informative to learn how directed mutations that raise or lower the nucleosome positioning score without affecting transcription factor binding sites within an enhancer alter the function of these ubiquitous, but still elusive regulatory elements.

References

1. Johnson, D.S., Mortazavi, A., Myers, R.M., and Wold, B. (2007). Genome-wide mapping of *in vivo* protein-DNA interactions. Science *316*, 1497–1502.
2. Zinzen, R.P., Girardot, C., Gagneur, J., Braun, M., and Furlong, E.E. (2009). Combinatorial binding predicts spatio-temporal *cis*-regulatory activity. Nature *462*, 65–70.
3. Segal, E., Raveh-Sadka, T., Schroeder, M., Unnerstall, U., and Gaul, U. (2008). Predicting expression patterns from regulatory sequence in *Drosophila* segmentation. Nature *451*, 535–540.
4. Fakhouri, W.D., Ay, A., Sayal, R., Dresch, J., Dayringer, E., and Arnosti, D.N. (2010). Deciphering a transcriptional regulatory code: modeling short-range repression in the *Drosophila* embryo. Mol. Syst. Biol. *6*, 341.
5. Khoueiry, P., Rothbächer, U., Ohtsuka, Y., Daian, F., Frangulian, E., Roure, A., Dubchak, I., and Lemaire, P. (2010). A local genomic cis-regulatory signature that complements the presence of transcription factor binding sites in ascidian and fly enhancers. Curr. Biol. *20*, 792–802.
6. Arnosti, D.N., and Kulkarni, M.M. (2005). Transcriptional enhancers: intelligent enhanceosomes or flexible billboards? J. Cell. Biochem. *94*, 890–898.
7. Schones, D.E., Cui, K., Cuddapah, S., Roh, T.Y., Barski, A., Wang, Z., Wei, G., and Zhao, K. (2008). Dynamic regulation of nucleosome positioning in the human genome. Cell *132*, 887–898.
8. Wolffe, A.P., and Brown, D.D. (1986). DNA replication *in vitro* erases a *Xenopus* 5S RNA gene transcription complex. Cell *47*, 217–227.
9. Tanaka, Y., and Nakai, K. (2009). An assessment of prediction algorithms for nucleosome positioning. Genome Inform. *23*, 169–178.
10. Ozsolak, F., Song, J.S., Liu, X.S., and Fisher, D.E. (2007). High-throughput mapping of the chromatin structure of human promoters. Nat. Biotechnol. *25*, 244–248.
11. Mavrich, T.N., Jiang, C., Ioshikhes, I.P., Li, X., Venters, B.J., Zanton, S.J., Tomsho, L.P., Qi, J., Glaser, R.L., Schuster, S.C., *et al*. (2008). Nucleosome organization in the *Drosophila* genome. Nature *453*, 358–362.
12. Valouev, A., Ichikawa, J., Tonthat, T., Stuart, J., Ranade, S., Peckham, H., Zeng, K., Malek, J.A., Costa, G., McKernan, K., *et al*. (2008). A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. Genome Res. *18*, 1051–1063.
13. Kaplan, N., Moore, I.K., Fondufe-Mittendorf, Y., Gossett, A.J., Tillo, D., Field, Y., LeProust, E.M., Hughes, T.R., Lieb, J.D., Widom, J., *et al*. (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. Nature *458*, 362–366.
14. Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thåström, A., Field, Y., Moore, I.K., Wang, J.Z., and Widom, J. (2006). A genomic code for nucleosome positioning. Nature *442*, 772–778.
15. Visel, A., Minovitsky, S., Dubchak, I., and Pennacchio, L.A. (2007). VISTA Enhancer Browser — a database of tissue-specific human enhancers. Nucleic Acids Res. *35*, D88–D92.
16. Yuan, G.C., and Liu, J.S. (2008). Genomic sequence is highly predictive of local nucleosome depletion. PLoS Comput. Biol. *4*, e13.
17. Field, Y., Kaplan, N., Fondufe-Mittendorf, Y., Moore, I.K., Sharon, E., Lubling, Y., Widom, J., and Segal, E. (2008). Distinct modes of regulation by chromatin encoded through nucleosome positioning signals. PLoS Comput. Biol. *4*, e1000216.
18. Miele, V., Vaillant, C., d'Aubenton-Carafa, Y., Thermes, C., and Grange, T. (2008). DNA physical properties determine nucleosome occupancy from yeast to fly. Nucleic Acids Res. *36*, 3746–3756.
19. Gupta, S., Dennis, J., Thurman, R.E., Kingston, R., Stamatoyannopoulos, J.A., and Noble, W.S. (2008). Predicting human nucleosome occupancy from primary sequence. PLoS Comput. Biol. *4*, e1000134.

