Aging Revealed!

by Gregory M. Fahy, Ph.D.

The Life Extension Foundation interviews Drs. Richard Weindruch and Tomas Prolla, the first researchers to use DNA chips to lay bare the secrets of aging-and pave the way to the rapid development of sweeping new treatments for aging.

With the abruptness of a single publication, a radically new era of aging research and aging modification has begun. With the release of their report in the August 27th issue of *Science* ("Gene Expression Profile of Aging and its Retardation by Caloric Restriction"), Tom Prolla and Rick Weindruch have sweepingly changed the field of aging research forever. So great is the power of the method they have demonstrated, that virtually all biomedical research on aging will be affected by their method and by their results. The prospect of tens of thousands of gerontologists and clinicians applying the new methodology to aging implies an awesomely rapid explosion in our knowledge of the fundamental causes of aging, and in our knowledge of what can slow down or reverse aging at the deepest level. The Life Extension Foundation interviewed these two pioneering gerontologists at their laboratory on August 8, 1999. What follows is an edited transcript of that interview. **Note:** readers not familiar with some of the terms used in this interview should consult the accompanying glossary.

Life Extension Foundation: Dr. Prolla and Dr. Weindruch, how would you describe the breakthrough you've just made?

Tomas Prolla: We have used the new technology of DNA microarrays to examine the expression level of thousands of genes during the aging process. We did this by comparing five and 30-month-old mice. We decided to use the gastrocnemius muscle as our tissue of choice for this first study because it has a high rate of oxygen consumption and seemed ideal to test current views on mitochondria in aging and free radicals as a cause of aging. We have learned more in the last three months than in the last three years.

LEF: What is a DNA microarray?

TP: There are several different varieties of DNA microarrays. Basically they are small glass slides that have thousands of genes attached to them in a regular array or layout. The genes can be present either as full genes or as small gene fragments known as oligonucleotides. The

particular array that we used is the Affymetrix system, where each gene is represented by 20 oligonucleotides, and 6347 genes are represented in all.

LEF: How can a DNA microarray give new and useful information about aging?

TP: By extracting RNA from the tissue of an animal or human, you can assay for the expression level of thousands of genes at the same time. First we label the RNA with a fluorescent probe. Then we hybridize the RNA from the animal to the microarray. The array is then scanned with a laser that allows quantitation of fluorescence coming from each different RNA that sticks to the array. Each RNA type that sticks is identified by its position in two dimensions on the array. This information is converted into a data file having the expression level of the different genes. One can do this for different ages and see by comparison what differences show up with aging. It can also be used to monitor any disease process.

The final goal here, what we would like to have, is a battery of genes that increases to such an extent with aging that we can actually get, let's say, a six month window in the life span and actually tell if something slows down aging during that time. We want to get away from having to wait 30 months, so if we can find markers that change 50-fold with aging, then we can order 12-month-old mice from NIH and study them from 12 to 18 and test whether a compound works during that period. This is for preliminary screening. If it looks good, then we can go back and do a full life span study. But the point is, we want to screen for compounds that affect aging quickly. We don't want to wait for the mouse's whole life span.

LEF: The power of DNA chips was illustrated in a paper in *Science* about a year ago. The investigators exposed skin cells (fibroblasts) to serum after they've been deprived of serum. They found what they were expecting, but they also saw another whole set of genes turning on related to wound repair, and they realized after awhile, oh yeah, if you're a fibroblast in skin and you're exposed to serum, that means the skin has been wounded, so you have to up-regulate your wound repair systems. So they understood then at a glance the biology of the fibroblast much better, because they could see everything, not only what they were originally looking for.

TP: The major advantage with DNA chips is that we're looking at basically all known genes, all the genes that have been well characterized. We start the experiment without the assumption that this gene or that gene will go up or down with aging. We just test them all at once and see what the result is. We get a result that is not biased by preconceived notions. I

mean, the only starting hypothesis is that there will be some changes with aging. What the changes are, we only find after we do the experiment.

