Higher gene expression stability during aging in long-lived giant molerats than in short-lived rats

Arne Sahm¹, Martin Bens¹, Yoshiyuki Henning^{2,3}, Christiane Vole², Marco Groth¹, Matthias Schwab⁴, Steve Hoffmann¹, Matthias Platzer^{1,*}, Karol Szafranski^{1,*}, Philip Dammann^{2,5,*}

¹Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany
 ²Department of General Zoology, Faculty of Biology, University of Duisburg-Essen, Essen, Germany
 ³Institute of Physiology, University of Duisburg-Essen, 45147 Essen, Germany
 ⁴Department of Neurology, Jena University Hospital-Friedrich Schiller University, Jena, Germany
 ⁵University Hospital, Central Animal Laboratory, University of Duisburg-Essen, Essen, Germany
 *Shared senior authorship

Correspondence to: Arne Sahm; email: arne.sahm@leibniz-fli.deKeywords: Fukomys, Bathyergidae, differential gene expression, longevity, collagenReceived: July 27, 2018Accepted: November 22, 2018Published: December 16, 2018

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ABSTRACT

Many aging-associated physiological changes are known to occur in short- and long-lived species with different trajectories. Emerging evidence suggests that numerous life history trait differences between species are based on interspecies variations in gene expression. Little information is available, however, about differences in transcriptome changes during aging between mammals with diverging lifespans. For this reason, we studied the transcriptomes of five tissue types and two age cohorts of two similarly sized rodent species with very different lifespans: laboratory rats (*Rattus norvegicus*) and giant mole-rats (*Fukomys mechowii*), with maximum lifespans of 3.8 and more than 20 years, respectively. Our findings show that giant mole-rats exhibit higher gene expression stability during aging than rats. Although well-known aging signatures were detected in all tissue types of rats, they were found in only one tissue type of giant mole-rats. Furthermore, many differentially expressed genes that were found in both species were regulated in opposite directions during aging. This suggests that expression changes which cause aging in short-lived species are counteracted in long-lived species. Taken together, we conclude that expression stability in giant mole rats (and potentially in African mole-rats in general) may be one key factor for their long and healthy life.

INTRODUCTION

Compared to short-lived mammals, long-lived mammals have repeatedly been shown to exhibit fewer age-associated changes in numerous physiological parameters related to the functional decline during aging [1-4]. Recent RNA-seq studies have suggested that much of the remarkable lifespan diversity among mammals is based on interspecies differences in gene expression [5, 6]. However, those studies focused on identifying particular genes and pathways that are

differentially expressed between species with divergent longevities. Whether short- and long-lived species differ at the transcript level with respect to their amount of differentially expressed genes (DEGs) during aging (hereinafter referred to as "gene expression stability") has, to the best of our knowledge, not been explored yet.

Here, we examined age associated transcriptome changes in two similarly sized rodent species with different longevities: the laboratory rat (*Rattus norvegicus*), which has a maximum lifespan of 3.8 years



Figure 1. Comparative transcriptomics in giant mole-rats and laboratory rats of elderly vs. young individuals. (A) Counts of differentially expressed genes (DEGs) during aging in five tissue types from laboratory rats (*Rattus norvegicus*) and giant mole-rat (*Fukomys mechowii*). Only orthologous genes in both transcript catalogs were counted (n=14,062). (B) Numbers of biological processes (Gene Ontology) enriched for DEGs during aging in five tissue types from laboratory rats (*R. norvegicus*) and giant mole-rats (*F. mechowii*).

[7], and the giant mole-rat (Fukomys mechowii), which has a maximum lifespan of more than 20 years ([8] and own unpublished data). In giant mole-rats, longevity is significantly correlated with the reproductive status. Breeding animals outlive non-breeders by far [8]. In the current study, we examined only non-breeding males. Male non-breeding giant mole-rats have a maximum lifespan of approximately 10 years and an average lifespan of approximately 6 years, still clearly exceeding the life expectancy of the laboratory rat [8]. For both species, we performed RNA-seq on tissue samples from five organs (blood, heart, kidney, liver, and skin; hereinafter called simply tissues) of young and elderly adults. The tissues were collected from young and elderly cohorts of laboratory rats (0.5 and 2.0 years) and giant mole-rats (young: approximately 1.5 years at average; elderly: approximately 6.8 years at average; see Tables S1-S3 for details). For both species, the first time points were chosen to sample young, sexually mature adults. The second time points correspond to an age-associated survival rate of less than 40% in rats and giant mole-rats (Tables S1-S3) [8, 9]. For each species, we determined DEGs between the two respective time points and searched for enriched functional categories.

RESULTS

The giant mole-rat transcriptomes changed much less during aging (Tables S4-S13). In four of five tissue types, the number of orthologous DEGs in the giant mole-rats was only a fraction of the respective number in the laboratory rats (0.6%-19.0\%; Fig. 1a). The number of DEGs was similar only in the blood of both species but still was 40% lower in blood from the giant mole-rats than in blood from the laboratory rats. Across tissues, the giant mole-rat transcriptomes contained significantly fewer DEGs during aging than did the laboratory rats (P = 0.016, Wilcoxon signed-rank test).

