

People & Ideas

Anne Brunet: Gracefully studying how we age

Brunet studies longevity, aging, and epigenetics in established and novel model systems.

Why do we age? Is it inevitable? Lifespans vary across species as much as 150,000-fold, yet all sexually reproducing species eventually experience loss of function, sometimes sliding into senescence before succumbing to death. Research has pointed to genetic, epigenetic, metabolic, and environmental factors that figure in the aging process. However, the reasons behind organismal aging remain an enduring and somewhat terrifying mystery.

Anne Brunet is not afraid to tackle this mystery, even if it means venturing into the unknown. Her postdoctoral work on the “pro-longevity” FOXO transcription factors (1, 2), and more recent studies on epigenetic (3) and metabolic (4) determinants of aging, have helped frame the kinds of questions we should be asking if we want to solve this mystery. Now, Brunet’s lab at Stanford University is leveraging new technologies—and even a new model animal (5)—to probe these questions in more depth. We called her to learn more.

SIREN SONG OF SCIENCE

When did you first become interested in science?

I grew up in the French Alps, and as a child I loved music and the arts. Music is still very important to me today. But in high school I had great teachers who made me realize that science was beautiful too, just like the arts.

In college I had a wonderful mentor, Philippe Ascher, who suggested I go to graduate school in Nice, to work in the lab of Jacques Pouysségur. Jacques is someone who is really passionate about science, so I had an amazing experience in graduate school. At the time, little was known about signaling pathways. MAP kinase had been cloned and discovered, but how it connected to environmental stimuli was unknown. I worked on MAP

kinase then, and it was neat going into the unknown to try to puzzle out how the pathway functions.

How did you first encounter FOXOs?

I was interested in the brain and wanted to study neuroscience. I saw one of Michael Greenberg’s postdocs give a talk at a meeting and thought, “This is perfect! I can study signaling in the brain.”

That was a good choice because Mike is an awesome scientist and a very supportive mentor. His lab had recently made the intriguing observation that the PI 3-kinase/Akt signaling pathway is important to prevent cell death and regulate neuronal survival. We were hunting for potential Akt substrates that could mediate this effect. At the same time, when I had just arrived in Mike’s lab, there were a couple of papers from Cynthia Kenyon and Gary Ruvkun’s labs, indicating that a forkhead transcription factor was downstream of the insulin pathway in worms. That raised the possibility that this family of transcription factors could be direct substrates of Akt. I tested this in mammalian cells, and it worked. It was a really beautiful example

of the scientific method working as it is supposed to.

Akt very potently phosphorylates a forkhead transcription factor, FOXO3, at three sites, which causes the protein to be retained in the cytoplasm, away from its nuclear targets. Because FOXO3 is a transcription factor that can regulate thousands of genes, its downstream effects can be difficult

to tease apart, but in neurons FOXO3 can potently trigger apoptosis.

There are also many layers of regulation on FOXOs...

Yes, that’s right. In fact, the predicted molecular weight of FOXO3 is about 70 kDa, but when you look at it on a western blot,



Anne Brunet

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it runs at about 100 kDa. It’s heavily post-translationally modified. We and others have identified acetylation sites, methylation and ubiquitination sites and many phosphorylation sites in addition to the Akt sites. FOXOs are mainly regulated by Akt, but the other modifications seem to modulate its function, and may actually be important for targeting it to specific genes or perhaps even subregions of the cell.

A BIGGER MYSTERY

What brought you to studying longevity and aging?

After my postdoc I got a job at Stanford, which was very exciting but also very scary. I was thinking that starting my own lab could be a turning point: I could focus just on FOXO, or I could also study an important problem, a big mystery that would be very interesting to crack. This was really scary because up until then I had only worked with cell lines, but aging is an organismal problem. We had a molecular handle on it with FOXO, and we could use adult stem cells, which have regeneration potential and are useful for studying mechanisms that preserve this potential. But I felt we really needed to work with organisms because one cannot understand aging just by studying cell death. My first graduate student, Eric Greer, set up worms as a model system. Later we also set up studies with mice.