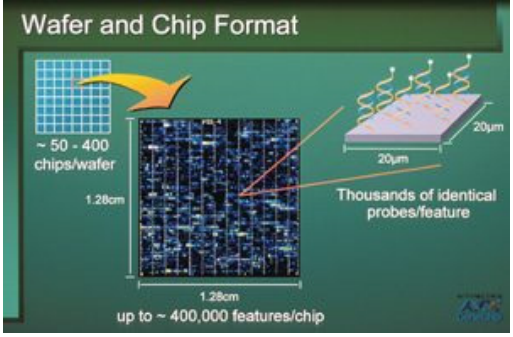
LEF: So conventional biology is like the old myth of the three blind men trying to understand what an elephant is like. One of them grabs the trunk and says an elephant is like a snake, and another grabs the leg and says no, the elephant is like a tree, and the last guy grabs the tusk and says oh, the elephant is like a spear. But your technique doesn't restrict you to one hypothesis. You see the whole elephant all at one time.

TP: Yes. Also, these changes will allow us to test specific hypotheses. We can determine if some of the genes involved are actually genes that control the aging process by making transgenic animals or developing compounds that mimic the actions of the genes. Then, by examining the alterations in gene expression with aging we can rapidly check if the transgene or compound in fact retards aging. The other very important part of the technique is that we can look at aging in an organ-specific manner. Life span studies, for example, look at the survival of a whole population of animals over several years. However, each laboratory mouse strain usually develops a specific disease pattern, such as cancer or kidney failure, which ends up limiting the life span. Therefore, the usefulness of life span studies is limited. We collect non-diseased organs from mice at different ages, and we examine at the molecular level if they're aging faster or slower, and this is independent of their survival rates.

LEF: The organs you are studying now were collected from different populations of animals and frozen for later analysis, is that correct?

Richard Weindruch: Yes, and these are tissues from small numbers of animals. One of the fascinating things about the technology is how little animal-to-animal variation we observe. We describe this statistically in our *Science* paper. The numbers of animals we use are small for aging studies, three animals per group. But when you compare young to old, for example, or old restricted to old control, you generate nine pairwise comparisons for each such group comparison, and that's how the data are analyzed. To have four animals per group would lead to a huge explosion of data that statistically is unnecessary based on the quality of the data that we're getting.

LEF: This is quite remarkable because the variation in aging between individuals in a given cohort is notorious.



RW: Right. I would argue that perhaps this is a reflection of the fact that this analysis of gene expression is upstream of many subsequent alterations. For many of these root genetic kinds of alterations, when you get way out in the pathway and among different animals, you might see a lot more noise than you do at the genetic level.

TP: Right. How the changes we see result in disease or other secondary end points might vary a lot between individuals in the population, but the point is that at the gene expression level, the mice were remarkably similar.

LEF: It suggests that if you have a 10% difference in gene expression between mouse A and mouse B, accumulated over 30 months, you get a huge phenotypic (physiological) difference but in fact, genetically there's a very minor difference between them.

RW: That may well be the case. Also, I think that our data, in large part, will validate the importance of several current areas of inquiry in biogerontology, but will also open up several others that have not been considered perhaps as important as they may well be.

TP: The technique is really a breakthrough in terms of understanding diseases. But also, and most importantly, it's a way of measuring the aging process. The only way you can actually interfere with aging is if you have a way to measure it, and we think that with this finding, we finally do.

LEF: Others have attempted to measure aging by measuring so-called "biomarkers" of aging, elements that change with age in a way that characterizes aging itself. In the past, how many age-related changes have been put forth as serious candidate biomarkers for aging?

RW: I think that depends. The answer to that question is strongly influenced by the person answering it.

TP: It also depends on whether you mean a molecular biomarker, as opposed to a physiological or a behavioral biomarker.

LEF: Tell us some of the problems of identifying good biomarkers of aging and how your method can help in the discovery of truly good biomarkers of aging.

