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RNA SURVEILLANCE– AN EMERGING ROLE FOR RNA REGULATORY NETWORKS IN AGING

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Abstract

In this review, we describe recent advances in the field of RNA regulatory biology and relate these advances to aging science. We introduce a new term, RNA surveillance, an RNA regulatory process that is conserved in metazoans, and describe how RNA surveillance represents molecular cross-talk between two emerging RNA regulatory systems – RNA interference and RNA editing. We discuss how RNA surveillance mechanisms influence mRNA and microRNA expression and activity during lifespan. Additionally, we summarize recent data from our own laboratory linking the RNA editor, ADAR, with exceptional longevity in humans and lifespan in *C. elegans*. We present data showing that transcriptional knockdown of RNA interference restores lifespan losses in the context of RNA editing defects, further suggesting that interaction between these two systems influences lifespan. Finally, we discuss the implications of RNA surveillance for sarcopenia and muscle maintenance, as frailty is a universal feature of aging. We end with a discussion of RNA surveillance as a robust regulatory system that can change in response to environmental stressors and represents a novel axis in aging science.

1. Introduction

An emerging theme in the biology of aging over the last decade is the prominent role gene regulatory pathways that are conserved among metazoans ((Partridge and Gems, 2002; Tissenbaum and Guarente, 2002). For example, caloric restriction, insulin-signaling pathways, and stress response pathways use common homologous genes to modulate longevity in nematodes, flies, and mammals. Age-associated declines in muscle mass and muscle strength (Sarcopenia) (Fisher, 2004; Karakelides and Nair, 2005) are also a common feature in metazoan lifespan (Herndon et al., 2002). While post-translational protein-based mechanisms are broadly utilized in gene regulation and recognized to play a role in aging, notably Sirtuin deacetylases (Guarente 2007), transcription factors (e.g., FOXO/DAF16, Lee 2003 Science) and apolipoproteins (e.g., apoE, Louhija 1994; Seripa D, 2006), there is a growing interest in the role of RNA regulatory mechanisms that potentially influence aging. The role of the “RNA World” as encompassing more than a messenger of the DNA genetic code was first championed by Walter Gilbert in 1986 and has since been well established, with multiple RNA based regulatory and enzymatic activities now recognized. In this review we discuss, based on both published and our own preliminary data, how environmental perturbations (e.g., stress ligands, microenvironmental cues) can induce a regulatory system of RNA surveillance, which we define as the interaction between a class of RNA editing (RNAe) and RNA interference (RNAi) genes (Figure 1), and how this RNA regulatory system may represent a novel axis with influence on aging.

MicroRNAs are a component of the RNAi pathway and have been identified in organisms across the evolutionary spectrum, including plants, mammals, invertebrates, and viruses (Bushati and Cohen, 2007). Many previous studies evaluating microRNA expression have observed age-associated expression patterns in distinct organisms and tissue (Lund et al., 2002; Murphy et al., 2003; Weindruch et al., 2002), suggesting that changes in gene

expression are driven by evolutionarily conserved mechanisms of aging (see Figure 1). Although age-associated changes are apparent, there is very little information on the potential role for microRNAs in age-related declines in function, despite the growing literature on the role for microRNA in specific gene regulation programs. In addition to RNA interference (RNAi) mediated gene-silencing by microRNA, a second RNA regulatory system is RNA editing. RNA editing enzymatic activity has the capacity to generate functional diversity in proteins (e.g., altered translated product) and in microRNA (e.g., via edited miR sequence). This activity has been described to influence multiple biological processes that include synaptic transmission (Schmauss and Howe, 2002), innate immune response (Yang et al., 2003) and muscle function (Keegan et al., 2005). The biochemical mechanism for enzymatic modification of multiple RNA targets has been well described (reviewed in (Bass, 2002; Valente and Nishikura, 2005), including editing of miRNA (Yang et al., 2006). Interestingly, both loss-of-function and gain-of-function in levels of RNA editing activity are deleterious, suggesting that cell-type or organ specific set points in RNAe activity levels are highly regulated (Keegan et al., 2005). The predominant form of RNA editing is adenosine-to inosine (A-to-I) post-transcriptional modification and is mediated by the RNA editor Adenosine Deaminase Acting on RNA (ADAR), first identified in *Xenopus* (Bass and Weintraub, 1988). RNA editors are environmental sensors that function in chemo-sensing and stress response. This capacity to detect microenvironmental flux and stress provides an adaptive advantage because mutations that extend longevity typically lead to improved stress resistance. In human studies, change in RNA editing activity is associated with age-related cognitive dysfunction that include, but are not limited to, schizophrenia, dementia and amyotrophic lateral sclerosis (Maas et al., 2006). The process of RNA editing appears to be conserved in evolution, with evidence for homologues with similar enzymatic function in humans (Kim et al., 1994), *Drosophila* (Palladino et al., 2000), Zebrafish (Slavov et al., 2000), *Xenopus* (Hough and Bass, 1997) and in *C. elegans* (Morse et al., 2002).

