Elinav, E., Henao-Mejia, J., and Flavell, R.A. (2013). Mucosal Immunol. *6*, 4–13.

Harrison, O.J., Srinivasan, N., Pott, J., Schiering, C., Krausgruber, T., Ilott, N.E., and Maloy, K.J. (2015). Mucosal Immunol. *8*, 1226–1236.

Hooper, L.V., and Macpherson, A.J. (2010). Nat. Rev. Immunol. *10*, 159–169.

Levy, M., Thaiss, C.A., Zeevi, D., Dohnalová, L., Zilberman-Schapira, G., Mahdi, J.A., David, E., Savidor, A., Korem, T., and Herzig, Y. (2015). Cell *163*, this issue, 1428–1443.

Macia, L., Tan, J., Vieira, A.T., Leach, K., Stanley, D., Luong, S., Maruya, M., Ian McKenzie, C., Hijikata, A., Wong, C., et al. (2015). Nat. Commun. *6*, 6734.

Nowarski, R., Jackson, R., Gagliani, N., de Zoete, M.R., Palm, N.W., Bailis, W., Low, J.S., Harman, C.C.D., Graham, M., Elinav, E., and Flavell, R.A. (2015). Cell *163*, this issue, 1444–1456.

Wlodarska, M., Thaiss, C.A., Nowarski, R., Henao-Mejia, J., Zhang, J.P., Brown, E.M., Frankel, G., Levy, M., Katz, M.N., Philbrick, W.M., et al. (2014). Cell *156*, 1045–1059.

Genome Sequencing Fishes out Longevity Genes

Vanisha Lakhina[1] and Coleen T. Murphy[1,*]
[1]Department of Molecular Biology & LSI Genomics, Princeton University, Princeton NJ 08544, USA
*Correspondence: ctmurphy@princeton.edu
http://dx.doi.org/10.1016/j.cell.2015.11.040

Understanding the molecular basis underlying aging is critical if we are to fully understand how and why we age—and possibly how to delay the aging process. Up until now, most longevity pathways were discovered in invertebrates because of their short lifespans and availability of genetic tools. Now, Reichwald et al. and Valenzano et al. independently provide a reference genome for the short-lived African turquoise killifish, establishing its role as a vertebrate system for aging research.

Human aging is associated with reproductive and cognitive decline and an increased risk of cancer, diabetes, cardiovascular disease, and neurodegenerative disease. The discovery of long-lived mutants demonstrated that aging is a genetically regulated process. Most molecular insights into the biology of aging come from short-lived invertebrates, such as *C. elegans* and *Drosophila* (with lifespans of 3 weeks and 3 months, respectively), or single-celled organisms like *Saccharomyces cerevisiae*. For example, the insulin/IGF-1 signaling pathway, which plays a conserved role in lifespan regulation from yeast to humans, was first discovered in *C. elegans*; mutants of the *daf-2* insulin receptor double lifespan (Kenyon et al., 1993). Other pathways that modulate lifespan, including the nutrient-sensing TOR pathway, the HSF-1 heat shock pathway, and the JNK stress response pathway, were also discovered in these systems. Vertebrate model systems, such as zebrafish and mouse, have also been used to study aging and age-related decline, but their long lifespans (~3.5 and 5 years, respectively; Figure 1), make it difficult to rapidly conduct complex aging experiments. Thus, the establishment of a short-lived genetic and genomic model system would allow both testing of conserved pathways and the discovery of new longevity regulators. While the African turquoise killifish *Nothobranchius furzeri* has been recognized for its potential for aging research (Genade et al., 2005; Valenzano et al., 2006; Di Cicco et al., 2011, Kirschner et al., 2012, Harel et al., 2015), until now, its utility has been limited because of the lack of genomic resources. In this issue of *Cell*, two independent groups, Reichwald et al. (2015) and Valenzano et al. (2015), map the genome of this short-lived fish and bridge this gap.

Nothobranchius furzeri is a short-lived vertebrate that lives in seasonal freshwater ponds in Zimbabwe and Mozambique (Genade et al., 2005). In laboratory conditions, *N. furzeri* exhibit a maximal lifespan of 4—6 months, making them the shortest-lived vertebrate that can be bred in captivity. They also exhibit age-related declines in fertility and cognitive ability, as well as age-related telomere shortening, impaired mitochondrial function, and cancer (Genade et al., 2005; Valenzano et al., 2006; Di Cicco et al., 2011), making them an ideal model system for lifespan studies. Indeed, *N. furzeri* have previously been used for mapping quantitative trait loci that control lifespan (Kirschner et al., 2012).

Here, Reichwald et al. and Valenzano et al., describe their independent work to generate a reference genome for the highly inbred GRZ strain of *N. furzeri.* The authors then use the new genome information to provide novel insights into lifespan regulation and sex determination. Upon genome assembly and annotation, Valenzano et al. identified 497 genes under positive selection in the GRZ reference strain. These include genes associated with longevity in humans (IGFR1, INSRA, LMNA3, and XRCC5). Further, they found that distinct residues are under positive selection in humans versus turquoise killifish, suggesting that variants of the same gene might confer context-dependent short or long lifespans in turquoise killifish and humans, respectively. They also sequenced two longer-lived