TP: Many labs have claimed to have developed biomarkers of aging. The problem is that each of these biomarkers usually involves a different assay. Some of them are biochemical, some involve behavioral tests, some involve functional tests...

RW: They're usually one at a time.

TP: Yes, they're one at a time, and they look at one aspect of the aging process. Some of these are good biomarkers. But ideally one should be able to look at many such biomarkers at the same time, and we basically did not have the technology to do this until recently. Another condition for a good biomarker is that there must be a way to validate the biomarker. In other words, something might change with aging but may not be necessarily due to aging, or that change might not be causal. One way to test for this is to make sure that changes you see during normal aging are affected by caloric restriction, because caloric restriction is the only way to slow down aging in mammals. After we discovered these biomarkers, we looked to see how they were affected in calorically restricted mice, and we found that a large fraction of them are prevented by caloric restriction, which validates them as biomarkers. The bottom line is that we can now screen hundreds of biomarkers at the same time with the same technique.

RW: Also, I think this set of biomarkers is advantageous in that it is at the gene level. So not only is it molecular, but as I indicated earlier, it precedes many of the secondary changes in levels of protein or activities of pathways, so we think that not only will this allow a near primary global view of gene expression changes in aging, but also will move us closer to a better understanding of aging as we and our colleagues are able to sort through these various changes and conduct experiments based on this information to try to get at which of these changes may be causal.

LEF: How do you decide if a change is significant, and what percentage of the genome changes significantly? What degree of change is considered significant?

TP: One response to that question is that about 1% of the genes that we examine show about a two-fold change or higher.

LEF: That's two-fold up or down, is that right?

RW: Right. About 0.9% percent each way, or around 2% of the ones we've looked at. Of the 6500 or so genes we've looked at, about 58 to 60 genes were two-fold higher in activity, another 58 or 60 were two-fold lower in activity as a result of aging.

TP: Right, but we only screened probably one tenth of the mouse genes, so the real number is probably close to 600 genes going up or down.

LEF: What fraction of the genes increase by more than that, or decrease by more than that ratio? In other words, by ten-fold or five-fold instead of two-fold?

RW: It may be a very small number, but it may be larger than we know.

TP: We can't really address that question right now because some of the largest fold changes probably represent genes that were absent in one state and present in the other, and there may be many of them. Those could be very good biomarkers.

RW: There's a technical issue, which is that if the gene is not expressed in a young animal and is expressed in an old animal, the machine may be telling us not to accept those data. We may need to follow those up using PCR-based approaches (see glossary -Ed.), which we are starting to do so that we will not miss many of these.

TP: Although there are gene transcripts that the machine calls "absent" in young animals and "present" in old animals, and the other way around, it's very unlikely that the activity is really entirely absent, it's just that there's a sensitivity limit. If the machine can't detect it accurately enough, it calls it "absent." The detection ability of the machine, for example, is not as good as something like quantitative PCR, which we're doing now. We will go back to those markers where, for example, the machine says "nothing" in young and "something" in old but reports something like a five-fold change. That so-called five-fold change doesn't mean anything. It might be that there actually is something in the young, but at a very low level, and then there's something like 50-fold more in the old. So we're doing some independent tests. A 50-fold change would be better. There are hundreds of genes for each tissue going up and down in expression that can be used as biomarkers. The ones that are going to be particularly good, in my view, are the ones that increase linearly with aging and show large increases, because what that means is that we can probably examine animals during a portion of their life span, such as six months or a year, to determine if some compound is affecting aging, as opposed to waiting 30 months to do a full life span study.

RW: Which would be complicated by the presence of diseases. So perhaps the optimal assay to test a candidate compound might take, say, five months and occur at between 20 and 25 months of age in a mouse. I'm just thinking out loud, it need not be precisely that way.

TP: The goal is to reduce the amount of time that's needed to evaluate if some experimental approach, including some drug manipulation or genetic manipulation, affects aging. We need to find biomarkers that change significantly on a monthly basis so that in a few months we can know if a drug works.