To ensure that the low number of identified DEGs in the giant mole-rat was not caused by a low statistical power compared to the rat, we examined the statistical power



Figure 2. REVIGO treemap summary of gene ontology processes that are significantly enriched (false discovery rate [FDR] < 0.05) for differentially expressed genes during aging. For each species and tissue, the superclusters, *i.e.*, the highest summarization level of gene ontology processes, as identified by REVIGO [13] are shown. Each rectangle painted with a unique color represents a supercluster. The colors only serve to distinguish superclusters. The size of the rectangles represents their p-value, *i.e.*, largest rectangles represent the most significant superclusters. For giant mole-rat skin, no treemap could be generated since no gene ontology process was significantly enriched (Fig. 1b). Corresponding REVIGO treemap summarizations are provided as high-resolution Figures S1-S9, showing also the clusters within the superclusters.

per species and tissue using the RNA-seq data dispersion [10]. We estimated a statistical power of 83-95% and 80-90% in giant mole-rat and rat, respectively, depending on the tissue (Table S14). Thus, this finding corroborates our evidence that the lower number of detected DEGs in the giant mole-rat might indeed reflect a greater expression stability during aging. Furthermore, we ensured that there was no relevant difference in the measured gene expression levels between the examined species (Fig. S10, all DEGs). Those genes, however, that were found to be differentially expressed in both species (Fig. S10, overlapping DEGs), tended to be lower expressed in the rat and higher expressed in the giant mole-rat, compared to median across all DEGs.

Upon Gene Ontology [11] analysis of the differentially expressed genes, we found typical molecular aging signatures across all examined tissues in the rat (Fig. 1b). For instance, altered expression levels of immune response genes (Gene Ontology [GO]:0006955; Tables S15-S24) and inflammatory response genes (GO:0006954) are known to be hallmarks of aging [12]. These, as well as many related processes, such as response to cytokine (GO:0034097) and leukocyte aggregation (GO:0070486), were consistently enriched for DEGs in all examined laboratory rat tissues. In the giant mole-rat, on the other hand, we found these signatures only in blood.

The clustering of DEG enriched biological processes with REVIGO [13] revealed that immune-related functions, immune process or regulation of immune process, determine the largest superclusters in four of five rat tissues (Fig. 2, Fig. S1-S9). Additional agingrelevant clusters found across rat tissues were apoptotic process (GO:0006915; all tissues except heart), coagulation (GO:0050817; all tissues) and oxidationreduction process (GO:0055114; all tissues except liver). Except in blood, the giant mole-rat did not exhibit the same (or similar) DEG enriched biological processes. These findings indicate typical agingdependent gene expression alterations are slowed down in several vital tissues of giant mole-rats.

On the single gene level, there was a modest but still statistically significant (P < 0.05; Fisher's exact test) overlap between the DEGs of laboratory rats and those of giant mole-rats in blood, heart, and skin tissues (Fig. 1a; Tables S25-S29). Common DEGs in the blood of laboratory rat and giant mole-rat were often regulated in the same direction (up or down) during aging in both species ($P = 3.3*10^{-31}$; Fisher's exact test based on regulation of all genes). This finding matches the shared aging signatures (DEG function analysis, see above) in this tissue. Interestingly, in skin samples we found a contrasting overrepresentation of DEGs, which are regulated in opposite directions (P = 0.005). This finding points to the intriguing possibility that, in some tissues, expression changes that cause aging in the laboratory rat are counteracted by opposite changes during aging in the giant mole-rat.

In kidney tissues, most shared DEGs were regulated in opposite directions between species during aging (Fig. 1a). As an example, collagen metabolic process (GO:0032963) is one of the seven processes that are enriched in the kidneys of both laboratory rats and giant mole-rats. Although the enrichment in the laboratory rat was based on 20 collagen genes that were significantly up-regulated and one that was down-regulated during aging, in the giant mole-rat this enrichment resulted from four collagens and two genes that code for potent collagenases (*CTSK* and *CTSS*), all of which were down-regulated during aging. Of these six collagenases and collagens, five overlapped with those, which were significantly up-regulated in laboratory rats. Collagen regulation reflects well the molecular aging process because decreasing collagen levels attenuate kidney diseases in rats [14], whereas increased collagen levels in the kidney have been shown to induce the development of cysts in rats with polycystic kidney disease [15]. At the same time, kidney diseases are an important cause of death in rats [16] and perhaps also in (naked) mole-rats [17]. The opposite collagen regulation pattern in the giant mole-rat can be interpreted as an anti-aging program rather than as a signature of the aging process.

DISCUSSION

The gene expression stability of giant mole-rats during aging that we show here concurs with a general pattern of stability. This has emerged from numerous molecular and physiological comparisons of the extremely longlived naked mole-rat (Heterocephalus glaber, a close relative of giant mole-rats) with shorter-lived species of mice or rats: For example, during aging, naked molerats maintain an unchanged membrane lipid composition [3], a fairly stable production of reactive oxygen species [18], and relatively stable levels of oxidative damage to lipids [2], as well as high protein stability and integrity [19]. At the same time, all of these variables, which are known to be among the key factors for lifespan and age-related diseases [20], change significantly in an unfavorable direction during aging in short-lived mice or rats. Naked mole-rats also exhibit minimal decline of physiological functions and maintain activity, fertility, and body composition into old age; they are also remarkably resistant to cancer, and their cancer-associated mortality rates do not increase substantially with age [1]. Given that nakedand giant mole-rats are closely related [21], our own husbandry experience with giant mole-rats leads us to assume that several of the aforementioned properties are shared by both species.

