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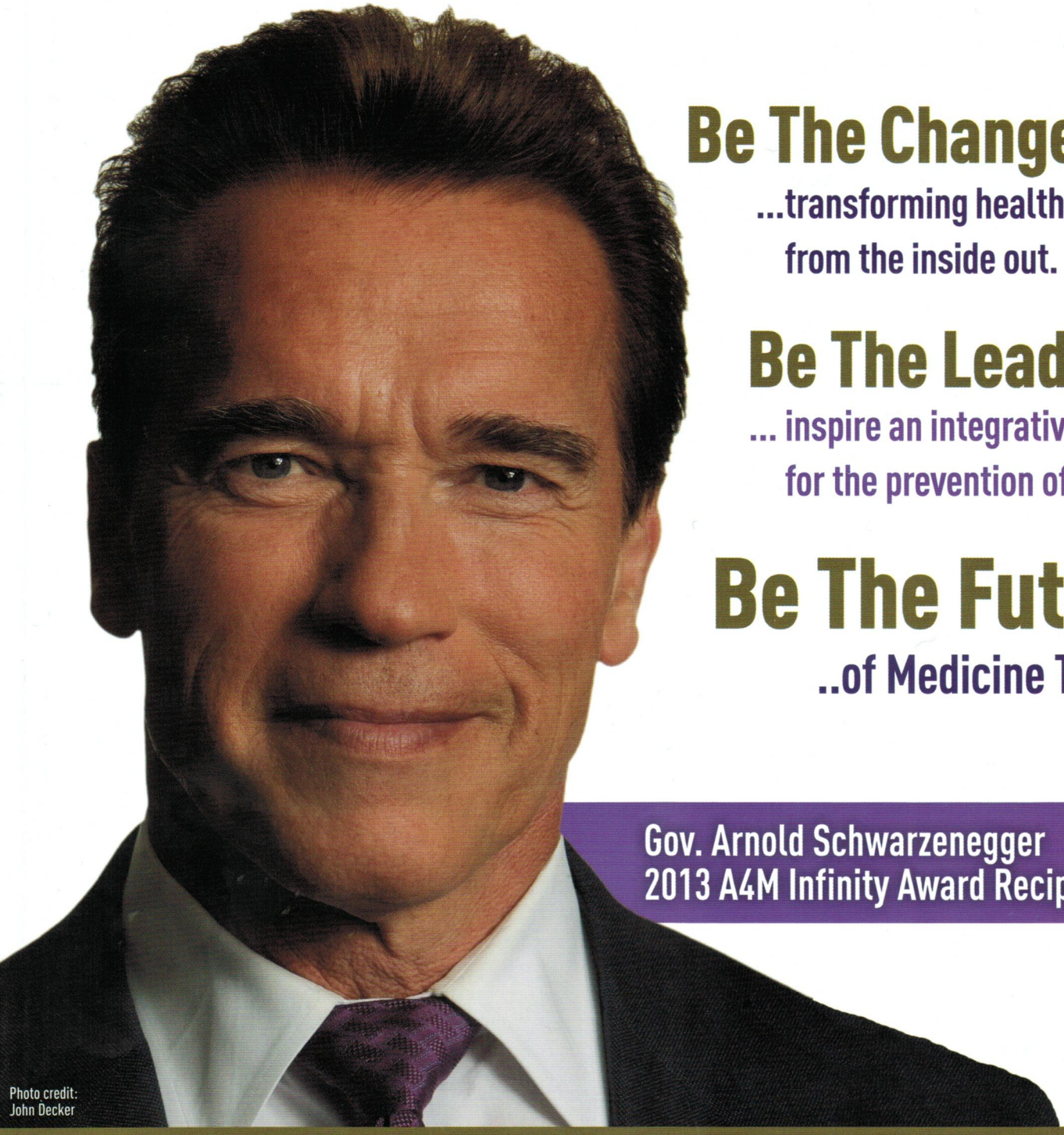

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A New Approach To Telomere Measurement

By- Dr. Marcelo Viegas, Ph.D.

From bacteria to humans all, living organisms are composed of cells which contain DNA (deoxyribonucleic acid), the molecules responsible for keeping the genetic information, the instructions for everything done directly or indirectly by the cells.

