotypes in the female mice which one kind of expected, but the biggest – the other big surprise was that there was infertility in the males lacking estrogen receptor and we know that part of that is sequestered to the testes and associated structures and it turns out that they have alterations in their spermatogenesis which contributes to the – and but the sperm that are developed in those male mice are not functional and we were fortunate to have a colleague here, Mitch Eddy who is a well recognized international expert on spermatogenesis and so he was helpful and we collaborated with him on a couple of the original papers.

And it turns out that there is a specific region of the male reproductive tract where estrogen receptor activity is critical for proper spermatogenesis. Again, the original hypothesis was that these were all androgen regulated functions and it turns out now that – when I lecture I like to use the term, “Nature in all of her wisdom decided that estrogen was going to have a critical role”. And this was important for toxicology also because it indicated that estrogenic chemicals that where we see male infertility and effects on male infertility that there is clearly a need for normal estrogen action in the male reproductive tract and when that’s altered or disrupted then you can elicit these types of effects. So, this clarified, to a certain extent, that it wasn’t just simply a high toxicological exposure, but there were normal physiological mechanisms that were required and going on in the male reproductive track.

So, that – and we’ve – people – we’ve sent these mice out to people all over the world and they’re all over the place. Some of the pharmaceutical companies have licensed the mice for testing compounds and stuff like that.

Interviewer: Have licensed –

Interviewee: – the use of the mice.

Interviewer: From?

Interviewee: From NIH.

Interviewer: OK.

Interviewee: Because NIH patented the estrogen receptor knockout mice.

Interviewer: OK and just for my clarification what – how are the ERKOs different enough from the patent that was given to Leader [spelled phonetically] and Harver [spelled phonetically] for the ONKO mouse?

Interviewee: Oh, that's a totally different gene.

Interviewer: OK.

Interviewee: It's a totally different concept. That mouse is over expressing an oncogenic gene that makes the tissue or the animal susceptible. This is where we've essentially removed a functional protein in the animal. So, it's a different concept.

Interviewer: And because NIH has the patent for the ERKO models they can be used by intramural researchers?

Interviewee: Oh, yes we've –

Interviewer: Can be used by researchers in extramural settings and it's only pharmaceutical companies or chemical companies that would need to get a license? Only commercial interest.

Interviewee: Right, exactly –

Interviewer: Okay.

Interviewee: Yeah, I mean they are sent out to – we breed – I mean when Harold Varmus was director when all of this happened and one of the things – he and I collaborated on a project with these mice, because the exact opposite question that we asked when we made that first mouse where we over-expressed the estrogen receptor the question was that if you don't have estrogen receptor does that effect or alter susceptibility? Do you still get toxicological effects – a carcinogenic effect?

We have been able to publish studies where we show that the toxicological effects of DES are essentially not observed or not seen in these mice that lack functional estrogen receptor alpha. So then – let's see I guess it's been now 6 years? Maybe it's been longer than that. I went to speak at this keystone symposium, which is a big symposium for all the nuclear receptor people. So, I got there and this fellow, Bert O'Malley, who's a very well known endocrine scientist, he said, "I've got to tell you something." He says that we had this guy by – the department gave this talk and he said, "He had this evidence that

there's a second estrogen receptor." So, everybody thought that there was one estrogen receptor and when we knocked it out that was it.

So, then it turns out that the fellow – that they group was Jan-Ake Gustafsson's group from Stockholm and so they discovered what is the second estrogen receptor, which is now called ER-β. It's a totally different protein on a separate gene, different chromosomes and everything. So, we then, in collaboration with Jan-Ake and again with Oliver, we knocked out ER-β and the question was: is it ER-β that's present?

So the first thing we did was we came back from the meeting and we checked out mice to see if they even made ER-β and they had the same levels of ER-β expression as the wild-type mice. So, they didn't have any alpha, but they had beta and we had all these effects. So, right away they were telling us that certain aspects of estrogen physiology are being mediated by ER-α and there are going to be others that may be through ER-β.

So, we knocked out ER-β and those mice have no beta, but they have wild-type levels of ER-α. So, you have two lines of mice each one with one specific form or the other.

Interviewer: And then are there any mice with both knocked out?

Interviewee: Right, so we crossed –

Interviewer: I'll let you go.

Interviewee: Okay, well, anyway, I don't know how long you want this story to go.

Interviewer: I want the whole story however long it takes.