2. RNA surveillance processing mechanisms: RNA interference and RNA editing

Recent advances in the understanding of RNA processing, particularly RNA interference (RNAi) and RNA editing (RNAe), have substantial implications for the molecular biology of aging. Much of our knowledge concerning RNAi, also known as RNA silencing or post-transcriptional gene silencing (PTSG), is based on early studies in plants and nematodes (Fire et al., 1998; Wightman et al., 1993). RNAi refers to a process wherein double-stranded RNA triggers a multistep process of RNA silencing that is sequence-dependent. The discovery of RNA silencing led to a Nobel prize in 2006 awarded to Andy Fire and Craig Mello. Our understanding of RNAi has now expanded to include small interfering RNA (siRNA) and microRNA (miRNA). The former, siRNA, is processed from short double-stranded RNA transcripts via RNA cleavage (i.e., through RNase III, Drosha and Dicer) in a process that tends to be induced by exogenous stimuli (e.g., RNA virus infection, stress ligands). RNAi therefore acts as a stress sensor in host response to exogenous RNA viral infection. The latter, microRNAs (miRNA) are small (~22 nucleotides) endogenously transcribed RNAs that are now recognized to be critical for post-translational gene expression and play important roles in numerous processes including cell proliferation, differentiation, apoptosis, cell fate determination, and immune regulation (Sonkoly et al., 2008).

Primary miRNAs are processed into short stem-loop pre-miRNA hairpin structures. These are then further cleaved to yield mature miRNA. MiRNAs regulate protein expression by binding to the 3' untranslated region (UTR) of a target mRNA, resulting in either mRNA destabilization, degradation, or inhibition of protein translation (Valencia-Sanchez et al.,

2006). Target specificity is primarily determined by the "seed sequence" of the miRNA, nucleotides 2–8 in the 5' end of the miRNA (Lewis et al., 2005; Valencia-Sanchez et al., 2006). Processing of dsRNA (both siRNA and miRNA) is guided by multiple protein classes (see Figure 1) that include: *dsRNA binding*. Proteins that bind dsRNA and recruit Dicer activity to facilitate the transfer of cleaved RNAs to the RISC complex. *Rnase III*. Dicer (cytoplasmic) and Drosha (nuclear) endoribonucleases cleave dsRNA and catalyze the first steps in the RNA interference pathway to initiate the formation of the RNA-induced silencing complex (RISC), thereby allowing argonaute proteins to degrade RNA targets. *Exportin (Xpo)*. Exportins facilitate nuclear transport of RNAs into the cytoplasm facilitating access to Dicer as part of the RNA silencing pathway. *Argonaute (Ago)*. Small RNAs such as siRNAs, miRNAs or Piwi-interacting RNAs (piRNAs) are bound by Argonaute proteins (e.g., Ago in humans, RDE in nematodes). On binding to a target mRNA, the Ago-siRNA or Ago-miRNA complex induces RNA degradation or translational inhibition. *RNA editing*. RNA editors modify RNA transcripts through A-to-I or C-to-U deamination. This can lead to altered protein sequence in RNAs that code for proteins, but RNA editing can also result in altered regulatory transcripts (e.g., microRNA) which can influence their target specificity. Notably, both siRNA and miRNA are further processed in an RNA-induced silencing complex (RISC), consisting of Argonaute and other proteins that mediate their mRNA targeted gene silencing, see Figure 1. In addition to the RNAi system, a second RNA processing regulatory system is RNA editing. RNA editing is mediated by conserved RNA editing enzymes (e.g., ADAR, APOBEC) whereby bases within RNA transcripts, both mRNA and miRNA, are deaminated (adenosine-to-inosine (A-to-I) or cytosine-to-uracil (C-to-U), respectively. Edited sequences, if translated, can result in protein diversification, or, in the case of miRNA, alter transcriptional silencing targets or susceptibility. Pre-miRNAs undergo A→I RNA editing (Luciano et al., 2004; Yang et al., 2006) and therefore may regulate the processing and expression of mature miRNAs (Luciano et al., 2004; Yang et al., 2006). Edited miRNA has profound implications for gene regulation.

