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# How the Snake Got Its Vertebrae

Snakes, fish, chickens, and humans all begin life in much the same way. Early in their transformation from an amorphous blob of cells into a fully developed animal, growing cells pinch off into a string of identical segments destined to become individual vertebrae, which will later sprout blood vessels, peripheral nerves, and muscle. These repeated segments ensure that the rod-like spinal column can hunch, arch, and twist.

The segmentation process also helps establish some key differences in the body plans of different organisms: while humans have 33 vertebrae, frogs have 10 or fewer, and snakes can have more than 300.

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> **- Olivier Pourquié**

Now, a team led by Howard Hughes Medical Institute researchers has uncovered the mechanism that guides vertebral development in the early embryo and makes sure each species ends up with the right number of vertebrae.

The team, led by Olivier Pourquié, an HHMI investigator at the Stowers Institute for Medical Research, reported its results on June 18, 2008, in an advanced online publication in the journal *Nature*.

“There is huge variability in the number of vertebrae among different animals, and virtually nothing [was] known about what controls this diversity,” said Pourquié. “What we do in the paper is provide an explanation.”

During development, vertebrate embryos grow from head to tail. As the body elongates, vertebral precursors known as somites, or segments, emerge from a group of immature cells called the presomitic mesoderm (PSM). These segments develop at regular intervals, much as a plant's stem forms branches at regular intervals as it grows.

“The way the body of a human or mouse or any vertebrate forms is from a growth zone - like the tip of a shoot - which is called the tail bud. First you produce the head, then the neck, then the thorax, and so on, until the tail,” said Pourquié. The long chain of vertebrae that defines a snake's body takes shape in about 23 days.

The regular emergence of segments from the PSM is driven by a cell signaling system known as a “clock and wavefront.” The clock is a simple set of signaling molecules that accumulate and disappear in rhythmic oscillations. Each time the clock molecules peak, a new vertebral segment is created.

Left to their own devices, the oscillating proteins would stay in one place, and create vertebrae one on top of another. But in developing embryos, the clock is whisked along the extending tail by another set of signaling molecules called the wavefront. It's a bit like an ambulance roaring down the street with its siren blaring; when the ambulance stops, the siren just blares in place. But when the ambulance is moving, every time the siren peaks, it's in a new place a little farther down the road. In the same way, every time the clock ticks, the wavefront has carried it a little further down the developing body to a new group of cells.

As a mouse or snake embryo begins to develop, the tailbud moves faster than the wavefront, leading to an increase of the PSM size. But then, probably due to a slowed down growth in the tail bud, the PSM starts to shrink. “The PSM shrinks until there is virtually no material left to make segments, and the process terminates,” said Pourquié. In mice, this happens after 65 segments have been produced. In snakes, over 300 segments are created before the PSM is exhausted.

Pourquié's group discovered this segmentation clock several years ago, and he and others have been working to identify and explain how the components of the clock and wavefront work together. But until now, no one understood how different species produced different numbers of vertebrae. The snake, with its unusually high number of vertebrae, served as a good organism for

Pourquié's team to study.

“The most intuitive way to think about it would be to say that the oscillator is going to move for a longer time period” to create so many more segments, said Pourquié. “You'd imagine that in chickens, where there are around 50 segments, the oscillator would move for a defined time period. In snakes, the oscillator would move for six times as long.”

Pourquié's group started out thinking that that was how snakes created all their myriad segments, but once they tracked the clock in a developing embryo, they rejected the idea. “What we see is that that is absolutely not the case,” he said. While it's true that snake embryos take several times as long as chicken embryos to develop, in fact this is because the development proceeds several times more slowly than it does in chickens. In relative terms, the time that a chicken and a snake have to produce somites is not very different.

Pourquié and his group discovered that the way a developing snake gets around this is to speed up their clock relative to their development rate. They compared a developing snake embryo to those of chickens and found that, after taking into account the different paces of development, the snake's clock ticks much faster relative to the movement of the wavefront, creating segments more often. They also compared the snake embryo to a lizard, because, said Pourquié, “lizards are like snakes in that they develop very slowly, but they produce many fewer somites than snakes.” The team found that, while the corn snake made a somite every 100 minutes or so, the lizard took four hours between somite formation.

Pourquié's work on vertebrae development has already begun to have an impact on medicine. “We're trying to investigate diseases that result from defects in the segmentation clock,” he said. Using their knowledge of how vertebrae develop, Pourquié's group compiled a list of genes they suspected might be involved in human diseases that result when this processs goes awry. Using this technique, they identified a recessive mutation responsible for Jarcho Levin syndrome, a congenital defect that results in malformation of the spine and ribcage. “Now we've developed a genetic test to identify the mutation, and so it's possible to do some genetic counseling,” said Pourquié.