In eukaryotes (including man) the DNA molecules are linear and highly packed inside the nuclei of the cells along with a complex group of proteins forming structures known as "chromosomes." To protect vital genetic information, cells have to replicate their chromosomes before dividing into two daughter cells, and thus preserving these instructions from generation to generation. Chromosome replication is a highly accurate process that ensures faithful copies of the DNA sequence. There is, however, one exception: the very tips of the chromosomes called "telomeres" which get shorter after each cell division *(Figure 1)*.

In the scientific jargon, this limitation is known as the "end-replication problem" and derives from the intrinsic nature of the DNA replication mechanism which cannot completely replicate the ends of a DNA molecule (and hence, the chromosomal ends).

At the DNA level, telomeres are repetitive sequences at the ends of linear chromosomes that serve as essential protective structures that maintain the integrity of the chromosomes. The whole structure is capped by a dynamic protein complex called a "shelterin," which further protects the chromosomal ends by impeding chromosome fusion[1].

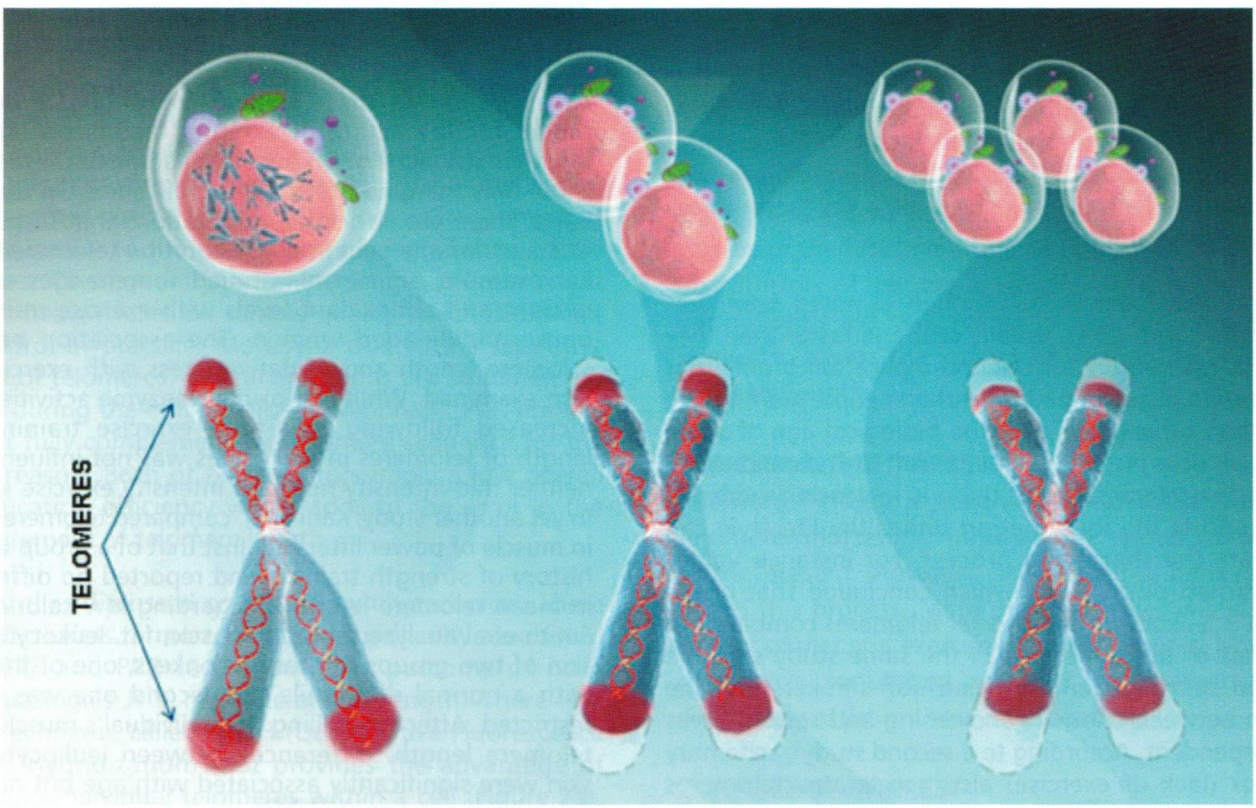

Figure 1 Liver detoxification pathways and supportive nutrients (used with permission from the Institute for Functional Medicine.)