In line with our results, an earlier study showed that gene expression in three types of tissue from naked mole-rats remains nearly unchanged during the first half of their lifespan [22]. However, the statistical power of this analysis was very limited because the study used only one replicate per age. Regarding laboratory rats, our results are in good agreement with the findings of the rat body map initiative [23]. This database shows many DEGs (491 to 14,062) across eleven types of tissue during rat aging; the time points used in this study are similar to ours (21 weeks vs. 2 years). The results of Kim et al. [22] and of the rat body map project cannot be directly compared with each other because those studies used different methods for sequencing and DEG detection. Therefore, in this study both species were examined with the same sequencing procedure and the same bioinformatic analyses. Thus, we confirmed that, the gene expression of a long-lived African mole-rat species - in contrast to those of a short-lived rodent indeed remains stable during aging from young to a elderly adulthood. Since gene expression is a basic regulatory process of the cell that determines many of the above-mentioned molecular phenotypes and physiological observations, we suggest that gene expression stability during aging is one of the key causal factors for the extraordinary long and healthy lifespan of this African mole-rat species, and potentially of the whole family.

In conclusion, we hypothesize that the higher gene expression stability observed in long-lived giant molerats compared to short-lived rats evolved under different evolutionary constraints and contributes to the considerably distinct life history traits of the short- and long-lived species: early onset and fast aging in one species, and delayed or slowed aging from youth to elderly adulthood in the other.

MATERIALS AND METHODS

Experimental design

This study compared the transcriptomes of young and elderly animals from two species: Wistar rats (Rattus norvegicus) and giant mole-rats (Fukomys mechowii). Samples from five tissues (blood, heart, kidney, liver, and skin) were taken from animals in both species and both age cohorts. All examined animals were nonbreeding males. Young and elderly laboratory rats were 6 and 24 months of age, respectively, and sampled in April, October and November 2016 (see table S1 for details). Library preparation and sequencing was performed for all but three rat samples in one batch in December 2016 - the remaining three were sequenced in January 2017 (table S3). Young mole-rats were 1.3 to 2.0 years old (grand mean across tissues: 1.5 years). whereas elderly mole-rats were 5.5 to 7.7 years old (grand mean across tissues: 6.8 years). Mole-rats were sampled in 5 distinct sampling sessions between February 2014 and December 2016 (table S1). Sequencing of mole-rat samples was performed in 7 runs across the same time frame (table S3).

We examined samples from 4 to 8 animals per tissue for each age cohort and species (Tables S1-S3). All animals were healthy at the time when they were sacrificed.

For tissue collection, rats were euthanized with CO₂. Mole-rats were anaesthetized with 6 mg/kg ketamine combined with 2.5 mg/kg xylazine and then euthanized by surgical decapitation. Immediately after dissection, tissue samples were transferred to tubes containing RNA-protective buffers and stored in -80°C until analysis.

For both species, the first age group consists of young, sexually mature adults. Their age was approximately one-fourth of the second group's age, which corresponds to a survival fraction of approximately 39% and 24% in rats and giant mole-rats, respectively (Tables S1-S3) [8, 9]. In relation to maximum lifespan the median age at the second time point represents 53% in rats (maximum lifespan in male Rattus norvegicus: 3.8 years) and 68% (maximum lifespan in Fukomys mechowii non-breeders: 10 years) [7, 8]. Thus, the chosen time points represent similar biological ages in the examined species with a wider age-range between the compared time points in giant mole-rats. The latter means that the observed smaller age-related changes of the transcriptomes in giant mole-rats compared to rats are conservative findings.

Animal housing and tissue collection was compliant with national and state legislation (breeding allowances 32-2-1180-71/328 (mole-rats) and 32-2-11-80-71/345 (rats), both Ordnungsamt Essen, Northrhine-Westfalia, Germany).

Transcript catalogue sequences

The giant mole-rat transcript catalog was assembled and annotated with human gene symbols on the basis of recently published read data [24] (European Nucleotide Archive [ENA] study PRJEB20584) and the assembly framework FRAMA [25] with default parameters. For laboratory rats, mRNA sequences were obtained from NCBI RefSeq. Ortholog relations between rat and human genes were downloaded from Ensembl Biomart. For both species, only the longest transcript isoform per gene was used, which is the method of choice for selecting a representative variant in large-scale experiments [26]. This resulted in 15,864 reference transcripts (genes) for the giant mole-rats and 23,479 reference transcripts (genes) for the laboratory rats of which 14,062 reference transcripts (genes) were annotated with the same human gene symbol.

RNA-seq, read mapping and quantification

Tissue samples were collected and stored in RNAlater (Qiagen, Venlo, Netherlands) after isolation. For all tissues except blood, RNA was purified with the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Blood samples (100 μ l) were collected in RNAprotect Animal Blood reagent (Qiagen). The resulting RNA was purified with the RNeasy Protect Animal Blood Kit (Qiagen). Kidney

and heart samples were treated with proteinase K before extraction, as recommended by the manufacturer. Poly(A) selection and preparation of the RNA-seq libraries was performed with the TruSeq RNA v2 kit (Illumina, San Diego, USA). RNA-seq was performed by single-end sequencing with 51 base pairs on a HiSeq 2500 sequencing system (Illumina) and with at least 17 million reads per sample, as described in Table S3. The reads were aligned to the respective reference – rat or giant mole-rat (see above) – with the BWA aln algorithm of the Burrows-Wheeler Aligner (BWA) [27], allowing no gaps and a maximum of two mismatches in the alignment. Only those reads that could be uniquely mapped to the respective gene were used for quantification.

