An Interview with Professor Michael Shapira

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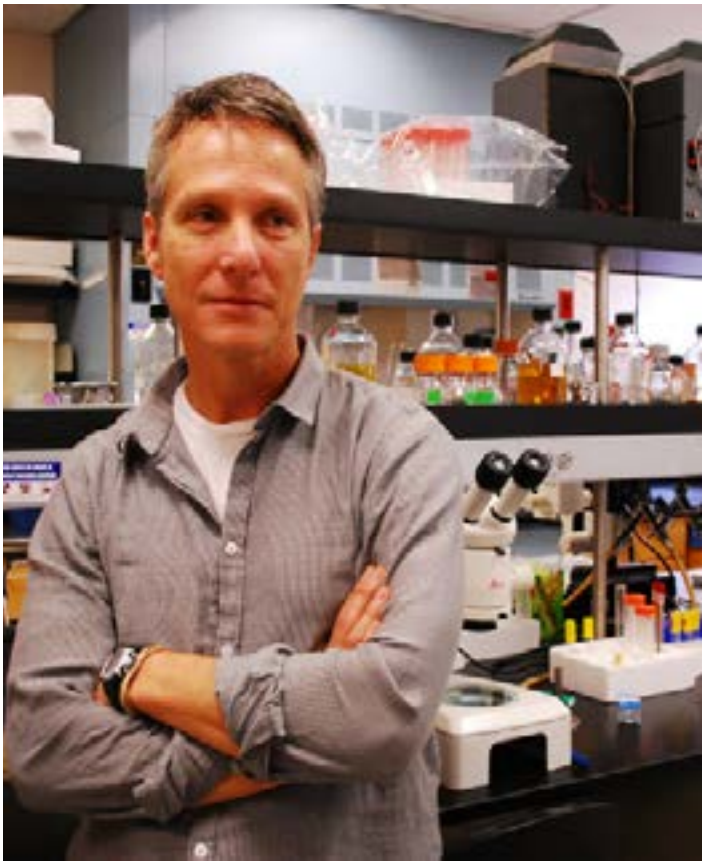


The BSJ Interviews Crew had the opportunity to interview Professor Michael Shapira. Professor Shapira received his B.Sc. and his Ph.D. in biochemistry and molecular biology from the Hebrew University in Jerusalem. Following receipt of his doctorate, Professor Shapira moved to the Genetics department at Stanford University School of Medicine, where he trained with David Botstein and Man-Wah Tan as a Life Sciences Research Foundation postdoctoral fellow. Currently, Professor Shapira's research is focused on understanding the fundamentals of host-pathogen interactions in the context of the whole organism. Through the use of his unique model organism, the soil nematode Caenorhabditis elegans, Professor Shapira's current point of interest at the lab is pathogen recognition.

BSJ: To start things off, how did you get interested in your research?

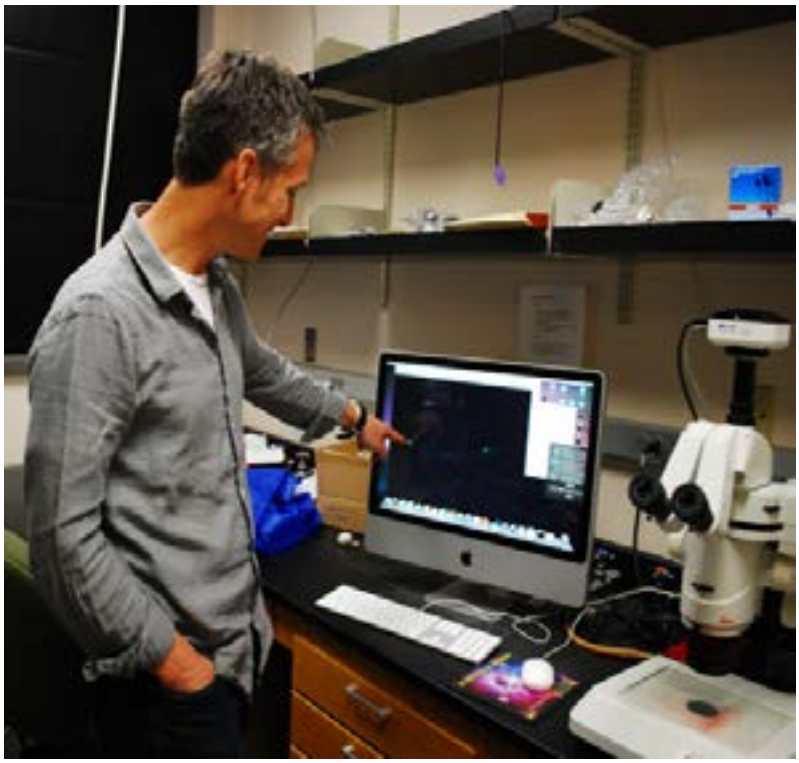
Prof. Shapira: Well, things have happened as they sometimes do in science. I wasn't always focusing on C. elegans; I used what seemed to be the most appropriate system at the time to answer a biological question. As my interests in stress and environmental stress conditions evolved to consider genome-wide responses, I moved to work with a eukaryotic unicellular model organism, in which analysis could be more complete, but actually, I was more interested in studying similar stress responses in multicellular organisms. By chance, a nearby lab was working with C. elegans, studying how it dealt with infection. I figured that that was a type of stress, and the system seemed overall very attractive: typically you study a one-sided response to stress, but when studying host-pathogen interactions, you have two sides that are responding to each other. I became increasingly fascinated with this host-pathogen interaction story. Again, nothing really stays the same, I started with stress and moved to the host-pathogen interactions, and before you know it, I'm back to stress.