It is believed that telomere sequences do not bear any genetic information but only support a structural function. Therefore, the progressive erosion of telomere ends does not imply any genetic loss. Nevertheless, telomeres are crucial for cells to live. So as cells replicate, telomeres shorten up to a point where the telomere length is critically short and the cells become senescent or apoptotic (i.e. they die). Therefore telomeres are like "biological timers" that count down after every cell division imposing a limit to the cell´s lifespan when they come to "zero". This property of the telomeres links telomere length with aging, the time-dependent functional decline of the organisms until death[2].

Interestingly, telomere shortening and aging have some remarkable exceptions that actually reinforce the correlation between these two processes. There exists a special enzyme called "telomerase" whose function is to elongate telomeres, thus counteracting normal telomere attrition. This enzyme is synthetized only by a few cell types, mostly germ-cells, stem-cells and cancer cells. It is also expressed in the morula-to-blastocyst transition in embryos. The expression of telomerase in both germ-cells and early embryos explains why our children are born young and with the same life expectancy that we have despite our own age. Stem-cells also express telomerase albeit at low levels. In these cells, the presence of telomerase allows a high mitotic index. Yet, even these telomerase levels are not sufficient to completely counteract telomere shortening. As a consequence, although stem-cells can replicate and live longer than somatic cells do, they also end-up dying after a certain number of divisions. Cancer cells probably represent the most striking example of the correlation between telomere length and aging. Telomerase is highly expressed in over 80% of all adult cancers[3]. This explains why these cells do not exhaust their replication capacity. Thus, telomere length turns out to be a key factor in cell replication and aging.

In normal aging somatic cells though, as noted, telomeres shorten inexorably. Yet, usually cells age faster than they would if it were only for the end-replication problem. It is a common observation that certain people seem to age faster than others. It is as if the biological age of some individuals does not fully correlate with their chronological age. At the telomere level, there is increasing evidence that supports the idea that an unhealthy life style can accelerate the shortening process. For instance Valdes et al. carried out a study which concluded that obese women´s leukocytes had shorter telomeres compared to lean ones of the same age. In the same study smokers also had shorter telomeres than non-smokers and the relation between telomere shortening and smoking was dose-dependent. According to a second study[5], sedentary behavior (lack of exercise) also accelerates telomeres shortening in leukocytes.

Even psychological stress seems to play a role on telomere length. Epel et al. compared healthy premenopausal women who were biological mothers of either a healthy child ("control mothers") or a chronically ill child ("caregiving mothers") who are subjected to significantly greater stress. While a direct comparison of these two groups did not show any significant difference in telomere length, the duration of the chronic stress (number of years spent as a caregiver) did have a significant correlation with telomere length. Indeed, within the caregiving group, the more years of caregiving, the shorter the mother's telomere length was after correcting for the mother's age[6]. Of greater concern, stress can also shorten telomeres in children according to a long-term study carried out in Romania that indicates that children who spent their early years in state-run orphanages have shorter telomeres than children grew up in foster care[7].

All in all, these studies illustrate basically the same underlying concept; namely, that there are existing mechanisms of telomere attrition that work on top of the basal shortening given by cell replication. So the question becomes: can we do something to stop further telomere shortening?

There is mounting evidence pointing toward an affirmative answer. Intake of certain vitamins, antioxidants and minerals has shown a positive correlation with telomere length[8,9]. Song et al. have demonstrated that duration of physical exercise correlates with telomere length. Exercise can reduce harmful fat and lead to reduced oxidative stress and preservation of DNA and telomeres[10]. Although not yet proved in humans, caloric restriction has a positive impact on telomere length in mice[11].