Department of Biochemistry and Molecular Biology, and Program in Quantitative Biology, Michigan State University, East Lansing, MI 48824, USA.
E-mail: ayahmet@msu.edu, arnosti@msu.edu

DOI: 10.1016/j.cub.2010.03.051

Evolution: Aging Up a Tree?

Evolutionary theories of aging predict that species in low-risk habitats will evolve longer lifespans. A new study comparing lifespan in arboreal and terrestrial mammals provides further support for this prediction. But is the prediction valid?

Jacob A. Moorad and Daniel E.L. Promislow

Imagine a hidden valley, populated by gentle herbivores and free of predators. Each individual faces some low but constant risk of mortality. This risk is independent of the animal's age, condition or density, as if a piano were suddenly to fall from the sky and crush the animal. A neighboring valley is identical except for the fact that here, pianos dispatch animals at twice the rate of the first valley. In his classic paper on the evolution of senescence, George C. Williams [1] would argue that populations in the second valley should evolve higher rates of senescence, due to the higher level of extrinsic mortality. A new study [2] compares maximum lifespan in nearly 800 species of arboreal and terrestrial mammals. In support of Williams' prediction, the authors find that arboreal mammals outlive their terrestrial counterparts. However, recent theoretical studies suggest that the explanation for such patterns may be more complex than previously thought.

G.C. Williams and his contemporary, Peter Medawar [3], argued that senescence, an age-related decline in survival and reproduction, was inevitable from an evolutionary perspective. Most offspring are born to relatively young parents; few are born to parents who have reached late age. An allele that increases the probability of death among young parents would be removed efficiently by natural selection. A late-acting allele that reduces survival would not be removed as readily, because a greater proportion of its carriers would be able to reproduce before dying. According to this logic, the force of natural selection will decline with age, and this in turn will allow late-acting deleterious alleles to accumulate, leading to senescence.

Williams believed that increased extrinsic mortality would exacerbate this effect by shifting the age distribution towards younger individuals. As a result, fewer old-aged individuals would live long enough to reproduce and transmit their genes to the next generation. Thus, extrinsic mortality would exaggerate the tendency for natural selection to weaken with age. This leads to the prediction that senescence should evolve to be more pronounced in environments with high risks of death. Conversely, populations that are protected from sources of extrinsic mortality should evolve longer lifespans.

Support for Williams' prediction comes from both laboratory and comparative studies. In the fruit fly *Drosophila melanogaster*, for example, Stearns *et al.* [4] found that flies evolved shorter lifespans when adults were exposed to high extrinsic mortality. In a series of comparative studies, researchers have found that, in general,

taxa that are protected from predators also appear to be long lived. Larger mammals live longer than smaller mammals [5], birds and bats live longer than similar-sized non-volant rodents [6], and species with obvious anti-predator defenses, like porcupines, live longer than their more vulnerable relatives [7]. Ants and bees offer a particularly impressive example. Among eusocial species, in which colonial life provides protection from both biotic and abiotic hazards, queens have evolved lifespans an average of 100 times that of less protected solitary species [8].

In a newly published study, Milena Shattuck and Scott Williams [2] set out to test G.C. Williams' 1957 prediction by comparing longevity in arboreal and terrestrial mammals. Importantly, they corrected for the fact that large mammals live longer than smaller ones by comparing residual values of lifespan after removing the confounding effects of body mass. At the same time, to control for the potentially confounding effects of phylogeny [9], they looked for a relationship between habitat and lifespan within phylogenetically independent groups. Arboreal or semi-arboreal groups always lived longer than terrestrial ones (Figure 1), with the exception of primates and marsupials. Just why primates and marsupials form exceptions is unclear, though Shattuck and Williams [2] suggest that at least for primates, it may have something to do with their long history of arboreality prior to coming down out of the trees, or with specialized anti-predator defenses.

G.C. Williams' conjecture has become such an important prediction from the evolutionary theory of aging that experiments that explicitly test the former are taken as evidence for the latter [10]. With Shattuck and Williams' [2] most recent results, is G.C. Williams' prediction one step closer to law? Not quite. While Shattuck and Williams' [2] results are robust, G.C. Williams' prediction regarding extrinsic mortality may turn out to be, well, up a tree.