Adding to the complexity of RNAi and RNAe interactions, evidence suggests that these two pathways compete for common dsRNA substrates (Bass, 2006). Furthermore, there is at least one mammalian ADAR that can sequester siRNAs from the RNAi pathway (Yang et al., 2006). Indeed, studies of ADAR-null strains of *C. elegans* indicate that RNA editing may counteract RNAi silencing of endogenous genes and transgenes (Nishikura, 2006). Therefore, complementary and interactive roles of RNAi and RNAe represent a powerful RNA regulatory system whose influence on lifespan and longevity remains to be fully investigated. All metazoa experience cellular stress. Cellular stressors, including dsRNA (e.g. viral infection), inflammatory cytokines, and bacterial lipopolysaccharide. Interestingly, stress induced apoptosis has also been associated with loss of ADAR, suggesting that ADAR may act to buffer cellular stressors (Hartner et al., 2009; Wang et al., 2004).

3. The relevance of RNA surveillance to aging, inflammation and muscle biology

3.1. RNA surveillance changes with normal aging

Published studies (e.g., (Lund et al., 2002)) and data from our laboratory (Montano et al., 2009) show that genes involved in RNA surveillance change with aging in nematodes, mice and human centenarians, suggesting that this regulatory axis is a common mechanism among evolutionarily distinct taxa. Whether this observation implies evolutionary selection to invasive RNA viruses or is an emergent phenotype of post-transcriptional gene regulation is unknown. Data in our laboratory are an example of how miRNA expression levels vary

substantially with age, in this case based on a comparison of ~400 miRNA expression levels in whole brain tissue obtained from 3 month-, 1 year- and 2 year-old mice (Figure 3). Whether there is a common set of mRNA targets that are regulated by miRNA during the aging process is unknown. Identifying and validating miRNA expression dynamics and targets within and between species will be highly informative towards clarifying the possibility of common regulatory signatures in the aging process. In addition to miRNA expression change with aging, our laboratory recently published compelling new data implicating RNA surveillance genes in human centenarians, and that implicate both RNA editing and RNA interference pathways in lifespan (Montano et al., 2009). In that study, we demonstrated that mutations discovered in human association studies dramatically influence lifespan in *C. elegans* and that expression levels for these RNA regulators decline in normal lifespan of these nematodes. RNAe also appears to be regulated with aging. In *C. elegans*, since *adr* expression levels rise early in adulthood then precipitously decline with advanced age (data not shown).

