On Mitochondria, Mutations, and Methodology

Whether mutations in mitochondrial DNA (mtDNA) are causal to, or merely correlated with, aging is an area of intense debate. Recently, this debate has intensified with the development of mitochondrial mutator mice. These mice carry an error-prone copy of DNA polymerase gamma (PolgA), the enzyme that replicates the mitochondrial genome. We found that heterozygous carriers of this error-prone allele display a >100-fold elevation in point mutation frequency without manifesting features of premature aging (Vermulst et al., 2007). These results imply that mtDNA point mutations do not limit the life span of normal mice.

Interestingly, homozygous carriers of this allele do display extensive mitochondrial dysfunction and suffer from a premature aging-like syndrome (Trifunovic et al., 2004). We found that this progeroid syndrome correlated best with a large increase in clonally expanded mtDNA mutations (which could be either point mutations or deletions) and a 10- to 90-fold increase in mtDNA deletions (Vermulst et al., 2008). MtDNA deletions are associated with a number of age-related pathologies, including muscle wasting and neuronal dysfunction. We reasoned that, if the frequency of deletions in WT animals is sufficient to perturb the function of aging neurons and muscle fibers, a substantial increase in their frequency should have a profound physiological impact. For instance, a much milder increase in mtDNA deletions results in late onset mitochondrial disease in Twinkle-deficient mice. Moreover, mtDNA deletions are suppressed in certain long-lived mice. A recent paper in *Cell Metabolism* by Edgar et al. (2009) now challenges the role of mtDNA deletions in the progeroid syndrome of mitochondrial mutator mice.

In their study, Edgar et al. find that protein supercomplexes in the electron transport chain are unstable in the progeroid mice, and they argue that this type of dysfunction could not have been generated by mtDNA deletions, which they find to be too infrequent. Thus, mtDNA deletions are not responsible for the progeroid syndrome of homozygous mutator mice.

We would like to point out that the instability of the supercomplexes is an interesting feature of the mutator mice, but Edgar et al. provide no experiments to show that this instability is indeed causal to their progeroid syndrome. Thus, it seems incorrect to argue that mtDNA deletions are not important for the phenotype of the mice because they have no role in supercomplex instability.

Second, the assays used to quantify mtDNA deletions and, thus, discard them as irrelevant are inappropriate for their detection. Previously, we demonstrated that mtDNA deletions in the mutator mice are generated randomly. As a result, the majority of deleted molecules are unique and of a different size. Although these molecules may clonally expand within single cells, this variability precludes their detection on a Southern blot, since no single-sized molecule predominates. Furthermore, random deletions are unlikely to be detected with the PCR assay used. To illustrate, if a thousand molecules are present in a PCR reaction and one hundred of these carry an identical deletion, this deletion is easily detected. However, if the size of each deletion is different, the signal of any deleted molecule will be too faint to detect. Importantly, the control experiments that demonstrate a 0.1% sensitivity of detection are done on clonally expanded mtDNA deletions and are thus inappropriate for the detection of random deletions. Similarly, due to their uneven distribution and location, it is also not expected that these deletions will cause a drop in protein levels, especially when a large group of cells is assayed simultaneously, and the signal of each individual cell is averaged out.

Finally, in human pathology, low numbers of deletions are thought to contribute significantly to muscle wasting and neuronal degradation. Thus, a low level of mtDNA deletions in mitochondrial mutator mice does not preclude an impact on animal physiology. If the hypothesis by Edgar et al. holds true though and point mutations are the sole cause of their progeroid syndrome, the mutator mice can no longer be regarded as an accurate model for normal aging, as point mutations do not cause aging in wild-type mice (Vermulst et al., 2007). Moreover, if a 10- to 90-fold increase in deletions is negligible in terms of pathology, this partially conflicts with the mitochondrial theory of aging. However, we conclude that the methodology used by Edgar et al. is insufficient to resolve this controversy (Kraytsberg et al., 2009).

Marc Vermulst,[1,*] Jonathan Wanagat,[2] and Lawrence A. Loeb[3]
[1]Department of Chemistry, University of North Carolina, Kenan Labs, Chapel Hill, NC 27599, USA
[2]Division of Geriatrics, David Geffen School of Medicine, University of California Los Angeles 10945 Le Conte Avenue, Los Angeles, CA 90095, USA
[3]Department of Pathology, University of Washington, 1959 NE Pacific Avenue, Seattle, WA 98195, USA
*Correspondence: vermulst@email.unc.edu
DOI 10.1016/j.cmet.2009.11.001

REFERENCES

Edgar, D., Shabalina, I., Camara, Y., Wredenberg, A., Calvaruso, M.A., Nijtmans, L., Nedergaard, J., Cannon, B., Larsson, N.G., and Trifunovic, A. (2009). Cell Metab. *10*, 131–138.

Kraytsberg, Y., Simon, D.K., Turnbull, D.M., and Khrapko, K. (2009). Aging Cell *8*, 502–506.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J.N., Rovio, A.T., Bruder, C.E., Bohlooly-Y, M., Gidlöf, S., Oldfors, A., Wibom, R., et al. (2004). Nature *429*, 417–423.

Vermulst, M., Bielas, J.H., Kujoth, G.C., Ladigies, W.C., Rabinovitch, P.S., Prolla, T.A., and Loeb, L.A. (2007). Nat. Genet. *39*, 540–543.

Vermulst, M., Wanagat, J., Kujoth, G.C., Bielas, J.H., Rabinovitch, P.S., Prolla, T.A., and Loeb, L.A. (2008). Nat. Genet. *40*, 392–394.