Interviewee: Well, anyway so what and a lot of – I thought that maybe ER-β was a developmental receptor and that was the receptor that got our alphas through this lethality window and well they were viable and so forth. And somewhat surprisingly they did not have – they had clear different or distinct phenotypes. They had very little phenotype actually and so the then question was the – as you've already asked is we then crossed the two lines together and we were characterizing the phenotypes of both the alphas and the betas and the one major phenotype that we saw in the betas was an effect on the ovary, which is the tissue with the highest expression of ER-β. Although there are interesting tissues like the lung which is not really thought of to be an estrogen although, clinically, there is a – for instance a female susceptibility or dimorphism towards asthma, things like that. So, there could be some role as far as estrogen or estrogen receptor in the lung, but it has never been very well investigated.

So, we crossed these two and this was another big surprise is that if you then have either receptor you still had viable animals and the question that I found very interesting is that the female reproductive tract has – let me say it this way. We know that the male reproductive tract needs androgen in order to develop because that is, in essence, what you get pseudo-hermaphrodism or Androgen Insensitivity Syndrome. So, these are males that look like females because their male genitalia and external sex organs do not develop

and we kind of expected without both the ERs now that there would be possibly a similar effect. That didn't happen. They develop a reproductive tract and all the organs. There's no difference in their morphology. There's alterations. They don't – the reproductive tract isn't able to be stimulated. It's very immature looking and things like that in the alpha knockouts.

And so we then observed or John Couse was looking at the ovary and he said, "You know these ovaries look like they have sertoli cells in them." So, sertoli cells are the cells that are in the testes that support spermatogenesis, so that's a male specific cell. It's never seen in the female and these ovaries had sertoli cells in them and so we published this paper in *Science* where we showed that it's a unique phenotype of the double knockout in the ovary where they have structures that start out looking like follicles, but then they – what we term transdifferentiate – they change into the male phenotype. So, I thought this was kind of interesting that the default is usually – in biology the default is female and in this case the default turns out to be male somewhat surprisingly – anyway that – so those mice and the other two lines have taken – I mean we're still experimenting with them, trying to understand why they have certain phenotypes and things like that, trying to understand what the effects are and stuff like – so, that's pretty extensive as far as what all we've identified or other people have using these mice have also shown, but that was the original goal was to just see where ER worked in the development and if you could make a knockout of it and whether it would be useful or and things like [inaudible].

So, I'm just very fortunate that I was able to work with a lot of – I have a really very good group of people working with me and that a couple of technicians that have been with me for a long time have helped with this aspect. So, now we're – we've crossed these mice with models that were involved with carcinogenesis to see what the role is of ER In developing like mammary cancer and that we've looked at the DES effects of the males and the females and we've now made some other lines and we're making now a line that what we can do with the new technology – what's called tissue specific knockouts so you can design your knockout in such a way so that you can only knock that receptor out, for instance, in one tissue and not in the others and that way you can start to look directly at the organ or the tissue effect when you lack that, but because there is an endocrine – this is a major endocrine signaling system, there's a lot of effects on a lot of other aspects of the physiology that one has to always keep in mind when you're evaluating the phenotypes and things like that and so we've – our group has taken a – we take more of a cautious conservative evaluation. We, for instance, there was this effect on the ovary and we found that that effect on the ovary was not due to an actual loss of ER-α in the ovary, but it was due to the lack of ER activity in the neuroendocrine, so there's no negative feedback and that high LH was actually producing the effect of the phenotype in the ovary and if you controlled the LH –

Interviewer: Which is luteinizing hormone?

Interviewee: Which is luteinizing hormone – then you don't develop the cystic follicles and the alterations.

Interviewer: Can you help me understand how developing a model to understand the function, say of estrogen receptorsm is similar to or different from developing a model to use as a carcinogen bioassay.

Interviewee: Well, the estrogen model allows you to look at – it gives you the opportunity to investigate normal physiology and biology which an oncogenic model is more limited but, in addition, the ERKO models do allow you to answer certain question regarding, for instance, carcinogenicity of tumorigenicity because you can ask, as we did in some of those studies, what is the role of estrogen receptor in mediating the tumorigenesis and things like that.

See we've found that, in fact, the question as you probably know in carcinogenesis is this two-step model which is a favored model. There's induction and then there's progression and what – in three different studies what the results demonstrated or showed is that, in fact, ER appears to be affecting progression not the induction of the tumor. The DES study spoke, to some extent, addressing the question of DES induction of cancer or toxicity and, in fact, the model showed that, in, fact that DES is not tumorigenic, toxic or carcinogenic in the absence of estrogen receptor alpha. It also told us that ER-β cannot replace ER-α and, second, that ER-β does not appear to be a – have the same activity or actions in the body that ER-α does. Now that – one might say, "Oh, well that was kind of intuitive. There's two receptors; they're probably doing something differently," but, in fact, until you experimentally test that hypothesis you don't – I mean, it's just a hypothesis. You have to prove it.

