can be in enhancing mating success. Males in many species seem unable to set aside their competitive differences to form bonds that could, over the long term, be of mutual beneficial. Why should male Assamese macaques have been able to navigate this impasse when males in so many other species remain caught in a 'bad bromance'?

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Aging: miRacles of Longevity?

The inventory of processes that miRNAs regulate has continued to expand since their relatively recent discovery. A new study reveals not only that the expression of miRNAs changes with age, but also that these miRNAs can act in both pro- and anti-longevity regulatory pathways.

Coleen T. Murphy

MicroRNAs (miRNAs), the endogenous 22-nucleotide non-coding RNAs that regulate expression through translational repression or RNA degradation, were first discovered through their roles in regulating developmental decisions in *Caenorhabditis elegans* [1]. Since then, miRNAs have been found to be remarkably well-conserved in plants and animals, including humans. The regulation of developmental timing, neuronal asymmetry, germline cell division, reprogramming of induced pluripotent stem cells, p53-induced cell senescence, and cancer progression are all controlled by miRNAs [2], and it is likely that even more functions of miRNAs will be discovered. In a paper published in this issue of *Current Biology* by de Lencastre *et al.* [3], miRNAs are shown to act in regulatory pathways that both extend and reduce lifespan, suggesting a more important role for miRNAs in the regulation of aging than had been previously appreciated.

The questions posed in the paper by de Lencastre *et al.* [3] are whether miRNA expression changes with age, and whether those miRNAs that change with age play a role in regulating longevity [3]. These two questions had been previously addressed in separate studies; Ibanez-Ventoso *et al.* [4] used microarrays to identify *C. elegans* miRNAs that change with age, while Boehm and Slack [5] showed that the heterochronic development circuit miRNAs *lin-4* and *lin-14* regulate longevity post-developmentally.

de Lencastre *et al.* [3] have elaborated on these concepts, using deep sequencing to identify miRNAs that change with age, examining young (day 0) and middle-aged (day 10) wild-type and long-lived *daf-2* insulin signaling mutants. Notably, the use of deep sequencing allowed the discovery of 11 new miRNAs, several of which share homology with miRNAs in higher eukaryotes.

Generally, miRNA expression declines with age. However, a small group of the small RNAs showed particularly large changes in expression, and a few were upregulated with age. Most of these miRNAs have not yet been characterized fully, but *let-7*, one of the founding miRNAs that is associated with both late larval development [6] and cancer [7], showed the greatest decrease with age. Fusion of the miRNA promoters to the *gfp* gene and analysis of GFP expression revealed that many of these age-regulated miRNAs are expressed primarily in the intestine, neurons, and somatic gonad — all tissues that have been previously associated with the regulation of aging [8].

Do these miRNAs actually regulate longevity, or are they merely passive markers of age? de Lencastre *et al.* [3] used *C. elegans* knock-out consortium deletion mutants to show that some of the miRNAs that were

upregulated with age also regulate longevity. Specifically, three of the miRNAs with large expression increases with age had significant effects on lifespan, including one, *miR-239*, that increased longevity. The loss of a gene that has deleterious effects on longevity (i.e., that is pro-aging) would be expected to extend lifespan. For example, loss-of-function mutants of *daf-2*, which normally functions to inhibit the pro-longevity activity of the FOXO transcription factor DAF-16, are long-lived. Thus, *miR-239* acts like *daf-2*, in that its loss extends lifespan and increases stress resistance, and its overexpression shortens lifespan.

The two miRNAs whose loss shortens lifespan are perhaps more unexpected. While one could chalk up the short lifespans of these *miR-71* and *miR-246* mutants simply to the induction of a sickly state, the authors went on to show that overexpression of these two miRNAs increased lifespan, suggesting that the two genes normally promote longevity and stress resistance. These miRNAs act similarly to heat shock genes, which are induced by stress and old age, and are also required for long lifespan. Thus, these miRNAs may be considered to be pro-longevity genes. Together, these data show that specific miRNAs can extend or shorten lifespan and act in stress resistance pathways.

