Commentary on
“Protein Carbonyl Accumulation in Aging Dauer Formation–Defective (daf) Mutants of Caenorhabditis elegans”

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In recent years, oxidative damage to macromolecules has gained popularity as the basis of the molecular mechanism of aging. Martin proposes oxidative damage to macromolecules as one of the major public mechanisms of aging (1). Interest in modifications of protein by reactive oxygen species in aging was apparently introduced by Stadtman (2). Although various types of oxidative modifications can occur in proteins, carbonyl residues believed to be generated by metal catalyzed reaction or otherwise introduced by lysine, arginine and/or proline residues in vivo are often used as a marker of direct or indirect protein oxidation.

The article by Yasuda and colleagues in this issue of the Journal of Gerontology: Biological Sciences focuses on genetic effects of protein oxidation using several age-mutants of nematode. The authors demonstrate that dauer single mutants daf-2 and daf-16 and double mutants daf-2;daf-12 and daf-2;daf-16 differing in life spans exhibit an inverse correlation with the accumulation of protein carbonyls in the whole body extracts as measured spectrophotometrically by 2,4-dinitrophenylhydrazine method. The same group of investigators has already shown that a long-lived mutant age-1 and a short-lived mutant mev-1 have lower and higher protein carbonyls respectively than the wild-type animal N2 (3). Their findings provide strong support for the causal relationship between accumulation of protein carbonyls and life span and/or aging rate. Although a large number of articles has claimed that protein carbonyls increase with age in mammalian models (4), it should be mentioned that this issue appears still controversial due largely to methodological problems (5). A considerable variation of the data (Figure 2 in the article), for instance, could partly be due to DNA in the extracts which can react with the reagent (6). Clearer results might have been obtained if post-nuclear extracts were used for the measurement. Western blot analysis of 2,4-dinitrophenylhydrazones of proteins could confirm the spectrophotometric measurement and would provide detailed information on the extent of carbonylation of individual proteins (5). In fact it was shown by immunoblot followed by amino acid sequencing that a major carbonylated protein in old nematodes is a vitellogenin, an egg yolk protein of which biological significance in older nematodes is discussed elsewhere (Nakamura et al., unpublished data).

An intriguing question is how the difference arises in the accumulated levels of protein carbonyl in the daf-mutants. Daf-2 encodes a mammalian insulin receptor homologue carrying a tyrosine kinase domain which could relay the signal to the nucleus in the same pathway as in the long-lived age-1 mutant that is defective in phosphatidylinositol 3 kinase. Recently, based on the investigation of age-related changes of parameters in mutants of C. elegans, Vanfleteren et al. (7) proposed that daf-2 is the major effector of metabolic activity during adult life and daf-12 stimulates oxygen consumption independent of daf-2. In view of these results it seems reasonable that the daf-2;daf-12 double mutant confers less oxidative damage to proteins than the wild-type animal and daf-2 mutant. It is possible that transcription of genes related to energy metabolism is influenced in some way or another in all of these age-mutants in such a way that oxidative damage to proteins are attenuated.

It should be noted that protein carbonyls are still very low at day 20 and daf-2;daf-12 at day 33 when the carbonyl contents are higher (see Figures 1 and 2 of the Yasuda et al. article). This might mean that what is important as the determinant of life span is oxidative damage of limited kinds of protein rather than that of protein in general. It will be interesting to see what kinds of protein are carbonylated and to what extent in short-lived and long-lived mutants.

The authors of the article argue that accumulation of protein carbonyls is one of the major determinants of life span and perhaps also of aging rates in the nematode. In support of this claim the gene responsible for short life span of mutant mev-1 that is highly sensitive to oxidative stress was recently identified by Ishii et al. as succinate dehydrogenase cytochrome b, a component of complex II of mitochondrial electron transport chain which is believed to be the major site of superoxide generation (8).

Finally, it should be mentioned that accumulation of oxidatively modified proteins must also be viewed as a possible result of lowering of protein turnover, which is well
documented in aging animals including nematodes (9,10). Further investigation of life-prolonging effects of daf age-mutations in nematodes will provide clues to better understand molecular mechanisms of aging.

REFERENCES