So, I think we are doing – we are finding out more. In organs where there weren't – in the alpha knockout where there were not possible effects it may be that those effects are now going to be seen in the beta knockouts and, in fact, one of the systems that we immediately wanted to evaluate was the immune system and, in the case of the immune system, the alpha knockouts don't really have any phenotype or compromise of immune function, and that was done, independently, in a couple of different collaborations and it was clear that isn't happening.

But the betas are showing a demonstrational role. The betas also have been shown to probably have a role in the GI tract, so there are a limited number of reports about estrogen and colon cancer and the highest expression of – or one of the high expressions of ER-β is actually in the gut, in the colon, and it turns out that Wyatt Pharmaceuticals has actually come out with a ER-β specific agonist for the treatment of colitis. And so it could be that this model – I mean they made their own ER-β knock out, but the whole concept of using these models for these types of things seems to be playing out.

Back to your question I think that the oncogenic models, not to be critical of them, but I think they are limited. But, on the other hand, the ERKO only allows you to ask a certain set of questions and not – coming back to the original question about if you have over-expression or if you have a tissue that doesn't normally express the receptor – this was one of the things that I was hoping we'd be able to do, and we could express it in a non-estrogen expressing tissue, would that tissue now start – would it behave as an estrogen target tissue? Would you be able to elicit certain gene responses and things like that and

we've never been able to – we're actually – I have a project where we are trying to actually do that and we think that we might be able to get around some of the obstacles of it and so we'll see if this develops – you know if we are able to develop that further.

Interviewer: I want to back up and ask you a couple of questions about how you came to NIHES. So, what's your education and training and what brought you here?

Interviewee: Okay, my training – I trained at the Medical College of Georgia and I got a PhD in endocrinology. It was one of the – I think there was only two schools in the U.S. that actually offers a degree in endocrinology, and I went to Boston for a postdoc and I did a postdoc at Harvard Medical School in the lab of reproductive biology and I was starting to look for a faculty position to get started and I was – I had a tentative offer at UVA in Charlottesville and then at University of South Carolina Medical School in Charlestown. And I was not un-inclined to come back south because I grew up in the South. My wife was from the South, families were down here, and then, when I was in graduate school I had – the guy who had done all of our centrifuge repair was always talking about this place called the Research Triangle Park and some of the faculty members down there were saying, "Oh yeah, there this place in North Carolina and they're trying to build this thing up as a research center and there are all these institutes" and this that and the other. So, the guy I was post-docing with, Lewis Engel who was the fellow who discovered the conversion of androgens to estrogens. It's called aromatization and he's the guy who discovered that process and the enzymes that were involved in it. And, so, he got this letter from the institute and they were interested in recruiting somebody who knew something about estrogen action and estrogen receptors.

And so I came down here and interviewed and a couple –

Interviewer: Did you interview with Bob?

Interviewee: No, no. I interviewed with the lab chief at the time that was hiring me was a fellow by the name of Bob Dickson [spelled phonetically] who is a male reproductive toxicologist, and he was trying to set up a group of reproductive people and I came here and I talked to three or four of the investigators and I said, "I'm not a toxicologist," and he said, "No, no you don't to be a toxicologist we want somebody who understands the endocrinology and the reproductive stuff and estrogens and stuff." And so I knew estrogen chemistry and stuff like that from my training and all that. And, so, I talked to my wife and, you know, there was this fact that you didn't have to – I didn't initially have to get a grant and I could just come and do work was very attractive to me and, so, I took the job and the position and I was here about 4-6 months or so and started to learn the government system is – you know the positives and negatives and all that. I remember talking to my old major professor and he said, "Well, how do you like it?" I said, "You know I feel like I'm in kind of a wasteland." There wasn't any other receptor or hormone action people around. There was one guy that they had hired from Baylor, Steve Harris and that, but he really wasn't a receptor or estrogen person.

So, I told him, I said, "Well, I'll probably be here three years and then I'll leave." So, that was twenty-eight years ago and I'm still leaving. So, it's – I mean things have changed, obviously, very dramatically; all this stuff on endocrine disruptors and

environmental estrogens. One of the guys that was my boss for a longtime, John McGlaughlin [spelled phonetically] was one of the kind of leaders in that area and he and I collaborated together for a number of years. And so, things have – I mean the area know is – you know, we have a number of people that are involved with receptor signaling of different types and we have a Receptor Mechanism Discussion Group that we started – I think it was like 22 years ago. We meet once a month. We have speakers from the institute and the surroundings universities and things like that. And then there's been more and more people that have come in that are endocrine or reproductive and that. So, the environment has changed dramatically.

