ger cullin complex with E2s from distantly related families (*7–12*).

The c-Cbl–UbcH7 structure shows how a region amino-terminal to the RING finger can contribute to the association of the E3 with its E2. The α-helical linker of c-Cbl (see the figure), which is not conserved among RING finger proteins, nonetheless establishes multiple contacts with an α-helix in UbcH7. Although not essential for E2 binding, the linker contributes to the affinity of c-Cbl for UbcH7 (*5*). Pavletich and colleagues propose that the interactions between the linker, the SH2 domain of c-Cbl, and the UbcH7 E2 may be important structurally, helping to properly orient other sections of this multiprotein ubiquitinating complex. In this regard, it has been suggested that phosphorylation of tyrosine 371 in the linker is essential for c-Cbl's E3 activity (*4*); however, according to the Pavletich structure, this tyrosine is packed against the SH2 domain and so is not readily available for phosphorylation. Certainly, the part played by the c-Cbl linker in the ubiquitination process requires further study.

In the absence of ubiquitin, there is no significant difference in the conformation of the free and c-Cbl–bound UbcH7 E2. Furthermore, none of the cysteines in the c-Cbl RING finger are likely to form an intermediate thioester bond with ubiquitin without disrupting the structure. The structure of the c-Cbl–UbcH7 complex is thus consistent with a model in which RING finger E3s act as adapters that bring the E2 and the protein substrate together. Pavletich and co-workers propose, however, that c-Cbl may play a more active role in ubiquitination than simply increasing the local concentration of reacting species. First, all the domains seem to be rigidly arranged. Second, and somewhat unexpectedly, the UbcH7 active-site cysteine is on the opposite side of the complex relative to the binding site for tyrosine-phosphorylated peptides. The provocative finding of a continuous surface "channel" extending between these two sites, whose lining residues are particularly well conserved among c-Cbl proteins, led the authors to speculate that this channel may guide the polypeptide chain of the protein substrate toward the E2 catalytic cysteine. Thus, c-Cbl and other RING E3s may serve as scaffolds that position and orient the protein substrate and E2 optimally for ubiquitin transfer.

We think that it may be possible to identify regions in RING finger proteins that independently confer E2 and substrate specificity (see the figure). Direct evidence for RING finger–substrate interactions in vitro has recently been reported. In one case, the RING finger of the apoptosis inhibitor cIAP2—but not those of c-Cbl or Apc11, a subunit of the anaphase-promoting complex (APC)—was sufficient for ubiquitination of caspase 7 (an enzyme that promotes apoptosis) (*13*). In the other case, Apc11—but not c-Cbl's RING—sufficed for ubiquitination of APC substrates (*14*, *15*). These in vitro assays use high protein concentrations, and so the specific RING-substrate interactions that they unmask are not necessarily the primary mechanisms for substrate recognition in the cell (see the figure). Indeed, in the cases where it is known, the primary interaction with a protein substrate involves distinct regions of the E3 (for example, the SH2 domain of c-Cbl). The primary substrate binding sites in E3s provide a high-affinity interaction, perhaps enabling the synthesis of a polyubiquitin chain to be completed without release of the substrate (polyubiquitin chains are more efficiently recognized and degraded by the proteasome). In turn, the weaker, secondary binding site in the RING may allow flexibility for changes in position and orientation between the substrate and the E2 catalytic site either as the synthesis of a polyubiquitin chain progresses or as multiple lysines in the substrate are modified with ubiquitin.

In the picture that we have now of a complex between c-Cbl's RING finger and UbcH7 E2, a critical ingredient is missing: ubiquitin. Future structural work on the complex engaged in the ubiquitination process should provide explanations for the other functions of RING finger E3 ligases in ubiquitination besides their E2 binding activity. Importantly, one would like to know how polyubiquitin chains are synthesized by apparently rigid E2-E3 complexes.