As appealing as these findings might be, a unifying model for the action of external factors on telomere length is still lacking. On one hand, some of these studies show only marginal statistical significance. On the other hand, there are also some publications that dispute the existence of any beneficial life habit on telomere health. For example, Shin et al. studied the changes of both oxidant and antioxidant levels with exercise training in obese middle-aged women. The association between telomere length and oxidative stress with exercise was also examined. While antioxidant enzyme activities were increased following long-term exercise training, the length of telomeres in leukocytes was not influenced by neither mid-intensity nor high intensity exercise stress[12]. In yet another study, Kadi et al. compared telomere length in muscle of power lifters against that of a group with no history of strength training and reported no differences in mean telomere length[13]. Regarding low calorie diets, Smith et al analyzed skeletal muscle, fat, leukocytes, and skin of two groups of rhesus monkeys, one of them fed with a normal diet while the second one was calorie restricted. After controlling the individual's muscle mean telomere length, differences between leukocytes and skin were significantly associated with age but not with calorie restriction[14].

What is the cause of these discrepant results? Undoubtedly, one of the problems is the use of different biological models that precludes inter-study comparisons. Additionally, many times the studies are based on relatively small groups and while the results can be statistically significant, the statistical power of the test applied turns out to be small.

But on top of all these issues, the methodology used to measure telomere length is of paramount importance. In the vast majority of these studies, telomeres are measured either by TRF or qPCR. The TRF (terminal restriction fragment) assay is the application of the classical Southern-blot technique to measure telomere length. DNA is purified from cells and digested with one or more restriction enzymes that do not cut into the telomere sequence. After blotting, a smear representing the distribution of telomere lengths in the total cell population is visualized. The final outcome of this test is an estimation of the mean telomere length in that cell population. However results depend on the total DNA amount, DNA integrity and electrophoretic conditions. The latter is of critical importance and while it is possible to fix the conditions in advance, the resolution of the assay will vary depending on the telomere length of the population under study qPCR (quantitative polymerase chain reaction) is a biochemical technology to amplify single or a few copies of a piece of DNA several orders of magnitude. Due to its high sensitivity and high-throughput capability it has become the technique of choice to quantify gene expression and gene copy number. However, telomere amplification poses a challenge to PCR. Not only telomeres but also one or more single-copy gene has to be amplified to control for DNA load in the reaction. Since the telomere sequence is repetitive, partially degraded DNA samples will not affect telomere amplification; however, the amplification of the genes used for normalization will be impaired proportionally to the percentage of degradation of the samples. Consequently, telomere length measurement by PCR heavily relies on sample quality which is difficult to control in practice. Moreover, due to the repetitive nature of telomeres, several amplicons are amplified per target during the PCR reaction; additionally, PCR primers cannot fully complement the telomere sequence for the same reason. Both of these factors imply a low overall amplification efficiency which adds to the error in the measurement of telomere length.

The hurdles discussed so far are purely technical. There is, nevertheless, a more conceptual difficulty associated with TRF and PCR techniques. The outcome of both methodologies is a mean telomere length. There is a third technique called qFISH (Quantitative Fluorescence In Situ Hybridization) that provides the advantage of detecting individual telomeres within a cell (Figure 2a). A number of studies based on qFISH showed that the actual distribution of telomere lengths in cells have a distribution similar to the one depicted in figure 2b (see [15,16] as examples). Far from being symmetrical, the distribution of telomere lengths is positively skewed. That implies that the mean is not a good estimation of the telomere length distribution for any small fluctuation in the tail of the distribution will affect the reading of the mean telomere length quite significantly.

Therefore the use of the median rather than the mean of the telomere length distribution should be preferred as it is a more robust and a representative estimator of telomere length. In addition, every study should include histograms like the one shown in figure 2b to be able to assess the quality of the data. A curve that strongly deviates from that of the figure could imply poor data (i.e. low number of telomeres tested) or some kind of artifact during the protocol. On the other hand, the whole distribution of telomere lengths is tremendously more informative than just one estimator. It is possible to analyze the percentage of short telomeres or the length of telomeres under a certain percentile to find effects on telomere length, not necessarily perceived by the median value. Moreover, the curve allows an analysis of the data dispersion as measured by the standard deviation. For instance, too high of a standard deviation could mean telomere lengthening through recombination rather than telomerase, which eventually could indicate deficient telomerase activity.