Interviewer: It's a different place than it –

Interviewee: Oh, yeah.

Interviewer: -- the place you would have left 25 years ago?

Interviewee: Exactly, exactly. So, that's how I guess – you know, I was a little nervous about the fact that I wasn't a toxicologist and I thought that was what – but it turned out not to be an issue at all.

Interviewer: Specifically because understanding toxicology requires understanding basic biology?

Interviewee: Right, you can't – I don't think you can understand toxicology and the effects on the toxins produced unless you understand where they are acting and what – how they're acting and what they're doing to the normal biology. And that's been one of the, I think, the founding concepts in that, but –

Interviewer: Founding concepts here or…?

Interviewee: Well, I think that there was a strong appreciation of that by the senior management at the time when I was a younger [laughs] investigator and things like that, but there needs to be a basic science background. I mean, if there wasn't these mice wouldn't have never been made, for instance, and I think it was – when Zerhouni was here Olden had been here about 6 to 8 months ago, or maybe longer. So, I had to give a little 10 or 15 minute talk about the ERKO mice and I told him that he had mentioned something about the intramural program should do difficult –

Interviewee: -- and I mentioned, I said, "You had said that intramural research should involve high risk and stuff that can't be done or it's hard to do on the outside." I said, "I think this is a perfect example of that where nobody on the outside was able to do this either because of the technology or they weren't able to get it funded.

Interviewer: Because the textbook said it wasn't possible.

Interviewee: Right, exactly, and study sections said, "Why go to the trouble of making this mouse if it was lethal?" Well, I mean, that's how early it was. Now we know that, in fact, a lot of knockouts are lethal, but it tells us – it uncovers a critical role for whatever the gene is in development and tells you where it works and things like that which you don't know.

You postulate but you don't have any clear experimental evidence for it to document it. So, anyway that was part of the opportunity here, but I have become, just from being here in that and some of the interactions and all that, I've become quite interested in understanding how certain environmental agents produce effects and whether they need to – whether it involves the receptors or certain signaling systems or not.

Interviewer: Can you tell me more about your research in that area?

Interviewee: So, we have looked at aspects of chemicals such as polychlorinated biphenyls and DES as a model of compound – just as a model compound because it has such potent effects that we could understand how other agents that have – it might be more difficult to investigate, because they have weaker effects or lower activities and all that. So, we've looked at receptor interactions and receptor signaling. We've just published a series of papers showing that certain environmental agents, when bound to one or the other of the receptors either alpha or beta, will have more than expected affinity to regulate certain genes than others suggesting that the gene itself is also important or critical for determining the activity of these agents.

And so it turns out that with ER-β that receptor has a preference for some of the – what I call natural [audio skip] like phytoestrogens and things like that. And so the initial indication might be is that receptor might be mediating some of these effects. We are planning studies to test that directly, by being able to use the knockouts for instance and we showed that genestin –

Interviewer: Which is in soy?

Interviewee: Which is a soy-based phytoestrogen. Genestin exposure early in development produces what are called multinucleated follicles. So, they have more than one oocyte in it. These obviously multiple oocytes are non-functional, but genestin for whatever reason specifically induces this. And so this was an observation that was known in and there's actually – we have a collaboration with a group here at the Institute as well as the woman who's actually looking at the molecular mechanisms of how this might occur and we decided to – because the cells that surround the oocyte in a follicle have a very high concentration of ER-β we asked the question could we test to see if ER-β is involved in mediating this genestin effect and, in fact, when you do that in the beta knockout animals you don't get the multi-oocyte follicle effect. So, that effect, however it may be occurring, appears to be involving – or ER-β is involved in it.

So, there's examples there of looking at the toxicological observation that we at least added some information towards how it may be occurring and the mechanism by which it's occurring. So that's – we're using environmental – examples of the different environmental agents to investigate certain mechanistic questions.

Interviewer: And are you in conversation with any of the regulatory agencies about this research?

Interviewee: Well, there – I mean a number of people over in the reproductive tox-group over at EPA are familiar with our studies. I'm actually supposed to go in a couple of weeks to a

workshop over at EPA that they've invited me as a participant to discuss estrogens as carcinogens and exposures in models and things like that.

