required for DNA replication [(84), including Supplementary data]. In higher eukaryotes, replication stress that impacts aging likely occurs in stem cells or their proliferating progeny when the constitutive activation of some growth-signaling pathways by mutations or other factors coincides with the downregulation of other growth-signaling pathways (in quiescent stem cells or during differentiation, for example) required for efficient DNA replication. This leads to apoptosis or the irreversible growth arrest in S phase with partially replicated chromosomes that characterizes OIS. Both outcomes would cause the reduced capacity for tissue renewal that underlies many age-related pathologies. Age-related replication stress also likely arises in post-mitotic neurons. As discussed above, inappropriate activation of growth signaling and ectopic entry into S phase after neuronal differentiation has occurred have been implicated in a variety of age-related neurodegenerative disorders.

This model also predicts that caloric restriction exerts some of its anti-aging (and anti-cancer) effects by protecting cells from replication stress. Protection from replication stress by caloric restriction is likely provided by the induction of energy-sensing 'metabolic checkpoints' that coordinately downregulate all growth-signaling pathways, leading to an efficient growth arrest in G1, rather than S phase. This might include growth-signaling pathways that have been constitutively activated by mutations upstream of cyclin-dependent kinase activity required for progression into S phase, which is likely inhibited by these checkpoints. Mutations in RecQ helicases and other proteins that respond to replication stress accelerate aging by amplifying the consequences of replication stress that develops during normal aging. Although this model can account for some of the failures of the free radical theory of aging to explain various aging phenotypes, it is not inconsistent with this theory. Most likely, the roles of oxidative stress-induced and replication stress-induced DNA damage in aging are inextricably linked by their common origin in deregulated growth signaling, perhaps including growth signaling by ROS produced in mitochondria (Figure 3).

A more complete understanding of how replication stress potentially impacts aging will require a better understanding of the non-dividing state induced by energy deprivation and during differentiation. Pathways that regulate the G1 to S phase transition in cycling populations of cells and their downregulation in response to DNA damage and other stresses have been extensively investigated. However, much less is known about how these pathways are downregulated during differentiation or in response to nutrient-limiting conditions that lead to quiescence, or how quiescence is maintained. The paucity of details concerning downregulation of these pathways in mammalian cells is reflected by the following fact: only recently was it determined that p53—one of the most thoroughly characterized mammalian proteins—induces a G1 arrest in response to low glucose, in addition to DNA damage.

Even less is known about how cells arrest in G1 in response to nutrient-limiting conditions in budding yeast,

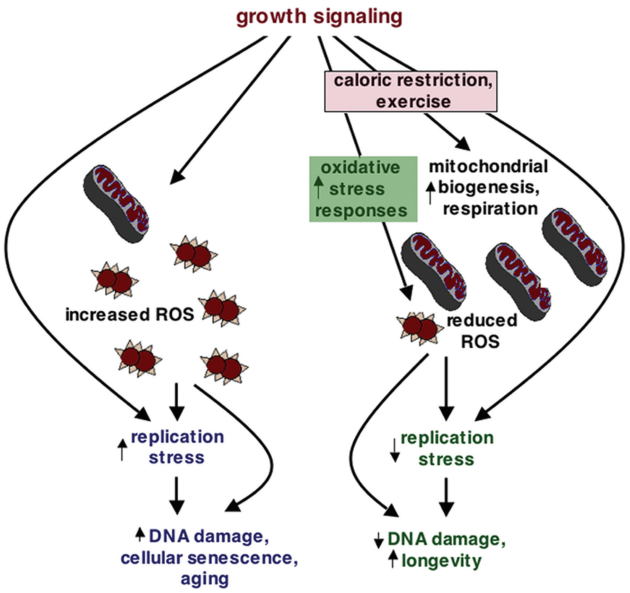

Figure 3. Replication stress model of aging. Growth signaling inhibits mitochondrial biogenesis and respiration and increases ROS, leading to replication stress, genome instability, cellular senescence and aging. Replication stress is likely enhanced by ROS-dependent growth signaling and by growth signaling that occurs independently of ROS. Caloric restriction and mutational inactivation of growth-signaling pathways stimulate mitochondrial biogenesis, increase respiration and reduce ROS. Reduced ROS-dependent and -independent growth signaling reduces replication stress and genome instability and promotes life span. In mammals, exercise also extends life span extension and promotes mitochondrial biogenesis and increased respiration (77,89). The effects of replication stress on aging likely occur in parallel with oxidative damage to DNA and other cellular constituents.

the organism that (together with fission yeast) was employed in numerous studies of growth in the presence of excess nutrients that provided the framework for understanding cell cycle regulation in all eukaryotes. For example, although the PUBMED search engine identifies (as of late October 2007) 3145 publications associated with the terms 'Cln' (G1 cyclins), Cdc28 (the cyclin-dependent kinase required for entry into S phase regulated by Clns) or 'Sic1' (an inhibitor of Cdc28), it identifies just 20 publications associated with these terms and the term 'stationary phase' (the growth-arrested state induced by nutrient depletion), most of which are not relevant to stationary phase G1 arrest.

This paucity of information exists despite the likelihood that in budding yeast, events associated with the down-regulation of growth during nutrient depletion—such as those detected in chronological aging experiments—more accurately model how replication stress arises and contributes to cancer and aging compared to models based on studies of cells dividing in the presence of excess nutrients. This view runs somewhat counter to the conventional wisdom that the chronological aging model of budding yeast is mostly relevant to aging of post-mitotic cells in higher eukaryotes. However, the chronological life span of this organism is clearly impacted by events during early stages of nutrient depletion that