3.2. Cross-regulatory role for RNA interference and RNA editing in lifespan

Because RNA editing modifies ribonucleotides in target RNAs, this creates the opportunity for altered recognition by miRNAs and modified susceptibility to the RNA interference (RNAi) machinery. This possibility has been elegantly addressed in a study by Brenda Bass and colleagues, evaluating the role of ADAR activity influence on chemotaxis in the *C. elegans* nematode (Tonkin and Bass, 2003; Tonkin et al., 2002). Several other studies have demonstrated that processing of microRNA from precursors (i.e., pri-miRNAs and pre-miRNAs) can produce substrates for ADAR-mediated editing (Habig et al., 2007; Kawahara et al., 2008). For example, editing of human pri-miR-142 blocks processing by the RNase Drosha (Yang et al., 2006) and leads to rapid degradation (Yang et al., 2006). A recent study identified 47 pri-miRNAs that were edited and subsequently blocked from reaching maturation, presumably through rapid degradation (Kawahara et al., 2008). ADAR mediated editing of both the mouse and human microRNA miR-151 prevents Dicer processing activity (Kawahara et al., 2007). ADAR editing also appears to change the specificity of target miRs. For example, both ADAR1 and ADAR2 edit miR-376, thereby creating a capacity to repress the PRPS1 gene (Kawahara et al., 2007). In muscle, a novel G to A transition in the 3' UTR of the myostatin gene in sheep generated a target site for miR-1 and miR-206 - both miRs that are highly expressed in muscle (Clop et al., 2006 fix ref). This mutation resulted in translational inhibition of myostatin RNA transcripts and phenotypically resembled myostatin knockouts with characteristic muscle hypertrophy. The ramifications of these ADAR mediated editing activities on microRNA and mRNA is now an active area of research and is likely to yield significant insights into post-transcriptional modification of gene expression. Whether ADAR mediated modification of microRNA processing and target specificity influences transcriptome patterns during aging is unknown. Although there are data on the temporal expression of miRNAs in *C. elegans* (Lund et al., 2002), the potential for miRNAs to functionally influence aging has yet to be systematically addressed either within or between species. A notable recent study elegantly demonstrated that the developmental timing regulator miRNA *lin-4* affects *C. elegans* lifespan (Boehm and Slack, 2005). Also, Ibanez-Ventoso and colleagues demonstrated that genome-wide expression of miRNAs change during adulthood and aging of normal *C. elegans* nematode worms (Ibanez-Ventoso et al., 2006). In that study they observed that roughly half of the nematode miRNAs exhibit changes in expression level during adult life. Included among those were *lin-4* and *let-7* miRNAs and miR-1, homologues of these genes are broadly expressed in *Drosophila*, Zebrafish, chick and mammalian muscle (Lee and Ambros, 2001; Mansfield et al., 2004; Zhao et al., 2005) to influence muscle development. These data implicate miRNA in aging and lifespan, with ramifications for critical age-dependent processes, such as sarcopenia. Since many age-regulated miRNAs discovered in lower

organisms are conserved, human homologues might be similarly regulated during aging. A noteworthy comparative study demonstrated that non-coding RNA in humans and *C. elegans* are targets of ADAR activity (Morse et al., 2002), suggesting that both RNAi and RNAe interact, thereby increasing the complexity of RNA regulatory mechanisms engaged during aging.

3.3. Variants in RNA editing genes are associated with exceptional longevity in multiple species

In a recent study of human centenarians by our group, the RNA-editing genes *ADARB1* (21q22.3), and *ADARB2* (10p15.3) emerged as candidate genes associated with exceptional longevity in a screening based on a new DNA pooling approach (Sebastiani et al., 2008). These preliminary data were then validated using individually genotyped data from four different centenarian cohorts for a larger number of SNPs and a total sample size (2105 subjects, 3044 controls). The consistent association of SNPs tagging regions of the genes *ADARB1* and *ADARB2* in multiple centenarian cohorts suggests a potential role for RNA editing genes in the survival to extreme old age (Montano et al., 2009).

To expand upon the statistical association of ADAR genes with longevity observed in humans, we evaluated a possible functional role for orthologues of these genes on influencing lifespan in *C. elegans*, a robust organism that is widely used for evaluation of lifespan and candidate gene discovery. To determine whether ADAR homologues in *C. elegans* (*adr-1*, *adr-2*) influence lifespan, we utilized ADAR mutant loss-of-function strains to conduct standard lifespan assays using synchronized L4 worms. There was a substantial ($p < 0.05$) decline in lifespan in *adr* mutants (see Figure 4a). Knockdown of *daf-2*, the worm homolog for the mammalian insulin-like growth factor (IGF) receptor, increased lifespan, but was also affected by loss of ADAR function (Figure 4a), possibly indicating that RNA editing is required for *daf-2* mediated gains in lifespan. To further determine whether RNAe and RNAi interact in this model of lifespan, we evaluated the RNAi defective Argonaute gene, *rde-1*, in the context of *adr* mutant strains. Provocatively, we observed that *rde-1* rescued the loss in lifespan due to *adr* (Figure 4b), in effect producing a phenocopy of normal lifespan. These data indicate genetic and possibly dynamic interactions between the RNAi and RNAe pathways to modulate lifespan (see model in Figure 4c).

