

LE Magazine November 1999

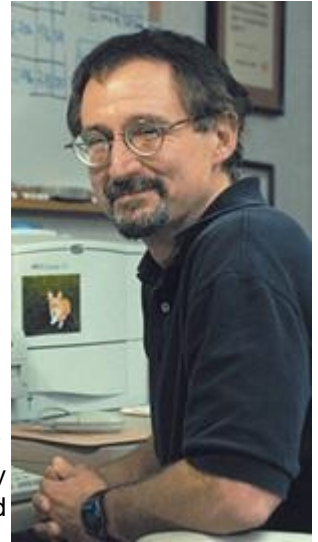
On The COVER

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.

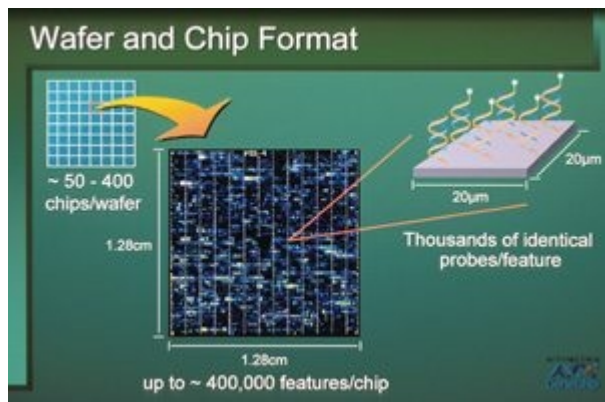
RW: Right. I would argue that perhaps this is a reflection of the fact that this analysis of gene expression is upstream of many subsequent alterations. For many of these root genetic kinds of alterations, when you get way out in the pathway and among different animals, you might see a lot more noise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

Continuation of "Aging Revealed" Interview on Page 2

[Back to the Magazine Forum](#)

your physician or other health care professional or any information contained on or in any product label or packaging. You should not use the information on this site for diagnosis or treatment of any health problem or for prescription of any medication or other treatment. You should consult with a healthcare professional before starting any diet, exercise or supplementation program, before taking any medication, or if you have or suspect you might have a health problem. You should not stop taking any medication without first consulting your physician.