RW: And we will also know whether it works on a tissue-specific basis, and that's a critical point. We'll be able to know very soon which systems are up-regulated or down-regulated

in each of the tissues that we look at and will be able to determine which of those are shared among tissues.

TP: Our goal right now, which we think should be the priority, is to examine three or four postmitotic tissues and look for changes in gene expression that are shared among tissues regarding aging. We postulate that those changes might be causal, and that they will therefore reveal the basic mechanisms of aging.

LEF: Can you make any predictions about how many core, causal changes in gene expression there might be?

RW: A response to this question really requires analysis of multiple tissues, so the answer should come in a few months.

LEF: If you see huge changes in gene expression between young and old, and if you could make these changes reverse by some sort of intervention, then you should expect to see large improvements, right?

RW: Only if the large changes are the most important ones. We don't know that. I want to emphasize that there's a whole range, say between 1.2-fold and 2-fold change up or down, where there are many genes, probably a couple hundred, that are influenced by aging. In terms of percent changes in normal physiology, that's where most of the action really is. So I think it would be a terrible mistake for us to focus entirely on the big changes.

TP: Right. So the genes that change a lot serve as very good biomarkers, but to get the big picture, a global picture, we don't restrict ourselves to those genes. We look for things that go up with aging significantly, and we cluster them into functional groups, to get a really global view. We don't focus simply on the genes that show the highest differences.

RW: As we have evolved in our ability to analyze the huge amount of data that is presented in front of us, we have been more attentive, in fact that's what I'm doing today, to the genes that are changing between 1.2- and 1.7-fold, either up or down, because it's providing very important information. While we've been sitting here, I've just discovered, for example, that the mRNA level for a gene called [censored] goes down by 30% with caloric restriction, and these are very high quality data. When we see 30-40% changes, we're very stringent about the signals.

LEF: In other words, that's a very certain change.

RW: Very certain. And so I find out about the gene, and I find out (reading from his computer monitor as the information comes in) it's a tumor necrosis factor receptor-associated

protein, and that it's involved in B cell signaling, and then I discover down here that it activates NF kappa B. So, there's another one! This is what we get all day long.

LEF: It's a great way to become a complete biologist isn't it?

RW: It is.

TP: This technique makes one think about aging in terms of pathways and how they connect to each other as opposed to focusing on one molecule, one hypothesis.

LEF: How do we apply this sort of technology to people?

RW: There's a human chip available. We're about to embark on a study of skeletal muscle from Rhesus monkeys varying in age and also in caloric intake. We will be looking at the effects of caloric restriction on the expression profile of the vastus lateralis (a major muscle group in the thigh). Technically, there will be the concern about the human chip not working for the Rhesus monkey, which is "only" 95-97% genetically similar to humans. We think, however, that probably won't be a problem, at least for many of the genes that may be most important, and those are the ancient, highly conserved kinds of molecules, such as heat shock factors and the like, which have emerged as being very important. We're collecting muscle biopsies now and should have those data in about three months if all goes well.

LEF: vHow big does a biopsy have to be to be analyzed?

RW: We need about 100 mg of tissue.

LEF: Different people have different genes. Can you determine what genes are the right genes? For example, can you compare a normal mouse to Peromyscus leucopus (an extremely long-lived type of mouse) and find out what genes are associated with longer life?

TP: The experiments we're doing don't actually address differences in genes among human beings because the DNA chip doesn't allow this. It just gives you the level of expression of different genes.

LEF: In other words, you're saying you can't vary the genes. They simply give you one choice.

RW: You could have a custom chip at some point.

LEF: So, let's say that I wanted to learn something about my own aging process. I come into a clinic, I have my blood drawn, and I get a pattern of what my genes are doing.

RW: We've done no work yet on blood, and that's obviously something that's on the list of things that needs to be done, and we anticipate starting that quite soon. But we have no

information on the amount of blood we would need to use for assays at this time. So if we were to assay someone's biological age now, all we know about so far is skeletal muscle. We're studying the brain now, but that's probably not a great site for getting a biopsy.