4. RNA surveillance in aging: skeletal muscle

4.1. Sarcopenia and aging in humans

Skeletal muscle accounts for 40–50% of total body mass, and is critical for both mobility and bioenergetic metabolism, for example see (Cerletti et al., 2008). Muscle homeostasis represents a dynamic balance between anabolism and catabolism of muscle, mediated by the actions of growth factors and cytokines, as well as their respective regulators (Yarasheski, 2003), with a potential role for vasculature in providing growth maintenance cues (e.g., insulin) (Fujita et al., 2007). The loss of muscle mass and strength is a universal feature of aging. In many cases, this impairs functional capacity leading to disability. The term sarcopenia is used to describe the wasting effects of age on skeletal muscle, characterized by a loss of muscle mass and function, metabolic dysregulation (Ryall et al., 2008), and an overall increase in vulnerability to stressors and in the capacity to maintain muscle homeostasis, particularly in the context of co-morbidities (Desquilbet et al., 2007; Desquilbet et al., 2009).

Regulatory RNAs such as miRNAs have been implicated in aging-associated loss of muscle mass. For example, Drummund, et al observed dysregulated miRNA expression in skeletal muscle of elderly men following anabolic stimuli compared with younger men, suggesting

that miRNA dysregulation (and potential targets) is correlated with a loss of muscle mass during aging (Drummond et al., 2008). However, further studies will be needed to establish a potential causal relationship. Circulating levels of inflammatory mediators (e.g., C-reactive protein, TNF-alpha, IL-1b and IL-6) also tend to increase with age (Finch and Morgan, 2007; Roubenoff et al., 2003). Indeed, elevated levels of IL-6 have been used to predict the development of sarcopenia in elderly men (Payette et al., 2003). Together these data suggest that alterations in miRNA expression and/or regulation of target mRNAs contribute to the age-associated inflammation observed in sarcopenia.

4.2. Myogenesis and the Muscle program

Skeletal muscle is composed of postmitotic, multinucleated, contractile myofibers formed through the process of myogenesis (Figure 5). Myogenesis is an ordered process whereby mononuclear muscle precursor cells exit the cell cycle, differentiate, and fuse with either existing myofibers or with each other to form new myofibers (Wagers and Conboy, 2005). Muscle precursor cells are derived from satellite cells, a myogenically committed population of cells which resides between the myofiber and the surrounding basal lamina (Bischoff, 1975; Mauro, 1961). Satellite cells are normally quiescent, but, upon exposure to appropriate growth stimuli, re-enter the cell cycle and give rise to muscle precursor cells (Charge and Rudnicki, 2004). It is these satellite cells that are primarily responsible for the regenerative capacity of skeletal muscle. Critical to their role as muscle stem cells, satellite cells are capable of undergoing self-renewal, thereby ensuring sufficient numbers of satellite cells for future regenerative needs (Cerletti et al., 2008).

Satellite cell activation occurs following muscle damage, although the nature of the events leading to re-entry of the cell cycle have not been fully elucidated. HGF, which is present in the basal lamina surrounding myofibers, has been shown to definitively activate satellite cells in vitro and in vivo (Bischoff, 1986; Jennische et al., 1993). Additionally, modulation of nitric oxide levels affects the activation state of satellite cells (Wozniak and Anderson, 2007). Following activation, satellite cells proliferate, and their progeny are referred to as myoblasts. These cells rapidly downregulate satellite cell markers (Pax7) and upregulate expression of the myogenic regulatory factors (MRFs) Myf5, which is required for myoblast proliferation, and MyoD, necessary for cell cycle arrest and differentiation. Two additional MRFs, myogenin and MRF4, are required during differentiation for expression of contractile proteins (Berkes and Tapscott, 2005; Pownall et al., 2002). Following differentiation, myoblasts undergo cell-cell fusion to form multinucleated myotubes. There are two separate phases of fusion. Initially, during embryogenesis and regeneration, mononucleated myoblasts fuse with each other to form a multinucleated cell. During the second phase, myoblasts fuse with existing myofibers to increase myofiber size and nuclear number. In addition to occurring during embryonic development and regeneration, this process of fusion is active during muscle growth following atrophy and muscle maintenance (Jansen and Pavlath, 2008).