BSJ: Why did you choose to work with C. elegans? Much of your research is based on this nematode. Many labs in Berkeley use the mouse or rat as their model organisms.

Prof. Shapira: First, the question is "how appropriate is the particular system to answer the biological question" and "what new insights can you get by using this system unlike other organisms that people have been using for a long time". In that respect, studying host pathogen interactions in C. elegans was new, and we thought we could get new insights by using this system. There were two other factors. Firstly, I did work with mice and human tissues, while I was completing my Ph.D.; I didn't like working with these models; I preferred to work with invertebrates -- to me it was cleaner. But the main reason was that I wanted to simplify the system I used. For my first postdoc I chose yeast to be able to study genome-wide stress responses. Microarray technology was new then, and with yeast, I felt that I could get the most. I could get into the nitty gritty of the mechanisms, be able to do serious bioinformatics and get new insights. It's a great system. Working with yeast made me grow affectionate with simple model organisms and the kind of things that you could do with them, both nasty things, but more importantly, the depth of information you can get from that. From yeast, moving to C. elegans was a step up to the 'real world' of multicellular organisms, and back to multi-tissue physiology. In a sense, it's the middle of the road between the well-defined and genetically tractable model and a complex organism.

BSJ: Could you elaborate a little more on your current research now and how it's related to stress?

Prof. Shapira: As I mentioned earlier, we started with infections and stumbled back into stress. We were initially aiming to examine a mechanism of the worm innate immune system. The adaptive immune system with all the antibodies and lymphocytes is something that is unique to vertebrates. It's a relatively recent invention. Innate immunity is something we vertebrates use alongside the adaptive immune system, but invertebrates are completely dependent on it. The innate immune system is quite similar in its function in all animals.



Professor Shapira in the lab showing C. elegans, the nematode he works with.

We were interested in a mechanism that is activated during exposure to a pathogen. We have a mutant strain, in which a disruption in a gene encoding a MAP kinase is unable to activate the protective response. MAP kinases are enzymes, which play roles in organismal and cellular signaling. They work as part of a 'pathway' in which one protein modifies the structure of another protein, which modifies the structure of another protein, leading to changes in the expression of a bunch of genes, many of them serving protective roles. We wanted to generate a situation where we have control over activation of that enzyme that was

regulating this protective response, a MAP kinase of the p38 family. By 'knocking-down' the expression of a negative regulator of the p38 MAP kinase, we were able to activate it. That's what nice about having C. elegans as a model organism. You can easily decrease the expression of any gene of interest to examine its function. When we knocked down the expression of the negative regulator, we increased the activation of the p38 MAP kinase and the worms were better protected from infection. However, to our surprise, this was true only in developing worms, and when the same knock-down was performed in young adults we saw the opposite effect: worms became more sensitive to infection! It turned out that the negative regulator we targeted regulated not only the p38 kinase, but also a second MAP kinase of the JNK family (pronounced "Junk"), called KGB-1, which when activated in adults showed a new and dominant detrimental contribution to infection resistance. The detrimental contribution of KGB-1 to stress resistance appearing in adults was not unique to infection resistance, but more general, affecting resistance to various environmental stress conditions and further shortening lifespan. Importantly, this experiment exposed an age-dependent reversal in the contribution of a stress-protective mechanism.