Life Length Inc. has been commercializing a powerful high-throughput qFISH test for a couple of years. This is the only company in the world that measures both short telomeres and the median telomere length in cells. Now the company has announced the launch of a new version of the test that also includes the whole frequency distribution of telomere lengths. The novel test will undoubtedly aid researchers in designing more robust studies to assess the impact of their subject matter on telomere length.

CONCLUDING REMARKS

Telomere biology is central to understanding aging. Telomere shortening (and aging) seems unavoidable at the present. However, some healthy behaviors may retard this process. More precise studies will clarify the impact of diet and exercise on our telomeres. Now a new commercial tool based on qFISH has emerged that will definitively assist in these investigations.

CONFLICT OF INTEREST

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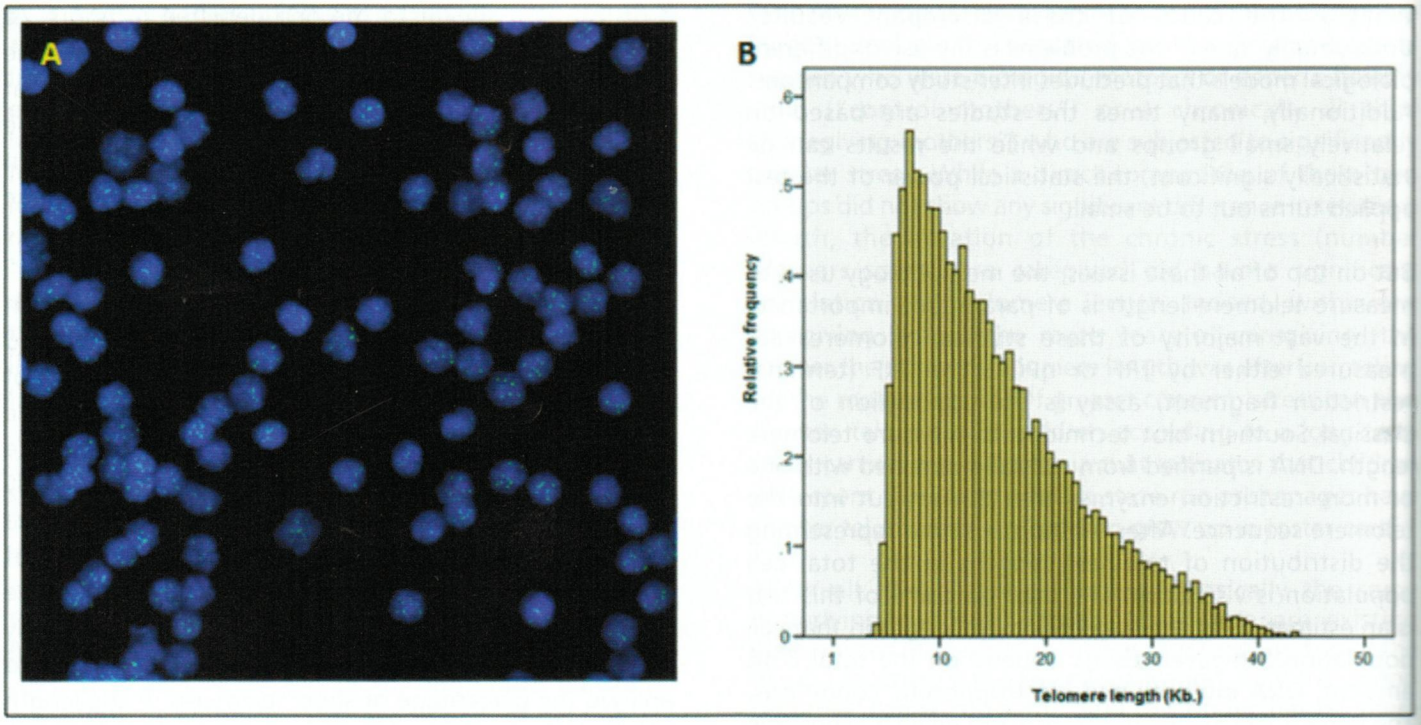

Fig. 2: qFISH image and frequency distribution of telomere length in cells. a) Image of qFISH done on purified lymphocytes. Nuclei were stained with DAPI (blue) and telomeres are visualized in green. b) Example of the distribution of telomere lengths in human lymphocytes.

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