Satellite cells and mature muscle fiber cells produce chemoattractants (e.g., MCP-1, Fractalkine, MDC, uPAR) that promote macrophage-mediated clearance of dead/apoptotic cells and myoblast proliferation through the release of local mediators (Goetsch et al., 2003; Massimino et al., 1997; Tidball, 2005). There is increasing data that inflammatory conditions in muscle are associated with infiltration of macrophages (for review, see (Tidball, 2005)) and evidence for muscle repair through cytokine-mediated signaling and cross-talk with muscle cells to promote myogenesis (Cantini and Carraro, 1995; Cantini et al., 1995; Massimino et al., 1997; Merly et al., 1999). In animal models the prevention of macrophage infiltration can blunt the repair process (Bondesen et al., 2004; Chazaud et al., 2003; Jejurikar and Kuzon, 2003; Lescaudron et al., 1999; Robertson et al., 1993; Summan et al., 2003).

4.3. RNA silencing, Myogenesis and Aging

RNA silencing by miRNAs plays an important role in skeletal muscle cell growth and differentiation. Three mRNAs, miR-1, miR-133, and miR-206 have emerged as key regulators of the myogenic program. While miR-1 and miR-206 promote differentiation of myogenic cells, miR-133 enhances proliferation while inhibiting differentiation (Chen et al., 2006; Kim et al., 2006). Additional evidence for the role of these miRNAs comes from the observations that expression of miR-206 is dysregulated in a mouse model of muscular dystrophy, while miR-1 and miR-133 expression is decreased during muscle hypertrophy (McCarthy, 2008; McCarthy and Esser, 2007). Conditional loss of microRNA biogenesis, wherein Dicer gene expression is selectively lost in skeletal muscle, results in perinatal lethality due to muscle hypoplasia, further supporting the importance of miRNAs in muscle development and function (O'Rourke et al., 2007). Since miRNA expression appears to change with age, it will be interesting to see how this activity influences muscle maintenance. The age-dependent decline in the ability of muscle to regenerate is primarily due to a decline in satellite cell function (Brack et al., 2007).

Satellite cell activation and proliferation are both impaired during regeneration of aged muscle relative to young muscle. Data from human studies suggest that satellite cell division declines with age (Renault et al., 2000), see Figure 6, and that the percentage of satellite cells in muscle decreases by as much as 50% between young (20s) to older (70s) (Kadi et al., 2004; Renault et al., 2002). Conboy and colleagues have attributed this decline to altered activity of the Notch/Delta/TGF β /Smad axis (Carlson et al., 2008a; Conboy et al., 2003). While the extent to which these changes are due to accumulation of genetic damage in aged satellite cells or age-related changes in the surrounding microenvironment has not been entirely resolved, evidence suggests that changes in the microenvironment play the more significant role (Bockhold et al., 1998; Brack and Rando, 2007; Schultz and Lipton, 1982). For example, heterochronic parabiosis experiments, a technique wherein there is surgical fusion of young mice with the vasculature of old mice dramatically demonstrate the importance of the microenvironment on satellite cell function. In these studies, either the young or old mouse sharing a circulatory system were exposed to hindlimb muscle injury compared with parabiotic fusion of young-young or old-old. By measuring the regenerative response it was determined that the effectiveness of satellite cell activation and muscle regeneration was dependent on the surrounding environment; regeneration in the old mouse was improved by exposure to a young environment, while regeneration in the young mouse was impaired by exposure to an aged environment (Conboy et al., 2003). Recent studies support a role for the Notch-Delta signaling and Smad transcriptional regulators in this process (Carlson et al., 2008a; Carlson et al., 2008b). These experiments underscore a critical role for the microenvironment in influencing the capacity for regeneration. Furthermore, satellite cells from old mice exhibit fibrogenic tendencies. Both these characteristics of old satellite cells are reversed when exposed to a young environment (Brack and Rando, 2007; Charge and Rudnicki, 2004). These data indicate not only the existence of factors that positively influence satellite cell function, but those that negatively impact their function, as well. Identification of these positive factors may result in valuable therapeutic treatments for sarcopenia.

