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REPORT

SECOND OF 2 PARTS

Companies and academic researchers are racing to find the control mechanism for aging, in order to block aging itself. In this second installment, covering an important conference on the topic, Dr. Gregory M. Fahy updates us on longevity genes, calorie restriction and telomeres.

A Dynamic Partnership in The Fight Against Aging

Powerful forces are being unleashed in the war against aging. The genes that control aging are rapidly being identified, drugs have been found that appear to be effective in reversing age-related changes without toxicity, and links to aging phenomena observed in widely divergent species, such as worms, yeast and humans, are becoming apparent.

Significantly, the economic implications of successful aging intervention are becoming real, meaning that more interest from Wall Street and other investors is forthcoming to bring products and techniques to market. Anti-aging research is no longer solely in the realm of "pure science," but is nearing a practical stage to be of benefit to most people living today.

Perhaps the most spectacular presentation at the Conference on Age-Related Diseases, held in Las Vegas in December, was given by Cynthia Kenyon, the Herbert Boyer Distinguished Professor of Biochemistry and Biophysics at the University of California, San Francisco. Describing the elements of a general scientific tour de force, she revealed that a special longevity gene in a nematode worm called *Caenorhabditis elegans* is normally triggered by calorie restriction, but can also be unleashed to double the life span of the normal adult. Further, the gene acts through a central signaling mechanism, and may function in a manner analogous to similar established mechanisms in human beings.

As detailed in previous issues of *Life Extension*, the basic way C. elegans usually balloons its life span under natural conditions is by blocking its own development. The worm usually goes through four larval stages prior to becoming an adult, but if its calorie intake is reduced or if overcrowding takes place, the worms go from the second larval stage into a state of arrested development, called the dauer stage. Worms in the dauer state live far longer than normal worms, allowing them to survive until food becomes more available or until crowding subsides, whereupon they complete development, become adults, and live out their 15-day life spans. (Aging postponement through developmental arrest has also been reported in insects, molluscs and mammals.)

Cynthia Kenyon of the University of California, San Francisco, described the elements of a general scientific tour de force: That a special longevity gene in the worm Caenorhabditis elegans, normally triggered by calorie restriction, can also be unleashed to double the life span of the normal adult. Further, the gene acts through a central signaling mechanism, and may function in a manner analogous to similar established mechanisms in human beings.

Kenyon explained that the genes called daf-2 and daf-16 (daf refers to "dauer formation") jointly govern life span in *C. elegans*. Daf-2 action normally causes maturation to adulthood and a normal life span, while daf-16 action tends to produce the dauer state and a long life span (causing Kenyon and her colleagues to affectionately call it "sweet 16"). Unless calorie restriction or crowding is imposed, daf-16 is silent and daf-2 imparts a normal life span. However, if mutations knock out daf-2, the result is a greatly extended life span or a permanent dauer state.

If the technology can ever be applied to humans, it conjures up images of people being six years old forever. The really interesting observation was that merely *weakening* daf-2 activity, rather than entirely knocking it out, allows daf-2 to facilitate the maturation of the worm, while also permitting daf-16 to produce long life spans-without arresting the worms in a pre-adult state.

Daf-2 activity is weakened by using a temperature-sensitive daf-2 mutant that permits maturation at low temperature, but then becomes inactive when the worms are warmed. When daf-2 becomes inactive, daf-16 is allowed to act in the adults, a very unusual situation. These adult worms appear normal, and do not exhibit most of the characteristics of dauer larvae.

Also of great importance is the fact that the metabolic rate of these super long-lived adults is the same as that of non calorie-restricted, normal adults, so the observed life span extension is not due to "living slower" or producing fewer free radicals. Something much more intriguing is involved. Daf-2 is a member of the insulin receptor and insulin-like growth factor-I receptor family. Therefore, the "death signal"-that is, the signal to mature and age at a normal rate-presumably is induced by the binding of the worm version of insulin to the daf-2 protein in response to increasing sugar levels following feeding. (Worm insulin-like molecules are known to exist.)

But daf-2 is no ordinary insulin receptor. Kenyon believes that daf-2, rather than acting locally, serves a signaling role mediated by neurons and endocrine cells. Using a special technique in which chromosome fragments that contain daf-2 are distributed at random throughout the cells of a daf-2-deficient worm as it develops, Kenyon tried to find out whether daf-2 acts only locally, or at a distance. What she found is that daf-2 in neurons and endocrine cells blocked dauer formation in cells elsewhere in the body, even though those cells lacked daf-2. Conversely, when daf-2 was missing in neurons and endocrine cells, the other cells went into a dauer state even when they had daf-2.

This means there must be a signal created by the daf-2 in neurons or endocrine cells that travels throughout the body and controls whether the animal as a whole goes into a dauer state or not. This signal is a kind of master anti-youth hormone, and it no doubt will be found soon.

