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Vax and Pax: Taking Turns to Build an Eye

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Opposing ball clubs don't take the field at the same time, and neither do teams of proteins responsible for creating the eye. While one team builds the retina, in adjacent cellular turf the opponents are busy constructing the cord that carries visual signals to the brain. And these guys aren't supposed to mingle.

That's why researchers at the Salk Institute for Biological Studies were surprised to find the respective team captains—Vax2, a protein that along with Vax1 builds the optic nerve cord, and Pax6, a protein that drives retinal fate—playing on the same field. That puzzle is explained in a forthcoming paper in *Genes and Development*.

Earlier studies from the laboratory of Greg Lemke, Ph.D., professor in Salk's Molecular Neurobiology Laboratory, had shown that Vax2 antagonized Pax6. "We knew that Vax1 and 2 acted together to inactivate Pax6. That's how you get an optic nerve—by preventing it from becoming a retina," explains Lemke. The only problem was that later on both Vax2 and Pax6 were co-expressed in the same cells. "If Vax2 was repressing Pax6 this seemed inconsistent," he says.

Both proteins bind DNA and function in a cell's nucleus to switch genes on and off. Pax6 regulates the development of the retina, while Vax2 ensures that the optic nerve gets built. Finding both proteins in the same nucleus would make about as much sense as having runners for the Giants and the Dodgers on base at the same time.

Analyzing eye development in both mouse and chick tissues, Lemke and

former postdoctoral fellow Jin Woo Kim, Ph.D., solved the mystery. Stina Mui, a former graduate student in the Lemke lab had originally observed Vax2 in the cytoplasm of cultured cell lines and Kim had taken on the task of figuring out why. He showed that Vax2 protein is indeed expressed in the same retinal cells as Pax6, but that Vax2 shuttles in and out of the nucleus in response to a signaling molecule known as Sonic hedgehog.

“Vax2 only entered the nucleus when its biological activity was needed,” says Kim. Once its job was done, Vax2 was apparently booted out of the nucleus into the cytoplasm where it remained in cellular time-out.

Kim and Lemke found that Vax2 shuttling was controlled by a chemical modification known as phosphorylation. Phosphorylation benched Vax2 in the cytoplasm, where it took a breather while Pax6 took over to form the retina. Kim then made a dramatic discovery. When he engineered a Vax2 protein that could not be phosphorylated—putting Pax6 permanently out of commission—and forced that protein into chick retinal precursor cells, the chicks had no eye.

“What you had was a chicken with just a big optic nerve,” says Lemke, noting with satisfaction that this was exactly the opposite outcome his group had observed when they genetically eliminated Vax2 and Vax1 genes from mice. “In that case you had no optic nerve but a giant eye. This basically says that you really have to get this protein out of the nucleus—if you keep it there you get no retina at all.”

But why doesn’t mother nature simply dispose of Vax2 when she’s finished with it? Most likely because it’s recycled for use again later in development. Explains Lemke, “This is a mechanism for pushing Vax2 aside—so it can’t do any damage by repressing Pax6—but keeping it close by so it can be quickly activated when it is needed again later on.”

“One consequence of this work is that we learn things ultimately important for medicine,” he continues. “The Sonic hedgehog pathway plays an important role during embryogenesis and also in the development of a series of cancers. Understanding the pathway is directly relevant to a whole spectrum of human diseases.”

Kim, who is now an assistant professor at Korea Advanced Institute of Science and Technology (KAIST) in Daejeon, South Korea, will continue collaborating with the Lemke lab by engineering a so-called “knock-in” mouse expressing the nonphosphorylatable protein in the normal developmental timeframe. The prediction is that, like the chicks, that mouse should have big problems making an eye. Stay tuned.

Source: Salk Institute for Biological Studies