LEF: How about skin? Do you have plans to look at skin? Do you think skin would be a valid tissue?

TP: We haven't looked at it. Right now we're focusing on the postmitotic tissues. We want to study lymphocytes and eventually replicative tissues, but right now we're just focusing on the postmitotic tissues because we think that that's where most of the damage is with aging.

RW: My lab has focused specifically on skeletal muscle for much of the last four or five years, for several reasons. One is that the postmitotic tissues such as brain, skeletal muscle and cardiac tissues share the characteristics of using high amounts of oxygen for their ATP production, and it's clear that these are major targets in aging. Alzheimer's and Parkinson's disease are linked to oxidative stress and damage, and cardiac myocyte loss may well be too. There's work ongoing here in Madison by Judd Aiken and Jon Wanagat, looking at the hypothesis in the context of mitochondrial dysfunction in the aging rat heart, and with sarcopenia (the loss of skeletal muscle mass with aging). It's a very dependable feature of advanced old age and an important component of physical frailty. So we've looked at this quite a bit in skeletal muscle from mice and rats and in the monkeys from the standpoint of testing the importance of oxidative stress of mitochondrial origin in causing sarcopenia, so that led us to initially look at skeletal muscle in our current report.

LEF: So, what can you now say about how biomarkers that relate to oxidative stress and energy production change with age?

TP: Aging results in a dramatic increase in the activity of genes that have to do with stress responses, and that includes oxidative stress and also responses that have to do with DNA damage.

RW: Yes, with skeletal muscle aging, there are signs of a general stress response. Indeed, that's one of the major findings of this report, and a portion of that stress can clearly be labeled as oxidative stress. Our major finding was that stress responses related to protein damage were induced. Also, there are signs of mitochondrial dysfunction and other problems in energy metabolism suggested by the gene profile that we saw with aging, and signs that all of the above changes were quite strongly attenuated by caloric restriction.

LEF: Are you able to pick up changes in mitochondrial gene activity with this method? If so, what did you see? Are mitochondrial gene transcripts dropping out or changing, or are they staying largely the same?

RW: We can detect the majority of mitochondrially related genes (the ones that are encoded in the nucleus). The activity of these genes declines with age.

LEF: That's a really important observation. However, the DNA microarray only allows you to detect mRNA production, and not protein production directly, and there are examples in biology in which mRNA production and protein production are not tied together well. To what extent do you think this problem complicates your results or their interpretation?

TP: Some of the changes in mRNA levels may not lead to an increase or decrease in protein levels. But these changes probably represent a small fraction of the total changes observed. Our conclusions are based on a global view.

LEF: You compared five-month-old animals to 30-month-old animals. Is a five-month-old mouse old enough to compare properly to an old mouse?

TP: Yes, a reviewer was concerned about the possibility that we were trying to compare immature mice to old mice. But we argued successfully to the editor that a five-month-old mouse is fully mature, and therefore that the changes we're seeing are most likely aging related and not something to do with maturation. Mice get sexually mature by eight weeks. Anyone who's kept mice around knows that a five-month-old mouse is mature.

LEF: How many animals would be dead at 30 months?

RW: About 75% of normally fed mice. But I do not use ad libitum feeding for my control group. I decided long ago to control the calorie intake of my controls to increase our chances of studying healthy old controls. The percent reduction in calories in these controls, compared to what they would normally eat, is about 10% or something like that.

TP: Normally, the 30-month-old mice that have been basically eating as much as they want are very obese, but our control animals are actually lean.

RW: And mobile.

TP: So they represent healthy aged mice. The data clearly suggest that even a modest amount of caloric restriction can have a major effect on aging, at least for muscle.

LEF: But you're saying that in terms of oxidative damage and so forth, putting them on slightly more severe calorie restriction (26% reduction in calories) greatly attenuated the changes in the biomarkers that you normally saw in your healthy controls?