First off, models have shown us that selection can, indeed, actually favor reduced lifespan in the face of increased mortality if young individuals suffer more from the negative effects of high population density [11], or if the effect of extrinsic stresses depends on an individual's intrinsic condition [12]. Predation risk is likely to depend both on population density and condition of the potential prey, so both are reasonable possibilities. However, if density dependence acts primarily at late age, the effects of extrinsic mortality are reversed. Perhaps more importantly, mathematical models of extrinsic mortality [11,13] show that, if survival is density-independent, extrinsic mortality might have no effect whatsoever on selection. How can this be?

Figure 1. Examples of closely related arboreal and terrestrial mammals.

Arboreal mammals: (A) the pale-throated three-toed sloth (order Xenarthra); (B) the binturong (family Viverridae). Closely-related terrestrial species: (C) the six-banded armadillo (order Xenarthra); (D) the Malabar civet (family Viverridae). Sources: three-toed sloth, http://www.dembsky.net/amazon/information4.html; six-banded armadillo, http://www.flickr.com/photos/pantaneiro/2377848149/; binturong, http://alamendah.wordpress.com/2009/07/25/satwa-indonesia-yang-dilindungi/; malabar civet, link to jpeg at http://www.animalinfo.org/species/carnivor/vivemega.htm#profile.

Imagine that we take a population with a huge number of individuals into a sufficiently large laboratory such that there is no density dependence. We allow the population to reach a stable equilibrium with respect to age-structure and gene frequencies. Now we take that population and apply extra mortality by removing half the individuals at random, regardless of age. After this extrinsic mortality event, absolutely nothing will have changed that can affect selection: the environment, reproductive output of survivors, age-distribution, phenotypes, and gene frequencies all stay the same. Assuming that protected environments, such as safe branches high atop trees, protect all age-classes equally, such environments can do nothing to promote selection for longevity.

Nevertheless, the patterns in nature are clear: species that live in protected environments live longer than those in riskier environments. Why might this be so? The first answer is obvious — all else being equal, less extrinsic mortality will lead to longer lifespans in a cohort. But what about the effects of extrinsic mortality over evolutionary time? As we noted earlier, if increased mortality in terrestrial habitats is density- or condition-dependent, slower aging could evolve. Alternatively, a model by Moorad and Promislow [14] suggests that greater longevity evolves more readily when survival depends on relatively few heritable traits. It could be that protected environments, such as arboreal habitats, greatly simplify

the problem of surviving by reducing the need for highly complex predator avoidance adaptations.

Twenty years of experimental and comparative studies support the prediction that longer lifespan will evolve in safer habitats. This latest study [2] provides yet further evidence for this pattern but theory suggests that the causal link between extrinsic mortality and innate lifespan is much more complex than previously thought. We have no doubt that the phenomenon is widespread. The challenge now is to figure out why.

References

1. Williams, G.C. (1957). Pleiotropy, natural selection, and the evolution of senescence. Evolution *11*, 398–411.
2. Shattuck, M.R., and Williams, S.A. (2010). Arboreality has allowed for the evolution of increased longevity in mammals. Proc. Natl. Acad. Sci. USA *107*, 4635–4639.
3. Medawar, P.B. (1946). Old age and natural death. Mod. Quart. *2*, 30–49.
4. Stearns, S.C., Ackermann, M., Doebeli, M., and Kaiser, M. (2000). Experimental evolution of aging, growth, and reproduction in fruit flies. Proc. Natl. Acad. Sci. USA *97*, 3309–3313.
5. Sacher, G.A. (1959). Relationship of lifespan to brain weight and body weight in mammals. In C.I.B.A. Foundation Symposium on the Lifespan of Animals, G.E.W. Wolstenholme and M. O'Connor, eds. (Boston, MA: Little, Brown and Co.), pp. 115–133.
6. Holmes, D.J., and Austad, S.N. (1994). Fly now, die later: life-history correlates of gliding and flying in mammals. J. Mammal. *75*, 224.
7. Gorbunova, V., Bozzella, M.J., and Seluanov, A. (2008). Rodents for comparative aging studies: from mice to beavers. Age *30*, 111–119.
8. Keller, L., and Genoud, M. (1997). Extraordinary lifespans in ants: a test of evolutionary theories of ageing. Nature *389*, 958–960.
9. Harvey, P.H., and Pagel, M.D. (1991). The Comparative Method in Evolutionary Biology (Oxford: Oxford University Press).
10. Reznick, D.N., Bryant, M.J., Roff, D., Ghalambor, C.K., and Ghalambor, D.E. (2004). Effect of extrinsic mortality on the evolution of senescence in guppies. Nature *431*, 1095–1099.
11. Abrams, P.A. (1993). Does increased mortality favor the evolution of more rapid senescence? Evolution *47*, 877–887.
12. Williams, P.D., and Day, T. (2003). Antagonistic pleiotropy, mortality source interactions, and the evolutionary theory of senescence. Evolution *57*, 1478–1488.
13. Caswell, H. (2007). Extrinsic mortality and the evolution of senescence. Trends Ecol. Evol. *22*, 173–174.
14. Moorad, J.A., and Promislow, D.E.L. (2008). A theory of age-dependent mutation and senescence. Genetics *179*, 2061–2073.