Read data for rats and giant mole-rats were deposited as ENA study PRJEB23955 (Table S3). Read counts per gene and sample can be found in Tables S30 and S31.

Method validation

To ensure the reliability of our RNA-seq results we determined pairwise Pearson correlation coefficients between all rat and all giant mole-rat samples, respectively, based on log-transformed read counts that were normalized for sample size (Tables S32, S33). For each species and tissue, we calculated the means and standard deviations (Table S34). The grand mean of the determined correlation coefficients across tissues was 0.96 and 0.97 for rat and giant mole-rat, respectively, and the mean standard deviation across tissues 0.02 for both species.

Furthermore, we estimated the statistical power of DESeq2 [28] based on the respective dispersion in our complete rat and giant mole-rat data sets, respectively, using the method of Ching et al. [10] and 10 simulation runs per species and tissue (Table S14).

Differential expression analysis

Differential expression analysis was performed with DeSeq2 [28]. In both species, the elderly animals were compared with their young conspecifics. Genes that showed a comparison p-value less than 0.05 after Benjamini-Hochberg correction for multiple testing were considered as DEGs. Initial numbers of DEGs per tissue and species were as follows: 4033 and 2002 (blood), 1506 and 227 (heart), 5015 and 57 (kidney), 635 and 94 (liver), 3231 and 18 (skin) DEGs were identified in rat and giant mole-rat, respectively (Tables S4-S13).

To acquire comparable numbers of DEGs and to determine the amount of DEGs that were found in both

species (overlap), only those genes were taken into account that were present in the transcript catalogs of both species based on human gene symbol annotation (Fig. 1a, n=14,062). The giant mole-rat transcript catalog was annotated against human (see above). Ortholog relations between rat and human were downloaded from Ensembl Biomart.

Biological processes that were enriched for DEGs were determined in both examined species by using the human gene symbol annotation of the DEGs (see above), their human gene ontology annotation (GO; annotation package: org.Hs.eg.db) and Fisher's exact test. The Benjamini-Hochberg method was used to correct the resulting *p*-values for multiple testing. Additionally, GO categories with a *p*-value of less than 0.05 after corrections for multiple testing were summarized with REVIGO (cutoff, 0.70; measure, SimRel; database, whole Uniprot) [13] (Fig. 2, Fig. S1-S9).

Abbreviations

DEG: differentially expressed gene; GO: gene ontology.

AUTHOR CONTRIBUTIONS

AS performed the sampling, the gene expression analysis and wrote the first draft of the paper. MB performed transcriptome assemblies. YH and CV performed giant mole-rat caretaking and sampling. MG oversaw the sequencing. MS oversaw rat caretaking and sampling. SH oversaw the statistics. MP acquired funding, wrote the first draft of the paper and supervised the project. KS performed the sampling, wrote the first draft of the paper and supervised the project. PD acquired funding, wrote the first draft of the paper, oversaw giant mole-rat caretaking, performed sampling and supervised the project. All authors have read and improved the first draft of the paper.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Edrey YH, Hanes M, Pinto M, Mele J, Buffenstein R. Successful aging and sustained good health in the naked mole rat: a long-lived mammalian model for biogerontology and biomedical research. ILAR J. 2011; 52:41–53. https://doi.org/10.1093/ilar.52.1.41
- Andziak B, Buffenstein R. Disparate patterns of agerelated changes in lipid peroxidation in long-lived naked mole-rats and shorter-lived mice. Aging Cell. 2006; 5:525–32. https://doi.org/10.1111/j.1474-9726.2006.00246.x
- Hulbert AJ, Faulks SC, Buffenstein R. Oxidationresistant membrane phospholipids can explain longevity differences among the longest-living rodents and similarly-sized mice. J Gerontol A Biol Sci Med Sci. 2006; 61:1009–18. https://doi.org/10.1093/gerona/61.10.1009
- Dammann P. Slow aging in mammals-Lessons from African mole-rats and bats. Semin Cell Dev Biol. 2017; 70:154–63. https://doi.org/10.1016/j.semcdb.2017.07.006
- Fushan AA, Turanov AA, Lee SG, Kim EB, Lobanov AV, Yim SH, Buffenstein R, Lee SR, Chang KT, Rhee H, Kim JS, Yang KS, Gladyshev VN. Gene expression defines natural changes in mammalian lifespan. Aging Cell. 2015; 14:352–65. https://doi.org/10.1111/acel.12283
- Malik A, Domankevich V, Lijuan H, Xiaodong F, Korol A, Avivi A, Shams I. Genome maintenance and bioenergetics of the long-lived hypoxia-tolerant and cancer-resistant blind mole rat, Spalax: a crossspecies analysis of brain transcriptome. Sci Rep. 2016; 6:38624. https://doi.org/10.1038/srep38624
- Tacutu R, Craig T, Budovsky A, Wuttke D, Lehmann G, Taranukha D, Costa J, Fraifeld VE, de Magalhães JP. Human Ageing Genomic Resources: integrated databases and tools for the biology and genetics of ageing. Nucleic Acids Res. 2013; 41:D1027–33. https://doi.org/10.1093/nar/gks1155
- Dammann P, Šumbera R, Massmann C, Scherag A, Burda H. Extended longevity of reproductives appears to be common in Fukomys mole-rats (Rodentia, Bathyergidae). PLoS One. 2011; 6:e18757. https://doi.org/10.1371/journal.pone.0018757
- 9. Carlus M, Elies L, Fouque MC, Maliver P, Schorsch F. Historical control data of neoplastic lesions in the