One important question, then, is what are the targets of these miRNAs that induce longevity-related cellular responses? *miR-239* and *miR-71* likely function in the insulin/IGF-1 signaling (IIS) pathway, because *miR-239*-mediated effects on longevity are dependent on *daf-16,* and loss of *daf-16* does not further shorten *miR-71*'s lifespan. The authors tested the expression of members of the insulin/IGF-1 signaling and cell-cycle checkpoint pathways, and found that expression of *pdk-1* and *cdc-25.1*, which both have predicted miRNA-binding sites in their 3′ untranslated regions, is altered in the *miR-71* mutant, while *miR-239* appears to regulate insulin/IGF-1 signaling pathway genes indirectly. *miR-71* may serve as a link between the insulin and DNA-damage checkpoint longevity pathways. In the future, it will be interesting to see whether other longevity pathways that were not examined (such as those involving TOR, heat shock factor, *daf-12*, dietary restriction, *sir-2,* mitochondrial function, and germline stem cells) are also affected, regulated, or linked by these miRNAs.

Is a developmental role necessary for miRNAs that affect longevity? Up to this point, *lin-4* and *lin-14* were the only miRNAs known to function in lifespan regulation, but these genes were first identified for their roles in developmental timing. The fact that *lin-4* and *lin-14* regulate longevity post-developmentally does not rule out the possibility that lifespan regulation and development are normally connected. In fact, *lin-4* was found to be regulated by *daf-16* in L1 arrest [9], further linking the two pathways. Therefore, it is important to ask whether every miRNA that affects longevity does so as a secondary role, while its primary role is developmental.

Previous studies had not uncovered any obvious developmental phenotypes for the miRNAs in question [10]; de Lencastre *et al.* [3] assayed the miRNA mutants in greater detail, but still found no obvious changes in developmental rates, reproductive timing, or progeny production. Although it is possible that there is a cell-specific phenotype yet to be uncovered, or that there is functional redundancy, the lack of a gross developmental change suggests the exciting possibility that these miRNAs specifically regulate aging independently of development. This would be novel, since even well-known longevity regulators (e.g., *daf-2*, *eat-2*, and mitochondrial mutants) have obvious developmental phenotypes.

These results beg the question of why post-reproductive aging would be regulated at all, a question that is currently unanswered for any pathway other than those that couple reproduction to lifespan [11]. The cellular and organismal mechanisms involved must also be determined; miRNAs might control the senescence of particular 'rate-limiting' cells or tissues, or may play a role in the coordination of the aging rates of different tissues.

Why would miRNAs be useful in the regulation of longevity? Controlling response robustness may be one role for miRNAs, and it has been proposed that robustness may generally decrease with age [12]. Understanding the kinetics of the responses may explain the advantage of adding a regulatory layer that involves small RNAs: the responses of bacterial small RNAs are less noisy [13] and have different sensitivities than protein-based regulators, which may make them more responsive than transcription factors to stressful conditions [14]. While the details of eukaryotic miRNA regulation might differ from those of prokaryotic small RNAs, the regulatory logic employed by the miRNAs and their targets may be conserved. It is interesting that prokaryotic small RNAs and eukaryotic miRNAs both regulate stress responses (and old age could be considered a stress), further suggesting parallels in their utility. Once the complement of upstream regulators of miRNA expression and the downstream targets of the miRNAs have been identified, mapping out these details could explain the significance of using miRNAs to regulate lifespan.

The high level of conservation from *C. elegans* through humans suggests miRNAs may regulate aging in other organisms as well. miRNAs now appear to play a role in biological decisions from the earliest to the latest stages of *C. elegans'* life. Given their high conservation and ubiquity, what is the likelihood that miRNAs don't play a similar role in humans as well?

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Eukaryotic Evolution: The Importance of Being Archaebacterial

Approximately half of all eukaryotic genes show signs of prokaryotic origin. Genes derived from eubacteria are more abundant than those from archaebacteria, but the latter are functionally more important. This supports archaebacteria as founding ancestors of the eukaryotic nucleus.