Department of Genetics, University of Georgia, Athens, GA 30602-7223, USA.
E-mail: jmoorad@uga.edu

DOI: 10.1016/j.cub.2010.03.016

Motor Proteins: Kinesin Drives with an Underhead Cam

A high-resolution cryo-EM structure of kinesin bound to its microtubule track allows for near-atomistic visualization of nucleotide-dependent conformational changes in this motor protein.

Matthew J. Lang[1] and Wonmuk Hwang[2]

As the smallest known motor protein that can walk processively, kinesin has been an important model system for understanding translocating motor proteins [1,2]. While many experimental, computational, and theoretical efforts have provided piecemeal information about kinesin motility, a clear atomistic-level picture of the process underlying the motility cycle is lacking. A key question is how ATP binding, hydrolysis, and product release control the motor head conformation and thereby the motility cycle. Although dozens of X-ray structures of kinesin are now available, far less is known about the structure of kinesin bound to its microtubule track. Kinesin's ATPase activity is known to be heavily influenced by the microtubule, and there are only a limited number of cryo-EM structures of the complex available [3–5]. Sindelar and Downing's new high-resolution (8–9 Å) cryo-EM structures of Kinesin-1 bound to the microtubule [6], together with their earlier work [5], provide native-like snapshots of the complex in various nucleotide-bound states: ADP-bound, no nucleotide, and ATP-analog-bound. Their findings indicate that the microtubule-bound motor head conformations are similar to those of the X-ray structures obtained in the absence of the microtubule, with the exception of the microtubule-binding domain and the nucleotide pocket. Although these results are consistent with previous cryo-EM studies of other members of the kinesin family [3,4,7], the higher resolution density maps reveal a vivid picture of nucleotide-dependent conformational changes of Kinesin-1, the 'conventional' kinesin.

Some important kinesin components include, from the amino terminus to the carboxyl terminus: the cover strand (β0), switch I and switch II loops, the microtubule-binding loop L11, the switch II helix (α4), α6, and the neck linker (β9 and β10) (Figure 1). Domains surrounding the switch I, II, L11, and the amino-terminal end of α4 process ATP binding and hydrolysis, while α4 and α6 control the behavior of the neck linker. The cover strand and the neck linker, respectively protruding from the amino and the carboxyl termini of the conserved motor head core, are involved in force generation.

The new work reveals that the motor action appears to be controlled in part through an 'underhead cam' region of the motor–track complex that is directly coupled to the power stroke (or crank shaft) motion that is responsible for kinesin stepping. In the nucleotide-free state, the switch I loop of the kinesin motor head is in the 'nucleotide-ejecting' conformation (Figure 1A, axial view). Binding of ATP (i.e. the ATP analog ADP·Al·F_x) leads to opening of the nucleotide pocket as the switch I loop changes to a tube-like conformation that Sindelar and Downing call the 'phosphate tube'. The retracted switch loops are stabilized in part by the amino-terminal end of the switch II helix α4, which interfaces with the microtubule and extends underneath the ATP pocket by several helical turns in all nucleotide states, a feature not observed in X-ray structures of kinesin in the absence of the microtubule (Figure 1A,B, hashed red region): note that the switch II helix corresponds to the relay helix in myosin [8].

Interestingly, the nucleotide-dependent conformations control the docking of kinesin's neck linker region, located near the carboxy-terminal end of the switch II helix [9,10], through a seesaw-like motion of the head. The seesaw action is not along the direction