Wistar Hannover Rat among eight 2-year carcinogenicity studies. Exp Toxicol Pathol. 2013; 65:243–53.

https://doi.org/10.1016/j.etp.2011.08.013

- Ching T, Huang S, Garmire LX. Power analysis and sample size estimation for RNA-Seq differential expression. RNA. 2014; 20:1684–96. https://doi.org/10.1261/rna.046011.114
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, et al, and The Gene Ontology Consortium. Gene ontology: tool for the unification of biology. Nat Genet. 2000; 25:25–29. https://doi.org/10.1038/75556
- de Magalhães JP, Curado J, Church GM. Metaanalysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics. 2009; 25:875–81. https://doi.org/10.1093/bioinformatics/btp073
- Supek F, Bošnjak M, Škunca N, Šmuc T. REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One. 2011; 6:e21800. https://doi.org/10.1371/journal.pone.0021800
- Liu B, Li C, Liu Z, Dai Z, Tao Y. Increasing extracellular matrix collagen level and MMP activity induces cyst development in polycystic kidney disease. BMC Nephrol. 2012; 13:109. https://doi.org/10.1186/1471-2369-13-109
- Gilbert RE, Zhang Y, Williams SJ, Zammit SC, Stapleton DI, Cox AJ, Krum H, Langham R, Kelly DJ. A purpose-synthesised anti-fibrotic agent attenuates experimental kidney diseases in the rat. PLoS One. 2012; 7:e47160. https://doi.org/10.1371/journal.pone.0047160
- Ettlin RA, Stirnimann P, Prentice DE. Causes of death in rodent toxicity and carcinogenicity studies. Toxicol Pathol. 1994; 22:165–78. https://doi.org/10.1177/019262339402200210
- Delaney MA, Kinsel MJ, Treuting PM. Renal pathology in a nontraditional aging model: the naked mole-rat (Heterocephalus glaber). Vet Pathol. 2016; 53:493–503.

https://doi.org/10.1177/0300985815612557

- Csiszar A, Labinskyy N, Orosz Z, Xiangmin Z, Buffenstein R, Ungvari Z. Vascular aging in the longest-living rodent, the naked mole rat. Am J Physiol Heart Circ Physiol. 2007; 293:H919–27. https://doi.org/10.1152/ajpheart.01287.2006
- Pérez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, Andziak B, Yang T, Edrey Y, Friguet B, Ward W, Richardson A, Chaudhuri A.

Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proc Natl Acad Sci USA. 2009; 106:3059–64. https://doi.org/10.1073/pnas.0809620106

- López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153:1194–217. https://doi.org/10.1016/j.cell.2013.05.039
- Faulkes CG, Bennett NC. Plasticity and constraints on social evolution in African mole-rats: ultimate and proximate factors. Philos Trans R Soc Lond B Biol Sci. 2013; 368:20120347. https://doi.org/10.1098/rstb.2012.0347
- 22. Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV, Han L, Marino SM, Sun X, Turanov AA, Yang P, Yim SH, Zhao X, Kasaikina MV, et al. Genome sequencing reveals insights into physiology and longevity of the naked mole rat. Nature. 2011; 479:223–27. https://doi.org/10.1038/nature10533
- Yu C, Li Y, Holmes A, Szafranski K, Faulkes CG, Coen CW, Buffenstein R, Platzer M, de Magalhães JP, Church GM. RNA sequencing reveals differential expression of mitochondrial and oxidation reduction genes in the long-lived naked mole-rat when compared to mice. PLoS One. 2011; 6:e26729. https://doi.org/10.1371/journal.pone.0026729

- Sahm A, Bens M, Szafranski K, Holtze S, Groth M, Görlach M, Calkhoven C, Müller C, Schwab M, Kestler HA, Cellerino A, Burda H, Hildebrandt TB, et al. Long-lived rodents reveal signatures of positive selection in genes associated with lifespan and eusociality. PLoS Genet. 2018; 14:e1007272. https://doi.org/10.1371/journal.pgen.1007272
- Bens M, Sahm A, Groth M, Jahn N, Morhart M, Holtze S, Hildebrandt TB, Platzer M, Szafranski K. FRAMA: from RNA-seq data to annotated mRNA assemblies. BMC Genomics. 2016; 17:54. https://doi.org/10.1186/s12864-015-2349-8
- Ezkurdia I, Rodriguez JM, Carrillo-de Santa Pau E, Vázquez J, Valencia A, Tress ML. Most highly expressed protein-coding genes have a single dominant isoform. J Proteome Res. 2015; 14:1880– 87. https://doi.org/10.1021/pr501286b
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25:1754–60. https://doi.org/10.1093/bioinformatics/btp324
- 28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014; 15(12):550. https://doi.org/10.1186/s13059-014-0550-8

SUPPLEMENTARY MATERIAL

Please browse the links in Full Text version of this manuscript to see Supplementary Tables.

