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Perspective

Cell Stress and Aging: New Emphasis on Multiplex Resistance Mechanisms

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Work, initially in *Caenorhabditis elegans* and then more recently in fruit flies and mice, has suggested that anti-aging mutations extend life span by simultaneous activation of pathways that protect cells from multiple forms of injury. This "multiplex stress resistance" theory suggests a number of new avenues for investigation of the genetic and cellular controls that influence organismic longevity within and among species, and that might lead to the development of pharmaceuticals that retard the aging process and, therefore, the entire panoply of age-dependent diseases and disabilities.

Key Words: Longevity—Cell injury—Overview—Comparative biology.

ONE hundred years from now, when historians of science look back on the revolution in preventive medicine, a revolution that will have used the insights of biogerontology to add a couple decades of active life to the proverbial threescore and ten, what will they point to as the "breakthrough" discovery, the set of observations that first switched the research locomotive to the right set of tracks? My alethiometer is back at the shop for repairs today, but if forced to bet, I would put my money on the realization, about 10 years ago, that mutations that slowed aging in *Caenorhabditis elegans* also induced resistance of the mutant worms to multiple forms of lethal injury. The key discovery was not that these long-lived worms were resistant to thermal injury, oxidative damage, or the ravages of heavy metal poisoning but that they were resistant to each and every one of these dangers. This initial discovery has since been firmed up and expanded by evidence that the proportion of life span augmentation is (roughly) proportional to the graded level of resistance to lethal injury; by discoveries of similar associations between longevity and stress resistance in yeast, flies, and cells of mice; and by gene expression analyses showing the interlaced networks of protective machinery induced by the anti-aging mutations.

This "multiplex stress resistance" model for determination of aging rate (1) imagines a series of evolutionary transitions. First, the model suggests that at an early point in evolutionary history, predating the splits among yeast, worms, flies, and vertebrates, single-celled organisms found a fitness advantage in circuits that could induce resistance, in parallel, to multiple forms of injury, presumably in response to environmental signals that rewarded stress resistance even at the cost of immediate reproductive gratification. Next, multicellular organisms, like worms and flies, developed neural or hormonal pathways, or both, to coordinate these preexisting cellular options, to render multiple cell types stress resistant when environmental conditions were poor for reproduction or hazardous for survival. It is a good bet that these coordinated responses were at least partly dependent on molecules in the insulin and insulin-like growth factor families (2). Leaving these stress resistance pathways in the "on" position may have diminished fitness in rich environments, or in conditions where abundant supplies of food and mating opportunities were sporadic (3), but having them in reserve would, according to this model, have allowed organisms to make it through particularly difficult, transient challenges. In the laboratory, manipulations that induce these antistress pathways (typically by mutational inactivation of their inhibitory components) extend mean and maximal life span. The frequency with which the pathways are called upon in natural conditions, the costs and benefits of making these organismic commitments, and the possible role of "partial" activation of these pathways, are still uncertain. Lastly, as organisms increased in size and complexity, the hormones and neural networks involved in coordinated stress resistance were co-opted for new activities in metabolic control, regulation of organismic size and growth rate, allocation of metabolic resources among tissues and among offspring, and other assignments that make it more complicated to tease out the links connecting hormones, cellular traits, and life span in mammals. The very deep evolutionary roots of the stress-hormone-aging connections make it possible to guess a lot about mammalian aging, and the mechanisms that time

late-life diseases, from studies of smaller, simpler, more tractable creatures, but also call for careful assessment as to which aspects of worm, fly, and yeast biology are guideposts for aging research, and which are merely distractions.

In the session of the Biology of Aging Summit dedicated to the issue of stress and stress responses, we tried to identify particularly attractive lines of experimentation on this topic. This article presents a synopsis of some of the key themes that emerged in the discussion, as a stimulus to further discussion and even, perhaps, action. The charge to the group asked for consideration of four basic problems: (a) What experiments can best define the molecular pathways that regulate cellular resistance to multiple forms of stress (as distinct from those that regulate resistance to one, but only one, variety of cellular damage)? (b) How can we best define pathways that link cellular stress resistance to organismic health, disease resistance, and longevity? (c) What are the strengths, and gaps, in models that propose that differences in stress resistance underlie interspecies differences in life span and possibly contribute to differences in life span among members of the same species? (d) How can we develop new approaches for evaluating resistance of internal cells and tissues (eg, endothelial cells, stem cells, kidney cells, neurons, and muscle and liver cells), and whole mammals, to various forms of cellular injury?