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PERSPECTIVES: AGING

Aging, Chromatin, and Food Restriction—Connecting the Dots

Judith Campisi

What causes aging? Current hypotheses generally fall into one of two categories. The first category invokes extrinsic or intrinsic factors that damage intracellular or extracellular molecules; the second invokes changes in gene expression that are either programmed or that are brought about by nonmutational changes in DNA structure. To what extent these hypotheses overlap or intersect is not known. Regardless of the hypothesis, however, caloric restriction (CR) has been an important tool for testing ideas about causes of aging in animals. Caloric restriction—reducing the food intake of animals (normally fed ad libitum) by 50 to 70%—reliably extends the mean and maximum life-spans of several species, including mammals (*1*). It postpones most age-related pathology and alters many, but not all, age-related processes. It is thought to do this primarily by reducing oxidative stress and damage caused by reactive oxygen species (*2*). Yet, despite more than half a decade of research, the major pathways through which CR acts remain enigmatic. Now, on page 2126 of this issue, Lin *et al.* (*3*) describe intriguing results that may link CR to the control of gene expression and to the suppression of DNA damage (loss or rearrangement of DNA) caused by mitotic recombination. These studies were carried out in a model organism, the yeast *Saccharomyces cerevisiae*, from which much basic cellular and molecular information has been gleaned, including several revelations about the genetics and physiology of aging (*4*).

Yeast undergo only a finite number of divisions, after which they die; thus, their life-span is defined by the number of divisions each cell completes (*4*). Lin *et al.* induced CR in yeast by limiting glucose availability or by genetically crippling their ability to sense and respond to glucose. Caloric restriction extended yeast longevity by 20 to 40%, similar to the relative life-span extension induced by CR in mammals. Of importance, this extension required the yeast genes *NPT1* and *SIR2*. *NPT1* encodes one of two enzymes that produce NAD (nicotinamide adenine dinu-

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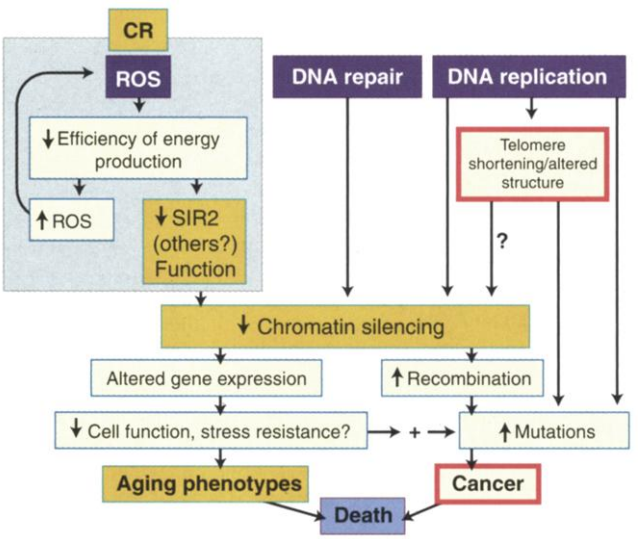
cleotide), a key intermediate in energy metabolism. *SIR2*—one of four silent information regulator genes—encodes a protein that promotes a compact chromatin structure, thereby preventing or silencing gene transcription at selected loci. As noted by Lin *et al.*, the yeast *SIR2* protein, Sir2p, is an NAD-dependent histone deacetylase, an enzyme that removes acetyl groups from the lysine residues of histone proteins (which are components of chromatin). This suggests that, through histone deacetylation, Sir2p may silence chromatin. In addition, the NAD requirement of Sir2p may serve to link its activity to the energy status of the cell. Thus, Sir2p may coordinate energy status with gene expression. Moreover, because by compressing chromatin Sir2p regulates the access of many nuclear proteins to the DNA, it represses homologous recombination at the highly repetitive ribosomal DNA (rDNA) locus with which it associates. The formation and accumulation of extrachromosomal rDNA circles (and possibly other DNA fragments) is a major cause of yeast aging. These circles are formed by homologous recombination during the cell cycle. Homologous recombination is important for repairing damaged DNA, but can inappropriately excise DNA fragments from regions of extensive homology, such as the rDNA locus. Sir2p modulates yeast life-span largely by suppressing rDNA circle formation. It is gratifying, then, that Lin *et al.* have discovered that CR also suppresses rDNA circle formation. Thus, at least in yeast, CR may extend life-span by modulating Sir2p activity and hence gene expression and recombination at silenced loci.