RW: Right. Please realize that we're not measuring perhaps more informative distal end points like levels of protein carbonyls and lipid peroxides, but we're really looking at genes such as antioxidant enzymes that we think would be induced by the presence of reactive oxygen species and the like. So that's the nature of our measurements related to oxidative stress and damage. It's a whole different profile than what people are used to thinking about.

LEF: Let's think about not only, let's say, competence to make energy and oxidative damage per se but also the repair of damage. I believe your study has shown interesting changes in the levels of repair systems for various kinds of damage and for DNA damage in particular.

TP: What the study suggests is that with aging there is more damage to DNA because we observed induction of a gene called gadd45, which is induced by various types of damage to DNA. It's interesting that caloric restriction, which some people thought might act by inducing DNA repair, actually results in a lower level of DNA repair enzymes. We think the reason is because the enzymes are made in response to DNA damage. The restricted mice apparently have lower endogenous DNA damage, so they don't need to synthesize repair enzymes as much as the controls do.

LEF: The genes you're able to screen for are the genes the company puts on the chip. Is it true that the functions of these genes are known, or are there many changes that you've observed that you don't understand yet because you don't know what the gene's function is?

RW: When we get a gene, we go to GenBank (see glossary -Ed.) with it. Now, some of the genes are what are called "homologues," meaning that the gene has a certain similarity to something that's in the GenBank, but it isn't the exact same gene as what has been described before. Other genes we find are the real McCoy (so to speak). For the homologues we need to determine how similar they are to known genes, and to which known genes they are truly homologous. It's often not what is stated on the information supplied by the chip maker.

TP: Right, because the information supplied to us relates to the date when the chip was made. So now new genes have been discovered, and some of the genes that were previously unknown now clearly match a known gene in the database so we have to search for all the genes on which we want to get information.

RW: And then after we have an idea of what the gene is or what its close homologue is, then we have to go to Medline to learn about the research that has been done on each of these so we understand what it does. Then we assign a functional class like, say, stress response or energy metabolism. So it is really a very exciting process of discovery to learn what the gene is and what it does.

LEF: It's also a lot of work. It sounds like the genes that are put on the chip are more or less random in a sense, or you don't get a road map of what each one of these six thousand dots represents, when you get the chip.

TP: The DNA chip that we have used has most of the known genes in the mouse and that includes most or almost all of the DNA repair genes, antioxidant genes, metabolic genes, heat shock response genes. . .

RW: A lot of the ubiquitin (protein turnover) pathways, too. And the factors for protein synthesis are well represented.

TP: So it does provide many representatives of each class of gene.

RW: The glycolytic pathway (which is responsible for breaking down blood sugar to extract energy from it), for example: we are seeing an up-regulation of glycolysis in skeletal muscle, and we see that for the four or five of the glycolytic enzymes that show up, the mRNA changes just cluster at the same fold change.

LEF: In other words, they're all being changed to the same degree by aging or calorie restriction. Very interesting. You mentioned changes in protein synthesis. Can you comment further on that? George Webster had an interesting observation about aging changes in protein synthesis being controlled by elongation factor 1. What have you found about proteins that may control the rate of protein synthesis and perhaps the rate of turnover of molecules in the body?

TP: In muscle, we confirmed a decrease in elongation factor 1 and we observed that there seemed to be a decline in the expression levels of genes that have to do with protein metabolism, including protein turnover. This decline in protein synthesis and turnover is happening at a time when it appears that misfolded or damaged proteins are accumulating. So it's really not a good combination.

LEF: It may be a cause and effect combination, actually.

TP: Right. In the calorically restricted mice, we saw that not only is the level of damaged proteins apparently low, as indicated by the decline in expression of genes that are involved in

refolding damaged proteins, but the protein turnover machinery seems to be activated, so it seems like a very good combination. The ubiquitin proteosome pathway of protein turnover is activated in muscle by caloric restriction, whereas, normally in aging it seems to be going down.