But daf-16 is the more interesting gene. Kenyon's search for other genes that act like daf-16 failed, indicating that this is a unique master gene for life-span extension. In fact, when daf-16 was essentially deleted from otherwise normal worms, they matured normally and had a normal life span. This means that the only thing daf-16 is naturally used for is increasing life span, in association with producing the dauer state. Kenyon is now investigating whether the effect of calorie restriction on life span *requires* daf-16.

Daf-16 is a member of the HNF3, or forkhead, family of transcription factor proteins. Therefore, the main function of daf-16 is to turn on other genes, something that is required for life span extension in the *C. elegans* worm. Finding which youth-preserving genes are turned on by daf-16 will obviously be of great interest.

The daf-16 gene resembles analogous mammalian HNF3 genes, including genes that affect the risk of cancer. Particularly tantalizing is the fact that there are examples in mammals in which insulin completely blocks the function of HNF3 forkhead proteins.

The obvious implication: The worm mechanisms also may apply to humans, and might directly lead to human life span-extension therapies, probably without the need for calorie restriction.

The thrust of almost all calorie-restriction research is to find the mechanisms by which calorie restriction works to extend life span, and then find ways for humans to do the same thing without having to undergo the discomfort of excessively reducing their food intake. Kenyon's worms seem especially close to showing us how this might be accomplished.

Underscoring such possibilities, Kenyon notes that human cells can use *C. elegans* genes and vice versa, including genes that govern many vital functions such as cell division, programmed cell death, cell migration, cell differentiation, and tissue pattern formation, with the "transplanted" genes working just fine in the radically alien host cells. This implies that a human analogue of daf-16 could have an active role in humans.

It is hard not to conclude from Kenyon's talk that gerontology is converging rapidly on some of the core mechanisms of both human aging and human life-span extension.

This is, of course, provided that calorie restriction actually does work in humans and other primates. This was addressed by Mark Lane, a senior staff fellow at the National Institute on Aging's Gerontology Research Center in Baltimore, Md. The NIA study on primate calorie restriction was started about 11 years ago and involves 200 rhesus monkeys (see "Calorie Restriction in Monkeys," July 1998, of which Dr. Lane was a co-author). Calories are restricted by 30 percent, a process phased in by reducing the calorie intake 10 percent per month over three months.

It is still too soon to know what is happening to aging per se, but the following observations have been made: First, the restricted animals are not emaciated, but are simply smaller. Secondly, restricted animals have lower cholesterol, triglycerides, blood pressure and fasting insulin (enhanced insulin sensitivity). Further, serum DHEA sulfate has fallen noticeably in the control monkeys, but not in the restricted ones.

In summary, 16 changes that have been induced by calorie restriction in rodents also have been replicated in these primates. Similar studies underway at the University of Wisconsin and at the University of Maryland are showing results consistent with those found in the NIA study. Lane concluded that calorie restriction appears to be reducing risk factors for aging and disease, and that obese animals respond in a manner similar to non-obese animals.

Previous studies have indicated that a common denominator is improvement in insulin sensitivity (glucose control). Lane further linked glucose metabolism to the calorie restriction effect by citing the link between insulin signaling and aging in models such as *C. elegans*. He also mentioned that the glucose analogue 2-deoxyglucose, which can't give rise to useful energy for the cell, mimics calorie restriction in that it reduces tumor growth, body temperature (even producing torpor) and cell cycling, and facilitates necessary programmed cell death (apoptosis).

In a pilot study, the diets of rodents were spiked with three doses of 2-deoxyglucose (0.2, 0.4, and 0.6 percent of food weight). These animals gained almost as much weight, and ate almost as much food, as did control animals, yet their insulin sensitivity was improved after three and six months of treatment. Other candidate glucose analogues also are under study.

Presentations were given by Mike West, previously of Geron Corp., left, Jan Vijg of the Harvard Medical School, and Minori Sugawara, of Agene Research Institute Co., bottom

At the end of the first morning of the IBC meeting, there was a panel discussion focused on central questions, including: If a gene is identified in a model system of aging, will it tell us anything about human disease? Are there universal mechanisms of aging? Is there a correlation between "in vitro aging" and other models of aging? Jack Egan, the senior director of pre-clinical research for Alteon Inc., a Ramsey, N.J.-based publically traded company, felt genes governing the ATP/ADP ratio and insulin resistance would be relevant to human disease, and he believes in the usefulness of in vitro models. Egan also said aging is a disease.

Kenyon said she felt her worms are not unique, that universal aging mechanisms do exist, and that random damage might be an effect of aging rather than a cause. Dr. Jan Vijg of the Harvard Medical School felt that aging was nothing more than pathology, and that in-vitro aging is not the cause of organismal aging.

Continuation of article

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