Table S1. Overview of examined animals.

Table S2. Mean ages in years (with standard deviations) of examined animals by species, age cohort and tissue.

 Table S3. Samples that were sequenced in this study.

Tables S4-S13. Result of DESeq2-analysis for differentially expressed genes during aging in laboratory rats and giant mole-rats (one table per species and tissue).

Table S14. Mean estimated power per tissue and species using the method of Ching et al., 2014 and 10 simulations runs.

Tables S15-S24. Biological process gene ontologies that are enriched for differentially expressed genes (DEGs) (false discovery rate [FDR] < 0.05) in laboratory rats and giant mole-rats (one table per species and tissue).

Tables S25-S29. Overlap of genes that are differentially expressed in laboratory rats and naked mole-rat blood (one table per tissue).

 Table S30/S31. Read counts per sample and gene in rats/giant mole-rats.

Table S32/S33. Pairwise Pearson correlation coefficients of rat/giant mole-rat samples based on normalized and logarithmized gene counts.

Table S34. Means and standard deviations of pairwise Pearson correlation coefficients between samples based on normalized and logarithmized gene counts.

Supplementary figures

Figures S1-S9. REVIGO treemaps of gene ontology processes that are significantly enriched (false discovery rate [FDR] < 0.05) one figure for tissue and species; figure for giant mole-rat skin is missing because the number of enriched terms was too small for summarizing).

Figure S10. Expression levels of examined genes.

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amide biosynthetic		anslaționa processi	initiation initiation: cycl ng compound catabolic	c regulation of biological	cellular protein modificatior	process cellular	organonitrogen	Iribonucleoside	electi transp	compound	macromolecula complex assembly	protei	x subunit o assem	bly assembly		ition of production
process	cellular macromolecule catabolic process		ation positive abolic regulation o	cytokine metabolic	process organic substance	macromolecule	metabolic process	process	cha	in process	cellular	comple biogene mitochon	sis comple biogene drial regula	x I assembly	regulatio cell dea	
organonitrogen compound biosynthetic	posttranscriptional regulation of	mRNA metabol	biological process ic gene	oellular nitrogen compound	biosyntheti process IRES-dependen translational	negative regulation of	cell activation	single-organism metabolic process	naladasa serta a yesi neksin naladir persa	mitochondrial ATP synthesis coupled proton transport	biogenesis	translatio elongati	Compo	nent disassem		lion
process	gene expression response to s		response to		initiation ie to	c receptor signaling pathway	intracellular transpor	cellular t localization		esicle-mediated transport	primary metabolic process primary me phosphorus	organic	immune proc		cellular component organization or biogenesis	nitrogen compound metabolism
ininune response			regulation	cellular	response to endoplasmi		establishment	cellular transport		organic substance	metabolic process	substance metabolic process			multi-organi	
	biotic stime		to stimulus to stress		reticulum stress production of	degranulation	protein localization	single-organ		transport			macroautophagy	metabolic metabolism process	catabolic catabolism process	cell-cell adhesion
defense response	regulation	of	organic kina substance		molecular mediator involve in inflammator response	activation	macromolecule	localization		endocytosis	interspecies i between or	ganisms			macromolecule metabolism	biosynthetic biosynthesis process
	defense resp	l intracellular		regulation of I-kappaB kinase/NF-kappaE signaling	external stimulus	to wounding	localization	phagocytosis		single-organism transport			autophagy	localization	biological adhesion	generation of precursor metabolites and energy

Figure S1. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat blood. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

					J.										
regulation of immune respon				response to stress	single-organism metabolic proces		organic acid metabolic process				tion-reduction process	fatty acid metabolic process	phosphorus metabolic process	proteolysis	protein phosphorylation
protein activation		response to anic substance blood coagulation		coagulation					acid metabolic		atory burst	hosphatklykthanolamine biosynthe6c process	protein phosphate-containing compound	phosphorylation protein metabolic process	and the Real Processing of the Procesing of the Processing of the Processing of the Processing of the
cascade	acute	regulation	positive regulation	of regulation	single-organism catabolic process		triglycerie	ide sm	small molecule biosynthetic			arboxylic acid biosynthetic process	metabolic process	regulation of cellular protein metabolic process	regulation of mitochondrial translation
response to wounding	inflammator	y of respons	e Rho protei	n of body fluid levels	small molecule	-			process bile acid	nucleol	base bile ad	id lipid	mitochondrial respiratory chain complex	enase of cellular	
cholesterol	ER-associated ubiquitin-dependent protein catabolic process	positive regulation of cellular component	regulation of molecular function	inflammatory response	metabolic proces	~	rine nucle tabolic pro		metabolic process		olic biosynth Proce	ss process	amitochondrial r mitochain complex respiratory chain complex		localization
homeostasis		organization intracellular	negative regulation	of			sin	ngle-organ	nism macror	nolecule	cellular	intracellular		rganization	
membrane depolarization	antigen processing and presentation of	signal transduction	mesenchymal to epithelial transition involved in metaneph morphogenesis	_{ros} organophosphorus	transport	vesicle-med transpor	Giated	localization			localization	transport	organonitrogen ammoni	proteinriorum	
during cardiac muscle cell action potential	peptide antigen via MHC class I	regulation of catalytic activi		y midbrain dopaminergic neuron			vesicle	-mediated transport	transport	otein lization	organic		process proces	rotioulum	folding
defense response	positive regulation of homeostatic process	response to external stimulus	assembly circulatory system proce	ss differentiation regulation of anatomical structure size	endocytosis	regulation endocyto		fatty acid transport		arboxylic ransport	regu	t localization Ilation of nigration	catabolism	immune system process	beta-amyloid metabolism