Theme 1—Studies of stress resistance in specific cells and tissues of intact mice are sorely needed: Although there is some evidence that cells, grown in vitro from long-lived mutant mice (4), or from long-lived species (5, 6), are resistant to many of the same forms of lethal injury to which long-lived mutant worms are relatively immune, critical evaluation of models linking cellular stress resistance properties to longevity, aging, and disease will require new methods to evaluate multiple cell types in intact organisms and particularly in experimental mice. The complexity of mammalian responses to cell injury, including changes in vascular, hormonal, metabolic, and immune networks, presents a particular challenge here. If the goal is to evaluate the resistance of, for example, a hepatocyte, cardiomyocyte, or pancreatic beta cell to a specific insult (say DNA damage or acute exposure to a toxic heavy metal), measurements made weeks or days or even hours after the initial cellular damage may reflect inflammatory responses, systemic changes in metal-binding proteins, or alterations in blood flow to the affected tissues, rather than the responses of the injured cells themselves. Disentangling these interconnections will require development and validation of single-cell end points that report early responses to cellular injury. Discovering and choosing among such reporters is likely to emerge from studies in model invertebrates as well as analyses of mammalian cells in culture. Differences, among intact animals, in the ability to detoxify, excrete, sequester, or buffer the effects of toxic agents will also complicate experimental designs aimed at learning more about cellular strategies for responses to individual, and multiple, forms of stress.

Methods to follow stress-induced changes in specific cell types in vivo will open a wide range of opportunities to address key issues in biogerontology and pathogenesis. There are now at least two dietary regimes, and over a dozen single-gene mutations, that can extend mean and maximal life span in mice, and only the barest hints of how these anti-aging interventions modulate resistance of cells and tissues to acute stress in vivo (7,8). Although there is decent evidence that aged animals (and people) are vulnerable to many kinds of risk (infections, heat and cold, many forms of toxic or metabolic perturbation), there is much less known about which specific kinds of injury affect which specific kinds of cells in an age-sensitive fashion. Similarly, there is almost nothing known, yet, at the cellular level, about the ways in which age-sensitive changes in responses to cellular injury contribute to the induction or progression of late-life illnesses and degenerative conditions. Changes in baseline levels of antistress defenses and changes in the speed and vigor of responses to challenges to cellular homeostasis are of equal interest, and exploration of these end points in animals aging at differing rates will be very informative, once studies of fibroblasts, worms, flies, and yeast cells give us good markers to tackle the more informative whole-animal problems.

Theme 2—The assumption that cell survival is the only useful metric of resistance to stress needs to be replaced by a more nuanced picture of cellular (and organismic) response patterns: The initial evidence of stress resistance in long-lived mutant worms used organismic death as the end point for assessment of resistance to injuries (9), and work on cultured mammalian cells (4–6, 10,11) has used cell death as the measured criterion. Yet, the continued health of an organism may depend on pathways that trigger apoptosis in cells where damage is of an extent, or a nature, that continued cellular survival could be harmful (12). Cells in which protein processing is blocked, leading to accumulation of unfolded protein in the endoplasmic reticulum (ER), can turn on two sets of pathways, one leading to reestablishment of cellular homeostasis and one that induces cell death if metabolism cannot be restored (13). How an individual cell makes the decision to give up on repair and thus commit to apoptosis is still being worked out, but there is now some preliminary evidence that the "set point" for this decision may be different in cells from long-lived versus short-lived mammals (11). Cells are likely to have a wider repertoire of responses to DNA damage, ER stress, lipid peroxidation, protein oxidation, and other dangers beyond the live-or-die rubric: Changes in display of surface macromolecules, secretion of paracrine and endocrine factors, induction of new gene expression, and metabolic strategy may all eventually be found to contribute to "stress" responses in age-sensitive ways or, more importantly, in patterns that help dictate the rate of aging and forge links of cellular properties to age-dependent illnesses.

Theme 3—Studies that work out the links among cellular stress resistance and physiological control networks are highly challenging but likely to be very rewarding: Discussions of "stress" (note the scare quotes) can be frustrating and nonproductive unless all participants are careful to avoid accidental conflation of the multiple biological meanings of this term: stress as acute encounter with a frightening situation (a snake in the sleeping bag), stress as longstanding immersion in difficult circumstances (a decade living in poverty, a week of final exams), stress as perturbation of organismic homeostasis (blood loss, viral infection, food poisoning, concussion, etc.), and, our current focus, stress as a generic term for agents that can cause cell injury. But although it is helpful to stay alert to these terminological ambiguities, it is also important to recognize, and start to investigate, the ways in which the stress resistance of cells can tweak, and in turn itself be tweaked by, the integrative control networks of the neuroendocrine system. This tiny article is not the place for a recapitulation of textbook passages about how the autonomic nervous system, higher central nervous system centers, endocrine organs, and immune circuits collaborate to adjust to acute, transient, longstanding, or intermittent challenges, some of which are appropriately, but confusingly, referred to as "stresses" in common and medical parlance. Here, though, it is worthwhile to note that improved understanding of how cellular resistance to stress, that is, stress resistance of the kind linked to aging rate in flies, worms, yeast, mutant and calorie-restricted mice, and across species, will represent only a first key step in working out the network of response and counterresponses that have long provided thorny problems for pathophysiologists and more recently to experts in systems biology. Experiments showing that individual worms, members of a genetically uniform clone, can be sorted into long-lived and short-lived classes based on inducibility of a single-chaperone protein (14) hint that useful simplifications may yet emerge from these intimidating complexities, as do observations that certain intracellular pathways, involving members of the forkhead, target of rapamycin, heat shock, and nrf-2 families, keep popping up repeatedly in studies of cell injury and aging in distant clades.