LEF: What happens to chaperone levels? (Note: chaperones are proteins that "chaperone" the way proteins fold up when they are made or when they are damaged by agents that change their shape. -Ed.)

TP: The chaperones are actually the major component of the stress response that we see. They're induced with aging, and they go down with caloric restriction. It's well known that these chaperones, including the heat shock proteins, are induced (raised) by oxidative damage and by damaged proteins.

LEF: Hubel and Wiesel won the Nobel Prize by mapping how the visual system works in the brain over many years. Today, there are techniques for looking at the brain that can visualize the same thing in less than five minutes. In like fashion, what you've just observed confirms for example some of the very painstaking work that Steve Spindler's group has done at the University of California at Riverside, using more traditional methods to look at the mechanisms of aging and of the calorie restriction effect. You guys get this for free essentially, as one part of the overall picture. It seems to be a good illustration of the incredible power of this technique.

RW: I would concur with your statement. The technique validates areas that are worthy of focused pursuit.

LEF: One interesting feature about calorie restriction and all interventions to aging that exist so far is that after you apply them the animals do much better, and then after a while longer they die anyway. What markers change with aging but are NOT affected by calorie restriction? Perhaps these reflect what kills calorically restricted animals in the end.

TP: We found that about 30% of the changes that happen with aging were not prevented by caloric restriction. Now there are two possibilities here. One is that those changes actually represent chronological aging as opposed to biological aging, meaning they're not truly biomarkers of biological age. The other possibility is that they in fact are biomarkers of aging that are not affected by caloric restriction and might be related to the fact that the animals die despite being on caloric restriction.

LEF: What would be an example of a chronological aging biomarker?

TP: You mean from this data set?

LEF: Yes.

TP: Well, one marker that we observed to be induced by aging was something called serum amyloid. Now serum amyloid is a gene that is known to be induced in a number of pathological states, and it's also known that amyloid deposits form in several tissues of mice and also in humans as a function of aging. It's a very poorly studied area, but actually there's a lot of evidence to suggest that there are amyloid deposits going on with aging. That change in gene expression was not affected by caloric restriction but we don't understand why.

RW: Actually, when we got done writing this manuscript for Science, we came to the conclusion that there were many more questions we could address with this data set.

TP: We could write 10 papers on this data. In fact, we're thinking about writing a paper just describing the relative levels of the different antioxidant enzymes, because for the first time in one experiment, we have measured them all, and we have some important and surprising discoveries about the abundance of different antioxidant enzymes.

RW: Or at least about the abundance of the messenger RNAs for these enzymes. But I would have to revisit the data for the details.

LEF:I guess there's too much in the treasure trove to reflect on each coin right now.

RW: Something like that.

LEF: Classically, most changes that take place with aging are relatively small but every once in a while you see examples of really huge changes. The classic example for me was the hormone receptor story, in which you may see a 70% reduction in the population of certain kinds of hormone receptors in cell membranes. Are hormone receptors among the biomarkers that you've seen change with age or with calorie restriction?

TP: Calorie restriction in muscle resulted in a lowering of the thyroid hormone receptor mRNA. One explanation for that is that the animals are reducing their metabolic rate in response to a lack of food.

LEF: Where do you see this technology going in the future?

TP: I think the greatest promise of the technology is to be able to examine aging on a tissue-specific level in a relatively short time frame. The technology will allow relatively rapid screening of drugs, nutritional compounds, or even genetic interventions for aging.

RW: In addition to hopefully speeding up the pace of drug discovery for retarding aging and for the development of other interventions, I think it will allow us to have a clearer

understanding of the root causes of aging, which will then positively interact with this other component of drug discovery so that we can more sharply target our interventions. This would apply to genetic interventions such as knockouts or transgenic animals that have the genes of choice as well as to metabolic, nutritional, hormonal and other types of interventions. So the two should synergize in a nice way.

LEF: Thank you both very much for your insights on this dramatic new step forward.