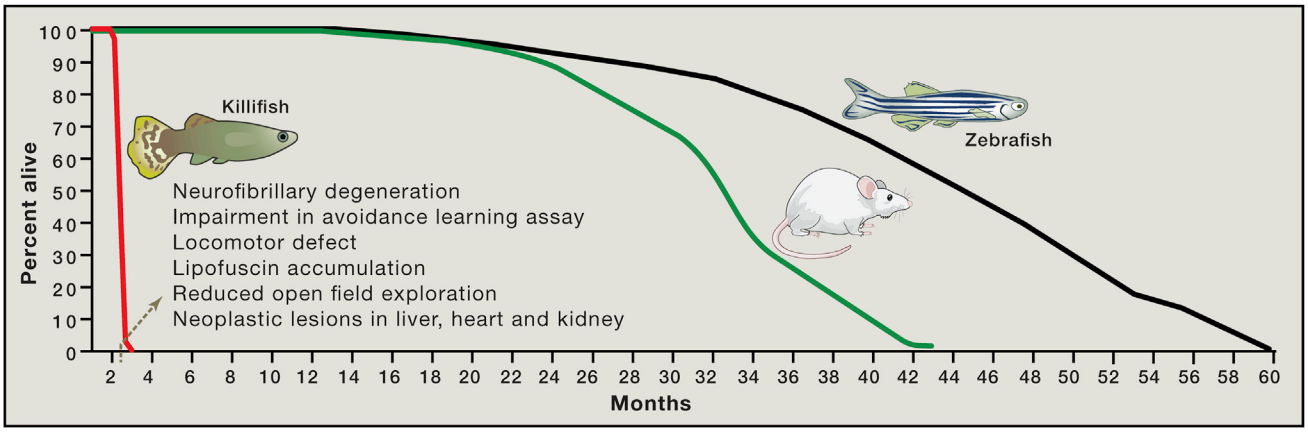

Figure 1. African Turquoise Killifish Are the Shortest-Lived Vertebrate Model System
Turquoise killifish live for about 3 months (red line) and exhibit age-related decline around 9 weeks (arrow). Their lifespan is significantly shorter than mouse (green) and zebrafish (black).

turquoise killifish strains and found SNP variants in genes encompassing all nine hallmarks of aging (López-Otín et al., 2013). Genetic linkage analysis using short- and long-lived turquoise killifish strains identified a lifespan QTL containing 31 genes, which is linked to, but distinct from, the sex-determining region.

In parallel, Reichwald et al. mapped the sex-determining region that is located on a male-specific region of the Y chromosome (MSY). The MSY differs in size between different turquoise killifish strains, yet the position of the variation peak is identical across strains. This suggests that MSYs arose from a common ancestor and that secondary events led to the generation of distinct strain-specific MSYs. Gdf-6, which encodes a TGF-β family growth differentiation factor, is the major sex-determining gene, though how it acts as a sex regulator in this context remains to be elucidated. Reichwald et al. also identified genes shared between aging and diapause (a state of developmental arrest that *N. furzeri* embryos enter in unfavorable environmental conditions), and found that these genes are distinct for different tissue types.

It is remarkable that both groups independently not only found aging/longevity genes, but also identified genomic regions enriched for these genes, through QTL analysis (Valenzano et al.) and positional gene enrichment (PGE; Reichwald et al.), reporting ten genomic regions of clustered age-related genes. Whether similar genomic clustering of aging/longevity genes exists in humans and other organisms remains to be determined.

Since turquoise killifish, like humans, have organs and organ systems (e.g., liver, kidneys, immune systems, and circulatory systems), this model system will provide valuable insights into how different tissues age, and whether cross-talk between tissues influences aging, as well. This work will be enabled by an abundance of available tools and resources: genomic engineering tools, such as CRISPR/Cas and transgenesis, are already available for the turquoise killifish (Valenzano et al., 2011; Harel et al., 2015), and methods developed by the zebrafish community can be adapted for the turquoise killifish. These approaches include high-throughput behavior testing and drug screening, electrophysiology, morpholino-mediated knockdown, and robotic injections of specific cell types. By combining these tools with the information that the new genome sequence provides, researchers will now be able to investigate the genetic and genomic bases of age-related declines and diseases, and identify biomarkers of aging, which may lead to therapeutics for age-related diseases.

REFERENCES

Di Cicco, E., Tozzini, E.T., Rossi, G., and Cellerino, A. (2011). Exp. Gerontol. *46*, 249–256.

Genade, T., Benedetti, M., Terzibasi, E., Roncaglia, P., Valenzano, D.R., Cattaneo, A., and Cellerino, A. (2005). Aging Cell *4*, 223–233.

Harel, I., Benayoun, B.A., Machado, B., Singh, P.P., Hu, C.K., Pech, M.F., Valenzano, D.R., Zhang, E., Sharp, S.C., Artandi, S.E., and Brunet, A. (2015). Cell *160*, 1013–1026.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). Nature *366*, 461–464.

Kirschner, J., Weber, D., Neuschl, C., Franke, A., Böttger, M., Zielke, L., Powalsky, E., Groth, M., Shagin, D., Petzold, A., et al. (2012). Aging Cell *11*, 252–261.

López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., and Kroemer, G. (2013). Cell *153*, 1194–1217.

Reichwald, K., Petzold, A., Koch, P., Downie, B.R., Hartmann, N., Pietsch, S., Baumgart, M., Chalopin, D., Felder, M., Bens, M., et al. (2015). Cell *163*, this issue, 1527–1538.

Valenzano, D.R., Terzibasi, E., Genade, T., Cattaneo, A., Domenici, L., and Cellerino, A. (2006). Curr. Biol. *16*, 296–300.

Valenzano, D.R., Sharp, S., and Brunet, A. (2011). G3 (Bethesda) *1*, 531–538.

Valenzano, D.R., Benayoun, B.A., Param, P.S., Zhang, E., Etter, P.D., Hu, C., Clément-Ziza, M., Willemsen, D., Cui, R., Harel, I., et al. (2015). Cell *163*, this issue, 1539–1554.