It turns out that JNK signaling seems to be doing similar things in mammals. It is generally protective, a stress activated protective mechanism, but it's also associated with a large array of diseases, most of them associated with old age; e.g. tissue damage after stroke, insulin resistance, and neurodegenerative diseases. We thought we might have a handle on conserved mechanisms that are affecting first, how we respond to stress; and second, aging and aging-associated pathologies. Therefore, this experiment has led us into a very basic mechanism of aging.

BSJ: So, is this a transregression mechanism wherein you suppress some of the immune systems first defense?

Prof. Shapira: No, not quite. It does suppress the ability to resist infection, but its effects are more general. Let me elaborate on that. Animals have two stress-activated MAP kinase pathways, the P38 and the JNK. In developing animals, both have protective effects. The p38 protects mostly against infection and oxidative stress, and JNK signaling, in C. elegans at least, is mostly for heavy metals and stress due to misfolded proteins, a fundamental problem in aging by the way. However, in young adults (which in worms means two days later), while the activation of the p38 pathway is still protective, activation of the KGB-1 JNK kinase becomes detrimental. It decreases heavy metal resistance, makes the worm more sensitive to infection and shortens life span. A reversal in the contribution of this important mechanism. The characteristics of the KGB-1 switch, responsible for age-dependent antagonistic contributions, were apparently described before, or rather predicted. More than 50 years ago, an evolutionary biologist called George Williams suggested a general theory for the evolution of aging, built on a conceptual mechanism he called Antagonistic Pleiotropy. We know that many proteins have pleiotropic effects, which means they can contribute to more than one biological process. Antagonistic Pleiotropy in turn suggests that some proteins are sometimes good and sometimes bad, specifically, good early in life, but bad late in life. It is possible that such mechanisms will be positively selected during evolution because the strength of natural selection decreases as we age. As the extreme, contributions of biological processes appearing past reproductive age have no effect on fitness. So, species can select for mechanisms that are good at the beginning of life although they may be really bad later. These mechanisms together would be the cause of aging.

BSJ: So along that line, do organisms that undergo stress tend to age more quickly? Or tend to have other side effects that you haven't talked about.

Prof. Shapira: Considering that our stress-activated KGB-1 has long-term detrimental effects (including lifespan reduction) when artificially activated in adult animals, it would seem so. This is of course in addition to the damage caused by the environmental stress itself, which is a part of the natural scenario.

BSJ: Could you elaborate on how JNK pathways and insulin signaling interactions affect stress resistance and lifespan?

Prof. Shapira: Yeah, that's the one million dollar question that we are interested in. The C. elegans insulin signaling is an important regulator of lifespan and aging, and its relevant human counterpart is the one responding to insulin-like growth factors. It is known that a C. elegans JNK homolog, expressed specifically in neurons (not our KGB-1), positively regulates a transcription factor called DAF-16, which is the main receiver of inputs from the insulin signaling pathway, driving it into the nucleus. This transcription factor can increase stress resistance and extend lifespan. In vertebrates, the homolog that serves similar functions is called FOXO-3A. FOXO-3A, as well as DAF-16, are the hallmarks of longevity regulation. They integrate many signals and accordingly induce many stress-protective genes: anti-oxidants, detoxifying enzymes, etc. The Insulin Signaling pathway keeps DAF-16 phosphorylated which prevents it from going into the nucleus. The C. elegans neuronal JNK homolog also phosphorylates DAF-16, but in a way that drives it into the nucleus, allowing it to increase protective capacities against oxidative stresses. What we found with the other JNK homolog, our KGB-1, is that it also promotes nuclear localization of DAF-16, but only in developing animals Going into adulthood, KGB-1 activation does the opposite, removing DAF-16 from the nucleus. We still don't understand how this age-dependent antagonistic regulation of DAF-16 occurs. But we have hypotheses, based on the interesting bit that the switch occurs when the animal enters into reproductive age. Initiation of reproduction is a junction point where decisions are made between reproduction and maintenance. In C. elegans, you can easily disrupt germline proliferation and generation of gametes. You have the somatic gonad, but you no longer have any eggs in it. What others have shown was that germline disruption leads to a huge increase in the secretion of steroid hormones from the somatic gonad. Results from the Kenyon, Ruvkun and Antebi labs showed that when these hormones are secreted, they extend lifespan tremendously, which makes some sense because if there is no reproduction there is more investment in maintenance i.e. stress resistance. So, the clue we were given was that KGB-1 activation can reverse some of the effects of these steroid hormones, which suggests that KGB-1 may interact with components of this hormonal signaling

Picture of the nematode

pathway.