John M. Logsdon, Jr.

How did eukaryotic cells arise from prokaryotic ancestors? In particular, from which lineage (or lineages) of prokaryotes can we trace the origin of the eukaryote nuclear genome? Such questions have puzzled biologists for decades. A recent study by Cotton and McInerney [1] takes a fresh look at the question by asking not only where eukaryotic genes came from, but also how functionally important these genes are in relation to which type of prokaryote — eubacteria or archaebacteria — they are derived from.

Initial hypotheses posited that the eukaryotic cell arose through endosymbioses among bacteria [2]. These hypotheses have been supported by early studies that confirmed that mitochondria and chloroplasts are derived from bacteria [3]. Archaebacteria — later recognized to be a prokaryotic group separate from the eubacteria [4] — were postulated as possible ancestors for the eukaryotic nucleus. In 1984, the privileged status of archaebacteria was elevated further, when Lake *et al.* [5] proposed that eukaryotes derived from a particular group of archaebacteria dubbed 'eocytes' (now referred to as 'crenarchaeotes').

Although additional data supported the eocyte hypothesis for eukaryotic origins [6-8], the ensuing two decades witnessed the widespread acceptance of a different view of the tree of life. This tree was now rooted by ancient gene duplications between the eubacteria and the archaebacteria and it indicated that eukaryotes had a sister relationship with archaebacteria, instead of being their descendants [9,10]. The hegemony of this so-called 'three domain' tree (Figure 1) even led to a renaming of these major domains [11]: Bacteria (eubacteria), Archaea (archaebacteria), and Eukarya (eukaryotes).

As both eukaryotic and prokaryotic genome sequences became available in the late 1990s, it looked as though these pressing questions of eukaryotic origins could be answered. If eukaryotes derived from an archaebacterium then many, if not most, eukaryotic genes should be traceable to archaebacteria. But this was not the case. Instead, of the many genes that could be traced to prokaryotic sources, most were derived from eubacteria [12]. A possible solution to this conundrum was that most eubacterial genes were derived from post-endosymbiotic gene transfer to the nucleus *via* the proteobacterial ancestor of the mitochondrion [13]. This explanation was at least consistent with previous phylogenetic studies indicating that most 'informational' genes in eukaryotes — *i.e.*, those functioning in transcription, translation and replication — were derived from archaebacterial sources, whereas the more abundant 'operational' genes, *e.g.*, those encoding metabolic functions, came primarily from eubacterial sources [14].

But how could so many, and seemingly functionally important, eubacterial genes take over an essentially archaebacterial cell? This conundrum led back to the ideas of endosymbiotic origins for eukaryotes. Instead of the mitochondrion representing a latecomer to an already established, post-archaebacterial, proto-eukaryotic lineage, perhaps the mitochondrial endosymbiosis was itself one — if not the — key initial event in eukaryote evolution. This view has gained ground following the clear rejection of the Archezoa hypothesis — the idea that some eukaryotic lineages diverged before the mitochondrial endosymbiosis — with data showing that all known eukaryotes either have or previously had a mitochondrion [15]. With the mitochondrion present in the common ancestor of eukaryotes, eukaryotic genomes would then easily be true chimeras: combining archaebacterial genetic infrastructure with metabolic machinery from eubacteria. These ideas have re-emerged as apparently synthetic views, exemplified by Lake's 'ring of life' hypothesis [16] that acknowledges multiple prokaryotic sources to the eukaryotic lineage. Even more recent phylogenetic analyses take us back to the eocyte hypothesis (now, 'two-domain hypothesis'; Figure 1B) and provide considerable (but perhaps not definitive) evidence that eukaryotes derive from within archaebacteria [17,18].

In the end, gene phylogenies, however methodologically rigorous, seem unable to definitively answer whether one particular and if so which prokaryotic lineage was the major foundation on which eukaryotes were built. By sheer numbers, eubacterial genes are more important. But the archaebacterial genes with their strong roles in the information economy of the cell are arguably more important. But