Figure S2. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat heart. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

immune response		defense re	sponse	cell surfa signalir	ace rece ng pathw		cell activation	on	cell m	igration	extrace mat organia	rix	extracellular structure organization	cytokine p	roduction		lation of productio	n ce	I adhesion	Tat
regulation of inflamm. response to stimulus			, , , , , , , , , , , , , , , , , , , ,		cellular response to chemical		vesicle-mediated transport	trans	transport endocytos		sis of loc	blishmen protein alization	t fatty acid oxidation	multicellu organism	multicellular coag organismal process		production igulation developments process		e system pro	ocess
					stimulu	s	movement of cell or				nism exc	gulated cytosis	of cellular component organization	blood vessel met		agen multicellular abolic organism metabolic process process				
response to stress	im response t biotic stimul	1.00000	nse to	positive regulation of response	regula of c		subcellular component	phago	cytosis	organi substan	e	acid m	netabolic ocess	n		program containing compared	phosphorus metabolic		esponse to	
				to external communica stimulus		nication	localization of cell			transpo macromole	modifica		platelet degranulation	biological adhesi		protein phosphorylation protein		stimulus	signa	aling
intracellular signal	cellular respo to stimulus		nse to ding	mediated signal	respon axygen-a compi	ontaining	positive	cello		localizatio	maun	rocess positi	ive			phosphorylation				
transduction		respor	ponse to transduc		Ras protein signal transduction by protein		regulation of biological	r	positive egulatio molecul function	n of a	apoptotic rocess	activi	dase process	single-organism	single-organisi metabolic	loca	lization	locomo		organism ocess
response to	regulation of		p	tigen processing and esentation of peptide or polysaccharide tigen via MHC class II	erzyme Erked	rosphorylation	regulation of				ogical p ological quality	fluid le	edy regulation of biological process	celular process cell proli	0000000		1		developmenta process	1
external stimulus	signaling	wound heal		MAPK cascade	IAPK poster signaling		molecular functi		ocalization inor		ganic io neostasi	LINUX DAY	-spectic of actin ding n tector filament-base			cell communicati n		proliferation bio	biological regulation	and the second

Figure S3. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat kidney. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

													negative	regulation of	,
response to external stimulus	respons purine-cor compo	ntaining	g response to chemical		oonse to rtokine	platelet degranulation	transport	vesicle-mediated transport	cell deat	h ce	II activation	coagulation	regulation of multicellular organismal process	endodermal cell differentiation	muscle adaptation
							secretion		regulation		egulation of		-coagulation	negative	regulation
response to	p response to regulation o			defense	e response	a di sala sala s		endocytosis	phagocyte	Ves	icle-mediated transport	energy homeostasis	blood circulation	regulation of developmenta process	of multicellular organismal
organic substance	organophospl	horus	nal stimulus		response	cell migration	localization of cell	lamellipodium assembly	single-organism transport		fatty acid transport				protein
protein activation	ctivation response response to respon		other orga	e to nism i	sponse to norganic ubstance	secretion by cell	actin filament-based	actin cytoskeletor	single-org		mellipodium rganization	single-organism metabolic process	lipid meta proces	s 📘	protein
Cascade					ubstance		process					lipid me monocarboxylic acid		anism	naturation
	response to	regulation	hyperosr		cellular sponse to	positive regulation	regulation of	regulation of	regulation		ell volume	metabolic process	catabolic p		eactive gardina and a set of the
immune response	endogenous stimulus	of respons to stimulus	respon	se m	echanical stimulus	of heterotypic cell-cell adhesio	n hydrolase activit	ly hormone levels	body fluid I	evels he	omeostasis	movement of cell or subcellular component	single-org biosynth proces	etic	
wound healing	response to oxygen-containing compound	response to mechanical stimulus	response carbohydr	to reg ate ERK	positive gulation of 1 and ERK2 cascade	regulation o ^{pc} hemostasis	equilation of apoptotic proces	heterotypic cell s homeostasis	negative -cell adhes ATPase act	ion by	lipid omeostasis	localization	response to stimulus	reactive oxygen species	leukocyte
response to metal ion	response to biotic stimulus	response to low-density lipoprotein particle	inflammatory response	response to netformir	of response	regulation of biological quality	positive regulation of steroid biosynthetic process	steroid metabolic process	regulation of reactive oxygen species metabolic process	regulation of localizatio	motabolio	locomotion	biological adhesion	retabolis FasL bi	

Figure S4. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat liver. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