BSJ: In an ideal organism, there is a mechanism whereby reproduction supersedes maintenance of the organism itself?

Prof. Shapira: It's always a trade-off and the trade-off has been well known for many years. There are exceptions, but typically, mutations that cause increased reproduction would come at the expense of lifespan or stress resistance. Long-lived mutants, in many cases, have smaller brood size. With limited resources, you have to balance how you use your resources. When germline proliferation is inhibited and animals live longer, there are at least two signaling mechanisms that are activated. There is a lot of redundancy in biology. One of the mechanisms is involves the increase in the hormone secretion from the somatic gonad. These hormones act both directly and indirectly, though mostly indirectly, to drive nuclear localization of DAF-16, thereby increasing lifespan. However, when we activated KGB-1, we were able to reverse that. DAF-16 was removed from the nucleus. So, we think that KGB-1may interact with the same signaling mechanism that first sent DAF-16 into the nucleus, downstream to gonadal signaling. This guides our current experiments.

BSJ: When a worm becomes reproductive, or it has the capability to reproduce, it's not in a stressed environment. It's in an environment where there is an appropriate amount of food, resources that it will be able to use to create the next generation. Thus, you have a greater number of the reproductive hormones in the worm. Would these reproductive hormones have a sort of negative feedback mechanism through which it would affect "the switch"?

Prof. Shapira: Well you are asking an interesting question, a little bit complicated because it assumes that we know everything, which we don't. I think that much of the mechanisms enabling such choices operate before reproduction, as adverse conditions rarely appear all of a sudden. In C. elegans, one of the first choices is made during early development, in the second of four larval stages. If conditions are not favorable, worms will leave the normal developmental course leading to adulthood and reproduction. They will go to an alternative larval stage called "Dauer", which is stress resistant and longer lived. Consider that the entire lifespan of the worm is about 2 weeks, Dauers can live more than a month. If the conditions become favorable again, it can return to the normal developmental course and continue to reproduction. The good decisions are typically made before reproduction. To a certain extent, once reproduction begins it is already a commitment.

BSJ: Pertaining to your work specific to C. elegans, do you have any aspirations for any clinical applications that could evolve to prevent aging?

Prof. Shapira: To connect our results to aging in humans, we need to know how relevant are mechanisms we're observing in C. elegans to those playing roles in human aging, or in aging-associated disease. We have a collaboration where we use mammalian cell cultures and human tissues to examine if there's a relationship between the age of individuals and the outcome of JNK activation, and whether some of the mechanisms that we exposed in C. elegans play a role in age-dependent outcomes in mammalian systems as well. Specifically we're looking at people with Alzheimer's Disease, where JNK activation was shown to be an exacerbating factor. But this is just the beginning, so I have nothing to report about it yet.

More generally, C. elegans is one of the best models to study aging, because it is a tractable genetic system and because it is short lived, but also because it turns out that many of the mechanisms that were characterized in C. elegans to affect aging

were also found to be very important for mammals. One of these is insulin signaling; certain variations in the insulin receptor affect the longevity of C. elegans. Genetic studies in centenarians, people around 100 years old, identified linkage between old age and mutations in genes that affect insulin signaling. So, studying the genetic programs of aging in C. elegans could unravel conserved mechanisms that play similar roles in human aging, and thus may have clinical applications. However, this is usually not an immediate translation. Nevertheless, all considered, I'm pretty sure that our work in this invertebrate model organism could provide valuable insights into our understanding of the process of aging.

BSJ: Where do you see your research going? What other facets are you looking into?

Prof. Shapira: We are still interested in host-pathogen interactions. A third project in the lab that I have not mentioned yet, focuses on the characterization of the C. elegans gut microbiota, how the host shapes it and what is the significance of microbiota members for their host. I would love to see the three projects come together: for us to understand the contribution of the microbiota to its host physiology – to infection resistance, to environmental stress resistance, and to aging; and also understand how priorities change with age. I believe that the host and its microbiota are one system, with its compound behavior determining the success of all system members. I also believe that aging disrupts relationships in this system leading to misrepresentation and malfunction. I hope that we could prevent, or slow down these changes and help improving the quality of life, now and later in life.

BSJ: Thank you again so much for your time.