One can now envision a model (see the figure) whereby the inevitable production of reactive oxygen species compromises mitochondrial efficiency, and eventually energy output, in a detrimental feedback loop. NAD levels may reflect energy status and influence chromatin silencing through the NAD requirement of Sir2p. Caloric restriction may ameliorate the impact of reactive oxygen species, including their indirect effect on the decline in energy production. Thus, by reducing the impact of reactive oxygen species and the resulting decrease in Sir2p activity, CR may postpone loss of chromatin silencing. But how could loss of chromatin silencing lead to aging? The state of chromatin is essential for maintaining optimal gene expression and for suppressing homologous recombination. Loss of chromatin silencing alters gene expression, which can compromise the cell's ability to function, and possibly its ability to withstand stressful stimuli. In addition, increased recombination leads to the lethal accumulation of rDNA circles, and possibly other detrimental mutations.

Life-span extension by CR delays but does not prevent aging in both yeast and mammals. Two processes—DNA replication and DNA repair—may alter chromatin silencing and recombination independently of Sir2p and NAD availability. In both processes, DNA is stripped, albeit transiently, of regulatory proteins, which must be rapidly reassembled. Mistakes or transient states in the reassembly process may leave chromatin susceptible to inappropriate transcription or recombination events. Because the probability of undergoing DNA replication and repair increases with the number of cell divisions, the probability of acquiring imperfectly silenced (or configured) chromatin will rise with age. Likewise, the probability that faulty DNA replication or error-prone repair will generate (or fix) mutations will rise with age. Thus, CR, or even perfect chromatin silencing, can postpone aging phenotypes, but cannot delay them indefinitely. This is consistent with the finding that CR reverses some, but not all, gene expression changes that accompany aging in rodents (*5*).

How pertinent might this model be to mammals? History tells us that we can learn a great deal about human biology from model organisms. Therefore, we may expect that chromatin silencing, or chromatin maintenance in general, will play a role in the development of aging phenotypes in mammals. Indeed, silenced genes on human X chromosomes (*6*) and other loci become reactivated with age, suggesting that age-related loss of silencing does occur in some mammalian cells. Moreover, preliminary studies suggest that CR will be effective in primates (*7*). Proteins such as Sir2p may well serve to link metabolism to chromatin state in mammals, including humans, although this idea has not yet been rigorously tested, even in yeast. However, owing to their complexity, mammals may engage multiple SIR2-like proteins, perhaps some that are tissue-specific. Finally, *WRN*, the gene responsible for Werner syndrome, a disease of premature aging in humans (*8*), is a member of a gene family that is likely to participate in recombination and other DNA repair pathways, suggesting that recombination and DNA repair may be important determinants of the rate of aging in mammals.

A fundamental difference between adult mammals and model organisms such as the yeast, the nematode, and the fruitfly is the prevalence of cancer in mammals, and essentially the lack of cancer in yeast, worms, and flies. In mammals, mutations, very likely coupled to the changes in cellular function that accompany aging (*9*), give rise to cancer, which poses an additional threat to longevity. In addition, most human cells undergo telomere attrition with successive cell divisions and aging (that is, the ends of chromosomes become progressively shorter) (*10*). The extent to which telomere-induced cellular senescence contributes to human aging is not yet clear (*9*, *10*), nor is it known how telomere length contributes to the senescent phenotype of cells. In yeast, telomeres increase the compactness of nearby chromatin, but we do not yet know if this process occurs in human cells. It is intriguing, however, that telomere shortening occurs more rapidly on human X chromosomes, which could contribute to the age-dependent reactivation of X chromosome loci (*6*). The state of chromatin is now at the center of several processes known or suspected to be important in mammalian aging, suggesting, once again, that model organisms have served us well.



Counting calories. The connection between caloric restriction (CR), the SIR2 protein, and chromatin silencing. The maintenance or silencing of chromatin may be at the center of processes leading to the aging of cells and development of cancer. Red outlined boxes indicate processes found in mammals but not in yeast. Dark yellow boxes indicate pathways in yeast that are connected and that contribute to aging (*3*). ROS, reactive oxygen species.

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