5. Concluding remarks

In this review, we present evidence that RNA surveillance, including RNA interference (RNAi, e.g., miRNA, siRNA) and RNA editing (RNAe, e.g., ADARs) are determinants of longevity and lifespan. We describe multiple studies that show microRNA expression patterns and RNA editing patterns change with age in nematodes, mice and humans. We describe recent data demonstrating that variants in stress response ADAR genes are linked to exceptional longevity in humans. We also show that loss of function in homologues of

ADAR reduce lifespan in *C. elegans*. In those studies, we have found that further knockdown of RNA interference argonaute genes, i.e., RDE-1 or RDE-4 restore lifespan, suggesting that RNAe and RNAi interact to modulate the RNA transcriptome, with effects on lifespan. Collectively, these results suggest that ADARs and microRNAs play an important role in longevity, potentially by buffering the response of cells to stressors. All metazoa experience cellular stress, and cellular stressors, including dsRNA (e.g. viral infection), inflammatory cytokines, and bacterial lipopolysaccharide. Interestingly, stress induced apoptosis has also been associated with loss of ADAR, suggesting that ADAR may act to buffer cellular stressors (Hartner et al., 2009; Wang et al., 2004). Because dsRNA-binding proteins can form heteromers, direct interaction between ADAR and PKR is possible, providing a mechanistic basis for the modulation of PKR activity and buffering of stress responses by ADAR. The notion that disease severity can be viewed as a form of stress management seems intuitively sensible and is consistent with our observations that centenarians appear to compress disease to their later years.

The majority of miRNAs are predicted to bind numerous targets and in many cases regulate a group of genes with similar functions. These aspects of miRNAs therefore provide of the capacity for complex transcriptional control. The functions of only a few miRNAs have been discerned, revealing that these small regulatory molecules control fundamental biological activities such as cell proliferation, cell differentiation, cell death, cell signaling, stress response and metabolism. The well recognized effects of aging on skeletal muscle, and the experimental tools available for this tissue render it highly relevant in the evaluation and identification of RNA surveillance factors involved in aging and the development of age-related frailty (Carlson and Conboy, 2007; Carlson et al., 2008b).

RNA surveillance may be an example of a robust dynamic system. The notion of robustness in biological systems has been described as the capacity to adapt to environmental cues and to maintain homeostatic stability in response to perturbation and stress (Kitano, 2007). A decline in robustness resembles the frailty phenotype, since frailty also represents a reduced capacity to manage environmental stressors to maintain tissue homeostasis (Desquilbet et al., 2007). The evolution of robust systems is not without comprise and trade-offs (Kitano, 2007). There is abundant evidence for trade-offs in lifespan studies. For example, increased expression of SIRT1, a deacetylase is associated with increased lifespan by mimicking caloric restriction. However, animals with increased SIRT1 are also hypersensitive to LPS and influenza infection (Imai, 2009).

The RNA surveillance system also is likely to represent a dynamic balance of trade-offs. Early studies demonstrated that RNAi provides clear innate immune protective effects; however, over expression of RNAi/microRNA is clearly deleterious (Grimm et al., 2006). Loss of RNA editors (e.g., ADAR) results in premature mortality (Montano et al., 2009), but over-expression of ADAR is associated with lethality (Keegan et al., 2005). These data reinforce the concept of “antagonistic pleiotropy” first proposed by GC Williams in 1957, to describe the duality of biologic features with selective advantage early in life that also have undesired features that are deleterious later in life (Williams, 1957). Such trade-offs may represent a robustness-frailty axis that underlies complex systems (Kitano, 2007) and innate regulatory pathways (Libert et al., 2006), with likely many more pathways to be discovered. Given the complexity and interconnectedness of the RNA surveillance system, any perturbation in this dynamic system to promote longevity, for example through therapeutic intervention, will undoubtedly have unexpected trade-offs that will need to be carefully evaluated to achieve longevity and a healthspan balance.