The idea that longevity extension produced by caloric restriction might represent a special case of hormesis—a modest stress turning on stress-resistance mechanisms—fits squarely into this rubric. There is evidence from flies (15) that very brief (nonlethal) exposure to high temperature can diminish mortality risks for a much longer period and that caloric restriction diets lead to mild, sustained elevation of corticosteroids thought to augment resistance to many forms of stress in rodents (16). In rodent fibroblasts, high susceptibility to death induced by ER stress accompanies resistance to many other forms of stress, although whether this association is causal or coincidental is uncertain. These, and related fragments of evidence reviewed by Rattan (17), should serve to motivate more attention to ways in which stressful stimuli, at the level of cell, tissue, or organism, might upregulate stress resistance pathways relevant to the pace of aging or to specific age-dependent diseases.

Theme 3.5—Interpretation of work on stress resistance and disease will need to make careful distinctions between the effects of acute and chronic exposure to specific modes of injury: This idea, hinted at several times in the previous sections, also deserves a nod in its own right. The simplest experimental designs, on which most of the extant models are based, involve exposing a worm or cell to a single dose of a threat and then watching to see if it survives. Results from experiments of this kind are extremely useful, providing information on molecular indexes, resistance pathways, genetic controls on resistance, distribution of resistance among cell types, and other questions of high interest. But more complex models, involving gradual adaptation to repeated or sustained stresses, under the influence of hormonal and other nonautonomous signals, including shifts in cellular composition and in the properties of cells that do not succumb to injury, are likely to be needed to construct a more accurate model of how stress resistance, at the cellular level, plays a role in disease resistance and the postponement of aging effects in long-lived individuals and long-lived species.

Themes 4, 5, 6, 7, and 8—A thematic structure, like a half-day meeting, leaves too many valuable ideas by the wayside, ideas each of which may deserve a review article on its own, but must here be consigned to a single question apiece: (a) There is some evidence that HSF (heat shock factor, a transcription factor that helps coordinate transcription of multiple chaperone genes) is less effective in cells from older animals. What is the molecular basis for this finding, how widely can it be generalized, and what are the consequences for cellular stress resistance? (b) Can a systems biology approach, focused on the topology and sensitivity of networks linking cells to supercellular controls, pose or answer questions about aging that are useful to physiologists and pharmacologists? (see also article by West & Aviv, [18]) (c) Why, in cellular and molecular terms, are not all cells from all species, long or short lived, as stress resistant as possible? In other words, what is given up by a cellular commitment to be as resistant as possible to injury? (d) Do age-dependent changes in autophagy pathways, and other cellular machinery for removing damaged structures for recycling, modulate resistance to cellular stresses, and, if so, is this a good thing or a bad thing for organismic health? (see also article by Morimoto & Cuervo, [19]) (e) Selection experiments to isolate mutants based on resistance to lethal stress produce a mixture of mutants, some with longer life span and some without; why is this?

Multiplex Stress Resistance: A New Wave in Aging Research?

Tsunami or mere sidestream? The bulk of the literature in the "stress and aging" pile was written by single-stress

enthusiasts: experts in DNA damage, lipid peroxidation, carbonyl biology, detoxification, or heat shock, each testing a case for or against the importance of the chosen stress in the aging process. The observation that alterations in daf-16 function can elevate resistance to multiple forms of stress in parallel, and, by the way, also dramatically extend life span, provides a big hint that we ought to back up and select a wider angle lens to view the elephant as a whole. It may be, for example, that the reason antioxidant drugs have so consistently failed to modify mammalian life span (20) is that successful (ie, slow) aging requires simultaneous adjustments in resistance to multiple forms of stress in tandem and not just one at a time. If the "multiplex" theory is correct, then the scary prospect of having to retard aging by juggling many balls at once, one for lipid oxidation, one for ER stress, one for protein denaturation, one (or five) for DNA repair, may be simpler than it seems, if evolution has indeed joined all these projectiles into a single bola (see http://en.wikipedia.org/wiki/Bolas).

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