How to learn new and interesting things from model systems based on "exotic" biological species

John M. Sedivy[1]

Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, 70 Ship Street, Providence, RI 02903

The famous quip of Monod that ". . . anything found to be true of *E*[*scherichia*] *coli* must also be true of elephants" reflects the widely held belief that the attention and resources of researchers are most effectively focused on a few wisely chosen "model systems" (1). Considerations such as short generation times, facility of genetic manipulation, and the economics of husbandry have strongly influenced these choices. The first half of the 20th century, dominated by studies of physiology and biochemistry, saw a relative diversity of model systems. In the modern era, the limitations imposed by the availability of precious molecular resources such as antibodies or gene clones (and until very recently, of whole genome sequences) have keenly sharpened the emphasis on a few "mainstream" model systems. For mammals, in addition to the obvious emphasis on human studies, the mouse has emerged as the premier model.

In this issue of PNAS Seluanov et al. (2) study naked mole rats and discover a new cancer resistance mechanism not yet documented in any other mammal. Naked mole rats (*Heterocephalus glaber*) are not rats at all, but hystricognath rodents more closely related to porcupines and guinea pigs than rats or mice. They are indigenous to east Africa where they lead subterranean lifestyles in extensive tunnel systems. They are almost completely sightless and hairless (Fig. 1 *Left*), although most other mole rats (there are some 22 species in the family *Bathyergidae*) are neither blind nor naked. In addition to their extreme adaptation to life underground, they initially attracted attention because of their eusocial behavior, having a single reproductively active "queen" female in a large colony of nonreproductive "workers" (the only other example of eusociality in all of mammalia is another mole rat, albeit a furry one).

More recently it came to light that *H. glaber* is the longest-lived rodent species on record (3, 4), with a maximum lifespan in captivity of >28 years. Such a long lifespan is especially striking given that naked mole rats have a body size very similar to mice, yet their longevity is seven times greater. Until the very last few years of their long lives these fascinating animals show no decline in fertility, age-associated acceleration of mortality (as seen in all other mammalian species examined to date), and only minimal age-related morphological and physiological changes.

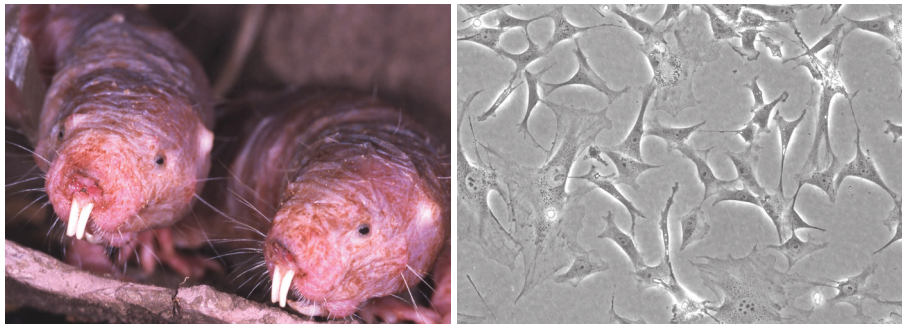

Fig. 1. Naked mole rats and their cells. (*Left*) Adult naked mole rats. (*Right*) Fibroblasts cultured from adult naked mole rat dermis in a state of early contact inhibition. A similar culture of mouse or human cells would continue to grow until the surface of the dish was completely covered with crowded cells. In contrast, the culture of naked mole rat cells shown here has completely ceased proliferation.

Also of considerable interest is the naked mole rat's remarkable resistance to cancer: not a single case of a spontaneously occurring neoplasm was documented in a large colony (>800 animals) observed over a period of many years (5). Their cancer incidence contrasts sharply with shorter-lived rodent such as mice or rats, whose predominant cause of death in captivity is cancer. Likewise humans, albeit much longer lived, sustain very significant cancer-caused deaths in advanced age.

In mammals the shortening of telomeres beyond a critical threshold triggers either cellular senescence or apoptosis and is believed to constitute a major tumor suppression mechanism (6). In humans, telomerase is expressed in early embryos, and with the exception of certain stem cells it is shut off in the vast majority of adult somatic cells (7). This situation seems to hold in primates and most large farm animals such as horses, cows, and sheep. Rodents and lagomorphs present a more complicated picture, with many species having very long telomeres that do not shorten significantly during their lifespans, not repressing telomerase in adulthood, or both (8, 9). These differences were first noted in laboratory mice (10), but are unlikely to have been caused by domestication, and recent studies of multiple species found that telomerase repression and in vitro replicative capacity correlate better with large body size than with long life span (9, 11).

Tissues of adult naked mole rats express telomerase, and cultures of fibroblasts proliferate continuously without any signs of replicative senescence (12). So how does this remarkable animal achieve both its impressive longevity and unprecedented cancer resistance? Either the naked mole rat's true longevity is much longer than 28 years (for example, their currently documented lifespans could be curtailed by unknown extrinsic mortality factors caused by husbandry conditions), or there is really something distinctive about their cancer resistance.