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PROTEIN CLASS	VERTEBRATES	NEMATODES	PLANTS	
Argonaute	Ago1/EIF2C1 Ago2/EIF2C2 Ago3/EIF2C3 Ago4/EIF2C4 PIWIL1 PILWIL2 PILWIL3 PILWIL4	ALG-1 & ALG-2 RDE-1 PPW-2 PPW-1	Ago1 Ago4	Binds small RNAs to induce RNA degradation or translational inhibition.
RNaseIII	Dicer Drosha (RNASEN)	DCR-1 Drosha	DCL1 DCL2 DCL3 DCL4	Endoribonucleases that cleave dsRNA thereby initiating the formation of RISC
dsRNA binding proteins	TRBP PACT Stau1 Stau2 DGCR8	RDE-4	HYI1 DRB4	Bind dsRNA and recruit Dicer. Facilitates transfer of cleaved RNA to RISC.
Exportins	Xpo-5 Xpo-1 Xpo-T	Xpo-1 (IMB-4) Xpo-T	Xpo-5 (HASTY) Xpo-1 Xpo-Y (PAUSED)	Transports RNAs to the cytoplasm where they are accessible to Dicer
RNA editing	Adar Adarb1 Adarb2 Apobec1 Apobec2 Apobec3	ADR-1 ADR-2		Modifies RNA via A to I or C to U deamination, leading to altered protein sequence or regulatory activity.

Figure 1. RNA surveillance genes

Composition of the RNA interference and RNA editing machinery with orthologues in vertebrates, nematodes and plants.

Figure 2. Schematic of RNA regulatory pathways
a) RNA interference (RNAi) pathway and, **b)** RNA editing (RNAe) pathway.

Figure 3. Age trend in microRNA expression during normal aging in mice

Brain tissue from young (3 months), adult (one year) and old (2 year) mice (C57BL6), left to right, was harvested and total RNA used to evaluate expression of 380 microRNAs using an Illumina chip platform. Hierarchical clustering was conducted yielding 8 clusters (see left of dendrogram), with clusters 1 and 2 displaying high expression in young, declining with age; and clusters 5 and 6 displaying low expression in young, increasing with age.

Figure 4. Effects of ADAR on lifespan in *C. elegans*

a) Lifespan using mutant strains for *adr-1;adr-2* in the context of dsRNA mediated gene inactivation of *daf-2*. Note decline in lifespan due to *adr-1; adr-2* compared with N2 wildtype. Also note increases in lifespan of both N2 and *adr-1; adr-2* in the presence of dsRNA for *daf-2*. days **b)** Lifespan using mutant strains for *adr-1;adr-2* and *adr-1; adr-2; rde-1* (grey solid) demonstrate declines in lifespan using mutant strains and full rescue of lifespan in an RNAi defective (*rde-1*) background. To synchronize worms for lifespan, eggs were isolated (N2, *adr-1, adr-2, adr-1;adr-2, rde-1, rde-4, adr-1;adr-2;rde-1*) and synchronized by hatching overnight in the absence of food at 20°C. Synchronized L1 larvae were counted and plated (10 worms/plate, n=60) on *Escherichia coli* bacterial lawns (OP50) on NGM media and allowed to develop to L4-stage larvae at 20°C. 5-fluorodeoxyuridine (FudR) solution was added to a final concentration of 0.1mg/ml to prevent reproduction. Worms were kept at 20°C and lifespan monitored by counting on alternate days. The *daf-2* dsRNA -expressing bacteria were grown overnight in LB with 50 ug/ml ampicillin and then

seeded onto RNAi NGM plates containing 5 mM isopropylthiogalactoside (IPTG). The RNAi bacteria were induced overnight at room temperature for dsRNA expression. About 30 synchronized L4-stage animals were added to each well and allowed to develop to adults, followed by the addition of FudR. Worms were kept at 20C, and their lifespan was monitored. c) Model interpretation of the lifespan data; wherein RNAe protects from RNAi mediated target RNA degradation.

QUIESCENCE → PROLIFERATION → DIFFERENTIATION → FUSION

Satellite cell → Myoblast → Fusion competent myoblast → Myotube (in vitro)
Myofiber (in vivo)

Figure 5. Schematic of Myogenic program

Figure 6. Decline in human muscle satellite cell division with age (*adapted from* (Renault et al., 2000)).