The battle of the morphogens: How to get ahead in the nervous system

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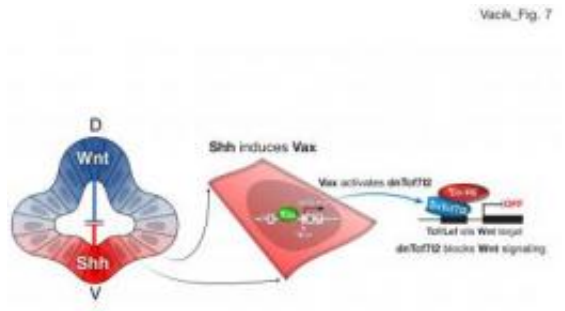

Wnt and Sonic hedgehog (Shh)are signaling proteins that pattern the dorsal (D) and ventral (V) ends - the North and South poles - of the embryonic brain. Wnt and Shh are in reciprocal competition in the embryo, and they strongly repress each other's activities. Vacik et al. describe a simple yet powerful new mechanism for this reciprocal repression: the Vax transcription factors, whose expression is activated by Shh, in turn activate the expression of dnTcf7l2, which functions as a potent inhibitor of Wnt signaling. This discovery is likely to have broad implications, since the competing Shh and Wnt pathways function in many other settings in biology, including the progression of colon cancer and the development of type II diabetes. Credit: Courtesy of the Salk Institute for Biological Studies

If you think today's political rhetoric is overheated, imagine what goes on inside a vertebrate embryo. There, two armies whose agendas are poles apart, engage in a battle with consequences much more dire than whether the economy will recover---- they are battling for whether you (or frogs or chickens) will have a forebrain.

In a study published in the August 19 online edition of *Genes & Development*, Salk Institute investigators led by Greg Lemke, Ph.D., professor in the Molecular Neurobiology Laboratory, reveals that a foot soldier of one army---- the ventralizers---deploys a weapon that disarms the other---- the dorsalizers---leaving the embryo free to develop a proper brain. Those findings define how the embryonic nervous system develops and could shed light on mechanisms underlying colon cancer.

The Lemke lab has a long-term interest in how different cell types emerging along the dorsal/ventral, or "top-to-bottom", axis of the nervous system are determined by competition between two secreted factors, or "morphogens"----the dorsalizer Wnt, trickling down from the brain or eye's "north pole" and its ventralizing opponent Sonic Hedgehog, creeping up from the "south".

"Opposing morphogen gradients regulate genes that must be expressed at either the top or the bottom of the brain for normal development to occur," says Lemke. "Those same signals must also be carefully controlled later on in mature tissues. An important example is provided by cancer, where over-active Wnt signaling is often linked to tumor formation."

The Lemke lab previously showed that a pair of Vax proteins, which bind DNA and regulate gene expression, are expressed in a gradient opposite to Wnts----high at the brain or eye's south, or ventral, pole and lower as you move north. This led them to propose that in response to Sonic Hedgehog signaling, Vax proteins ventralize tissues by blocking Wnt signals.

To test this idea they set up a genomic screen to search for Wnt inhibitors switched on by Vax. They found that Vax bound to a DNA sequence, or promoter, unusually positioned in the middle of a gene, rather than flanking it. And that gene---- designated Tcf7l2 ---- encoded

a transcription factor normally deployed by Wnt to dorsalize target tissue.

The paradox was explained when the group showed that Vax activated expression of a molecular decoy, namely a stump of Tcf7l2 protein missing its front end, the part required for it to activate gene expression. When bound to DNA the fragment would instead recruit repressor proteins to silence dorsalizing signals. "Cells expressing the inhibitor would be blind to Wnt signaling," says Lemke.

Full-length Tcf7l2 proteins pair with an activator called ß-catenin. But truncated Tcf7l2 lacks the ß-catenin interaction region, short-circuiting its function. Scientists call such interfering proteins "dominant negatives."

Tomas Vacik, Ph.D., a postdoctoral fellow in the Lemke lab and the study's first author, re-evaluated gene expression patterns in mice the lab had engineered to lack Vax2. "We found that Vax2 was necessary for expression of a group of Wnt antagonists in the mouse eye, including dominant negative Tcf7l2" he says.

Bioinformatics analysis of the Tcf7l2 DNA sequence bound by Vax revealed another surprise. Approximately 700 base pairs, or nucleotides, of the mouse genome around the Vax binding site in Tcf7l2 showed an astonishing 99% identity between mouse, humans, and chickens and 85% identity with fish, a conservation Lemke calls, "exceptional in the extreme."

"This means that over several million years of evolution, Mother Nature says you can't change a single nucleotide, " he says. "That tells you straightway that this DNA sequence fulfills a very important regulatory function."

Database searches revealed that truncated Tcf7l2 is expressed in human brain cells, and the group's own analysis revealed similar constructs in the heads of frog embryos. "These results suggest that dnTcf7l2 has been highly conserved during evolution for its ability to powerfully repress Wnt target genes," says Vacik.

Previously, other investigators have found that mice harboring mutations in Wnt inhibitor genes often exhibit severely truncated forebrains. So the group asked whether loss of the dominant negative Tcf7l2 would perturb head formation. To test that they injected frog embryos with a short inhibitory RNA designed to artificially degrade the frog version of truncated Tcf7l2.

The resulting embryos were essentially headless, showing complete loss of structures in front of the midbrain, the very point where Wnt signaling is brought to a grinding halt in normal mouse or frog embryos by the opposing ventral morphogen sonic hedgehog and its henchman Vax. This is the first study to provide an explanation for how this molecular line in the sand is drawn.

"Our results illustrate a very basic principle-that if you have the power to turn something on, you must have the ability to turn it off. Otherwise, you set up a situation of uncontrolled signaling," says Lemke.

The disastrous outcome of uncontrolled signaling is also illustrated by the fact that cancer cells often show aberrant reactivation of factors governing normal development. Some colon cancer tumors, for example, show high levels of Tcf7l2's partner ß-catenin and resulting unchecked Wnt signaling causes the disease. More intriguingly, the inability of tumor cells to make a short, inhibitory form of a factor related to Tcf7l2 is also associated with tumorigenicity.

"So Wnts and sonic hedgehog may be in competition in colon cancer just

like they are in the brain," Lemke says. "Our work could provide insight into how that happens mechanistically. "

Provided by Salk Institute