So, we have collaborations and interactions with a number of academic as well as NIH people. Not so much – I'm not sure if our studies necessarily speak towards the regulatory aspects. They would potentially be towards the mechanisms by which chemicals could act or something related to susceptibility, but I don't know if levels of the compounds and things like that – you know our studies don't really address that.

Interviewer: So, not so much quantitative risk assessment as understanding mechanisms.

Interviewee: Right.

Interviewer: And how would they contribute to understanding susceptibility?

Interviewee: If you're able to show that certain tissues, for instance, that have one form of the receptor – like the ovary. Well, that happened only in the ovaries within the beta knockouts and not in the alpha knockouts in that. And if it could, in the case of when we did for instance the DES effect, you would – you could show or you would show that it was a – that they're less susceptible and so…

Interviewer: You're the Director of the Environmental Diseases and Medicine Program.

Interviewee: -- and medicine program. Right.

Interviewer: Two questions about that and then one final question and then we'll let you go to lunch. Can you tell me if there are any significant uses of transgenic models in other aspects of that program?

Interviewee: Well, I mean for instance, in the pulmonary group they are using both transgenics as well as knockouts to investigate various pulmonary effects and pulmonary toxicology and also susceptibilities that are linked to genomic or genetic analysis due to exposures.

Interviewer: And if I were to talk to one person in that group about their work who would you recommend?

Interviewee: Well, there's the Lab Chief for pulmonary, Steve Klieberger [spelled phonetically] has a number of animal models involving pulmonary toxicological susceptibilities, but then another investigator there by the name of Darrel Zeldon [spelled phonetically] has some other models that are not really pulmonary as much as cardiovascular, so I think I would probably as an initial contact probably talk to Steve.

Interviewer: Okay.

Interviewee: Darrel has made transgenics of one of the – he was involved with discovering a cytochrome 2J5 which is a – metabolizes prostaglandins and appears to have a role both in the kidney as well as in the cardiovascular system as far as hypertension and things like that so he's, for instance, over-expressing it to see if these animals are more highly

susceptible to or sensitive to hypertension type conditions or challenges. There's Mitch Eddy in our group has developed animal models for studying the various effects and mechanisms in spermatogenesis. The major obstacles in studying spermatogenesis is there is no cell lines that you can study some of these things [unintelligible]. It's just a process that is an *in vivo* process, can't do anything. So, the really only way to address it is to – you can't use RNAi and things like that – is to do knockouts and so that's been the approach that he took several years ago. So, he's made knockouts of genes that he's identified that are critical in certain aspects of spermatogenesis that he's then also evaluating whether chemicals then would effect the activity of this gene or this aspect of the signaling that would then produce an alteration in spermatogenesis. So, he – I'm trying to think – I think as far as transgenics I guess Darrell and Steve are probably the ones that have –

Interviewer: And then our program that has the –

Interviewer: And then there other people working with knockouts?

Interviewee: And then there's several people that are working with knockouts.

Interviewer: Okay, and Mitch Eddy would be one of them?

Interviewee: Mitch Eddy would be one of them, Anton Jeten [spelled phonetically] who's also in pulmonary but has discovered a unique receptor-like protein that he's been investigating that works with retinoids. Retinoids do have a major physiological effect on the pulmonary system and so he's looking at a different aspect of it involving retinoid biology. So…then a couple of people have some knockouts underway and then I guess the major person is in our lab in LRDT is Yuji Mishina who we recruited here and he's a molecular developmental biologist and has not only outstanding experience and technology in the knockouts but also is interested in – he's looking at BMP molecules which are like – kind of like a cytokine factor. BMP3 and 5 and he made some of the original BMP knockouts when he was at Houston before he came here and he's – for instance, he has developed or identified a role for BMP in the actual development of the heart. The heart starts out as a tube and then it does this morphological change in that the folding over that produces the ventricle and so forth and this is I mean no one knew – really knew how that was happening that he showed that BMP5 was involved in this process and when you have it knocked out you get kind of the reverse condition and this happens clinically to very few people, but they're kind of internally reversed and this is based on his studies which suggests that there was some possibly alteration in the BMP mechanism. So, he's another – he would be I think a very informative person to talk to.

Interviewer: My last question is always is there anything I should have asked you about that we have not yet discussed? Anything I missed?

Interviewee: I don't think so. I think I've kind of given you the accounting of how all of that happened and tried to give you some of the highlights as far of what might be – the importance of what we might have done for you know for the contributiond and all of that.

Interviewer: That's great. Thank you.

Interviewee: You're welcome.

Interviewer: I'll turn this off.