Seluanov et al. (2) put their finger on exactly such a novel mechanism. They started by examining the propensity of naked mole rat fibroblasts for malignant transformation in cell culture by using a combination of known oncoproteins, RAS* and SV40 virus large T antigen (SV LT). Not surprisingly, whereas mouse cells were easily transformed, naked mole rat cells failed to do so. Thus, similar to human cells, naked mole rat cells require more "hits" for transformation, but in the absence of the telomeric mechanism, what could these be? The initial answer came from simple observation of the growth properties of naked mole rat cultures: the cells appeared to be hypersensitive to

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[1]E-mail: john_sedivy@brown.edu.

*Encoded by *HRAS* in the human genome.

contact inhibition, which Seluanov et al. termed "early contact inhibition," a phenomenon that has not been reported in any other species. Although cultures of human and mouse fibroblasts (and all other species on record) maintain proliferation until the cells are in close contact throughout the culture dish (often referred to as "confluence"), naked mole rat cells ceased growth as soon as initial cell–cell contacts were made (Fig. 1 *Right*).

The rest of the article by Seluanov et al. (2) goes on to fill in some intriguing mechanistic details. Early contact inhibition requires the activities of both the p53[†] and retinoblastoma (pRB[‡]) tumor suppressor pathways and is overcome when both are abrogated (by introduction of SV LT). Abrogating either the p53 or pRB pathway alone did not bypass early contact inhibition, but instead strongly promoted apoptosis, suggesting a mechanism by which cells sustaining a single oncogenic mutation in either pathway could be eliminated.

Contact inhibition in human and mouse cells is mediated by the up-regulation of the cyclin-dependent kinase (Cdk) inhibitor $p27^{Kip1}$ ($p27^{§}$). Early contact inhibition in naked mole rats was, however, associated with the up-regulation of a different Cdk inhibitor, $p16^{Ink4a}$ ($p16^{¶}$). Cells that bypassed early contact inhibition, either by introducing SV LT or sustaining a spontaneous (and as yet uncharacterized) mutation, underwent "normal contact inhibition" upon reaching confluence that was associated with up-regulation of p27. Naked mole rats thus apparently have two independent contact inhibition mechanisms. Interestingly, the mutant cells incapable of early contact inhibition displayed anchorage-independent growth, an important milestone on the path to full transformation.

In human cells p16 is a key regulator of replicative senescence, which once triggered is believed to be irreversible. This pathway appears to be of relatively minor importance in the mouse, which relies more exclusively on the p53 pathway, and may in part explain the high cancer susceptibility of this species. The naked mole rat, like the mouse, does not appear to use telomere-initiated replicative senescence for tumor suppression, but instead appears to have co-opted p16 into a new and hitherto completely unique role.

In this context it is intriguing to note that p16 is found only in mammals, having evolved by gene duplication from a neighboring Cdk inhibitor, $p15^{Ink4b}$ ($p15^{‖}$) found in all other vertebrates (13). In chicken cells, p15 appears to play the role of regulating cellular senescence, whereas in the human, p16 having taken over the senescence function, p15 has evolved to an effector in the TGF-β pathway. Examination of mammalian genome databases indicates that p16 is evolving rapidly, to the point that very few antibodies raised against the human protein cross-react with the mouse protein (and vice versa). Extrapolating from the evidence from Seluanov et al. (2), it seems that the regulation of the gene encoding p16 is likewise capable of impressive evolutionary bursts.

Those interested in the biology of aging have recognized for some time a key limitation of short-lived model systems, namely, that these species may not even possess the mechanisms that have allowed long-lived species, such as humans, to evolve (14, 15). The naked mole rat, recently shown to be the longest-lived rodent, has already attracted considerable attention (5, 16). One obvious (although obviously not completely satisfactory) shortcut with long-lived species is to study their cells in culture. The work of Seluanov et al. (2) elegantly demonstrates the value of this approach and, having discovered a new regulatory mechanism that simply does not appear to exist in mice, amply underscores the fears of those who have argued for long-lived models. The fact that the discovery is more in the area of cancer biology than aging should add fuel to the argument that valuable lessons in many fields are likely to emerge.

How important is this discovery likely to be for understanding human cancer, given that our cells do not seem to possess the early contact inhibition mechanism? Mechanisms of extreme cancer resistance are not only inherently intriguing, but can arguably lead to important practical insights. The anticancer defenses of the naked mole rat are the result of natural evolution and appear to be incredibly effective. Remarkably, these mechanisms use only the "known players," such as pRB, p53, p16, p27, etc., only they have apparently been reshuffled in a hitherto unknown fashion. As we learn more about these mechanisms we may find out how to tweak the entire network to develop new prevention strategies.

In 2006 a group of scientists studying the biology of aging argued for sequencing the genomes of long-lived species, ultimately leading to several grant applications to sequence the naked mole rat genome. Unfortunately, the naked mole rat was viewed to be such an extreme "boutique" model system as to be of insufficient "general" interest. "We were basically told to wait until the costs came down to a point that we could sequence the genome on someone's R01," said Joao de Magalhaes, the lead author of the applications.

The situation is in some ways reminiscent of the old joke of the drunk looking for his keys under the street lamp. The currently mainstream biological model systems sure shine a powerful light, but the keys to some really interesting (and important) questions may simply not be found under it.

[†]Encoded by *TP53* in the human genome.

[‡]Encoded by *RB1* in the human genome.

[§]Encoded by *CDKN1B* in the human genome.

[¶]Encoded by *CDKN2A* in the human genome.

[‖]Encoded by *CDKN2B* in the human genome.

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