									II molecule polic proce	•	xtracellul organiz		localization of cell	endocyt	of ce comp		regulation of hydrolase activity	e regulation of biological quality
immune respons	e	defense respo	onse	regulation of defense response		single–organi metabolic proc	metabolic process		activation	str	extracellular structure organization		single-org localizatio transport		of cellf si musi	cle cell	process	tion of e activity chemical
													phagocytosis	single-organism localization	regulation of localization	cell of	egulation leukocyte oliferation	homeostasis regulation of molecular
response to external stimulus	cellular respor chemical stin		onse to stimulus		response to ne stimulus	oxidation-reduction process	lipid meta proce	cell acti	mall molec vation biosynthet process	monoca	tabolic n	sterol netabolic process						function
	immune response		positive			organic acid metabolic process		lular lipid a atabolic		single-organism catabolic process	fatty aci		immune s	ystem (process		dhesion dhesion	response to stimulus
regulation of response to stimulus	cell surface receptor signaling path	activatio	regula	ation	response to wounding		proce	SS	catabolic process carboxyli secondary blosynt		id	process					cell mulation	
	positive regula	glial cell-derived neurotophic bictor receptor signaling pathway	purinergic receptor signaling pathway		" inflammatory response	single-organism biosynthetic process	single-org cellular pr	anism ocess n	alcohol netabolic process	process organophospha metabolic process	ATP	etic	biological adhesion		single-organi process		signalin	reactive oxygen species metabolism
response to stress	external stime response t	ulus cellular response	protein activation cascade	intracellul signal transducti	of phosphatidylinosito 3-kinase signaling		cytokine	secretion	reg mu	egative ulation of Iticellular ganismal	multicellula organism metabolic	r collagen metabolic process			cofactor	ataz	locomoti	on respiratory burst
response to other organism	chemical response t oxygen-conta compound	o wound healing	of MyD88-independent toll-like receptor signaling pathway signal transduction by protein	xenob metal proci cellu respon	bolic ess dar se to	cytokine production		f multicellular		rocess od vessel hogenesis	coagulation	renal	cofactor u bic cofactor biosynthe sulfur comp	esis ^{ss}	me process	olism	compound	pronitrogen compound netabolism

Figure S5. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in rat skin. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

immune response				ive regulationse to stir		cytokine p		regulation of catalytic activity	hemostas	sis of m	gulation protein etabolic process					
		defense response	cellular response to stimulus) stimulus	regulation of regulation of regulation		positive regulation of NF-kappaB transcription factor activity lation of cytokin coagulation	regulation of biological process e production	process	regulation of body fluid levels negative	immune	e system pro	ocess	response to stimulus	
								coaguration	of "	internet internet	regulation of cellular process					
regulation of response to stimulus	response to biotic stimulus	cell surface receptor signaling pathwa	regulation signaling		lation of cel nmunication	regulation of molecular	positive regulation of	regulation of autophagy	protein phosphorylatio regulation of	prosproryan	on single-organism localization	cell com	munication	s	gnaling	
		regulation of I-kappaB kinase/NF-kappaB	regulation	1 of	all GTPase diated signal	function	biological process	regulation of biological quality	phosphorus	vac	cuolar					
intracellular signal transduction				cellular	transmembrane				phagocyt	osis	ocalization of cell	leuko		cell proi cell lio proliferatio		
transduction	response to	signaling pathway	compound	esponse to chemical stimulus	tyrosine kinase signaling pathway			cell death		cytosolic		cell-cell adhesion		cellular proces	n multi-organism	
	external stimulu	response to	response to chemical	response to nitrogen	macrophage		cell activat	tion	pha endocytosis	ion transport				cytokine	process	
response to other organism	I-kappaB kinase/NF-kappa	aB		compound	d signal	apoptotic p	TOCOPS 0	novement of cell or nucleoside	regulation of	secretie	on transition metal ion	leukocyte proliferation	biological regulation			
	signaling	wound healing		response to peptidoglyca			S	ubcellular ^{salvage} omponent	vesicle-mediated transport	single-orga transpo	nism transpor rt			interspecies interaction between organis	ms localization	

Figure S6 REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat blood. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.

single-organism catabolic process	single-organism metabolic process	collagen catabolic process	organic acid catabolic process		agen organic acid in regulation of blood volume		response to amino acid	regulation of smooth muscle contraction	blood coagulation, fibrin clot formation	organic orgånic hydroxy
fatty acid metabolic process	ty acid metabolic process monocarbs(prices) metabolic process			dation-reduction process	negative regulation of cellular responders of the second system vascular endothelial growth factor stimulus	n proçe <u>şe involve</u> d in ı body fluid levels	egulation of blood vol detoxification	unegative regulation of lamellipodium assembly	compound metabolism process	
					multicellular organism metabolic process	aorta smooth muscle tissue morphogenesis		protein activation cascade		
	small molecule	leukotriene B4 catabolic	process	steroid			particle remodeling		cholesterol import	
lipid metabolic process	metabolic process	small molecule biosynthetic process		process	extracellular matrix or	galextracellular matrix	organizationular structu	re organization		

Figure S7. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat heart. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.



Figure S8. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat kidney. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.



Figure S9. REVIGO treemap of gene ontology processes that are significantly enriched (FDR<0.05) for gene ontology processes in giant mole-rat liver. Each rectangle is a single cluster representative. The representatives are joined into superclusters of loosely related terms, visualized with different colors. The size of the rectangles reflects the enrichment p-values of the covered GO-terms.



Figure S10. Expression levels of examined genes. Gene counts were first normalized for overall read number per sample. Then, separately for each tissue, across samples of, both, young and old animals, mean gene counts per kilobase transcript length were determined. Whiskers extend to the most extreme datum within 1.5 times inter quartile range. DEGs - differentially expressed genes. Overlapping DEGs - DEGs found, both, in rat and giant mole-rat.