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Young (rear) and old (front) African turquoise killifish

We're taught that inherited traits are driven by genetic makeup, but with your worms you discovered possible epigenetic influences on aging...

Chromatin states are long lasting but reversible, so we wanted to ask whether chromatin states can modulate the plasticity of aging, and whether this can be reversed by environmental stimuli. Eric asked this question by screening all the methyltransferases and demethylases that we could find in the worm genome. Often people use sterile worms to do screens because it's easier technically, but because reproductive activity influences aging we felt that it would be important to use fully fertile worms.

Eric found that the COMPASS complex, which trimethylates histone H3 at lysine 4, is very important for the regulation of lifespan. When it is knocked down in worms their lifespan increases significantly. Then he learned that this complex acts mostly in the germ line of the animal to regulate lifespan. He postulated that it might not only regulate the lifespan of the parents, but could also have an effect on descendants via a transgenerational, epigenetic mechanism. When he set out to test that, he found that when the COMPASS complex is knocked down in the parents but not the progeny, these wild-type progeny are still long-lived for a few generations before reverting to a normal lifespan. We don't yet understand the mechanism, but this is consistent with a transgenerational inheritance of lifespan that's set in motion by deficiency in the COMPASS complex. Eric has actually started his own lab to pursue transgenerational inheritance mechanisms, but several people in my lab are following up on other

aspects of epigenetic regulation of aging that we first encountered in that screen.

NEW MODELS

You've developed a new model organism for studying aging...

Not all aspects of aging can be studied in invertebrates, but studies with mice are very slow because they're relatively long-lived animals. We really needed a shorter-lived vertebrate model.

Then I met Dario Valenzano, a graduate student from Italy who was visiting Stanford for a course on fish genomics. He and his PI in Italy, Alessandro Cellerino, had done some work with a fish, the African killifish, that was known by hobbyists to be beautiful but very painful to love because they die so fast. Dario had done a lot to characterize their life and behavioral decline with aging, so he knew them well, but he didn't have the genetic and genomic tools that could turn it into a model that could be manipulated and studied.

I thought it would be cool to set the system up in my lab, so I offered Dario a postdoc position to do this. He set the fish up in a tiny equipment room and arranged some crosses to study their lifespan. We had some disasters because of a parasite infection, but he was very persistent and brave and finally set everything up to do genetic linkage maps. At first he was by himself but then several other postdocs got interested and joined him, sequenced the genome, and did great proof-of-concept experiments with CRISPR/Cas9 genomic editing. CRISPR/Cas9 is really going to help make this model very useful for testing genes that are important for aging.

It's taken about 10 years to reach this point, but now I think it's an excellent model for studying aging. It's a vertebrate with similar organs and systems to mammals, including an adaptive immune system. It's short-lived but encapsulates several features of aging such as declining muscle and cognitive function, and even cancer-like lesions. It also exhibits interesting behaviors such as an inducible diapause—basically a state of suspended animation, whose regulation we're studying.

We're very excited to use the killifish to test genes, drugs and compounds that can affect aging and longevity—especially those factors that cannot be studied in invertebrate systems. We're also very excited about the genetics of these fish. Different strains have different lifespans, and we think we can use this as a handle to study genes that affect lifespan not only within but between species. If we can connect this to our work in other organisms, we hope to gain insights into the lifespan strategies of those species. Several people

in my lab are now working with this model; it's a really exciting time for this fish!

What about FOXO?

FOXOs are my first love. There are four FOXOs in mammalian cells, and we've finally created a quadruple conditional knockout mouse for all of them. But everybody in my lab now wants to study epigenetics. I've been shopping the mouse around with no success. [Laughs] If you know anyone who wants to study FOXO, can you send them my way?

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2. Brunet, A., et al. 2004. *Science*. 303:2011–2015.
3. Greer, E.L., et al. 2011. *Nature*. 479:365–371.
4. Rafalski, V.A., E. Mancini, and A. Brunet. 2012. *J. Cell Sci.* 125:5597–5608.
5. Harel, I., et al. 2015. *Cell*. 160:1013–1026.



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The Brunet Lab at a Bastille Day party, getting ready to tackle the mystery of aging (or storm the Bastille)