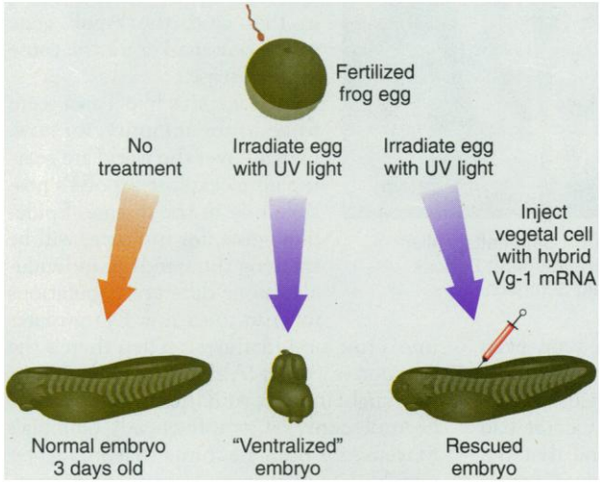
Protein Signals Frog Embryo To Start Getting a Head

The central issue in developmental biology is how an early, practically formless embryo differentiates into a complex organism. Among the many proteins that play a role in that process is a shadowy and elusive protein that has tantalized and baffled biologists for 8 years, since it was discovered. The molecule, known as Vg-1, appears in the frog embryo just when tissues that give rise to the head start to develop, making it seem likely that the protein signals those tissues to take shape. Try as they might, however, biologists had been unable to show that Vg-1 actually triggers that development.



Protein to the rescue. Damaged frog embryos injected with Vg-1 messenger RNA develop normal mesoderm features.

ILLUSTRATION: L. OWENS

Now the elusive protein has finally been caught in the act by a team of Harvard researchers. In today's issue of *Cell*, biologists Douglas Melton and Jerry Thomsen report that in embryos of *Xenopus laevis*, an African frog widely used as a model of vertebrate develoment, Vg-1 sends a crucial signal to cells that ultimately give rise to the head and other nearby organs. The protein can even "rescue" embryos that have been developmentally damaged and would otherwise become headless blobs.

The discovery is the reward for persistence. Melton discovered the messenger RNA coding for Vg-1 in 1985, and he has been pursuing the protein ever since. "Doug has been working on this for a while," says biologist James Smith of the National Institute for Medical Research in London. "He hasn't given up, and now it's come up trumps."

From the start, Melton's group thought Vg-1 was something special. Vg-1 is a maternal molecule, appearing in the unfertilized egg, and most types of maternal RNA are scattered evenly throughout the egg. But the team found that Vg-1 RNA collects exclusively in the half of the egg known as the vegetal hemisphere, the yolky portion that gives rise to intestinal tissue after fertilization.

The vegetal hemisphere does something else: It somehow signals cells in the other half of the embryo—the less yolky animal hemisphere—to form mesoderm, the tissue that gives rise to the head. Biologists had shown that with the vegetal half of a fertilized egg removed, those mesoderm-producing cells in the animal half only form the ectoderm, a different set of tissues that give rise to skin and nerves. Since no vegetal half meant no mesoderm, it seemed likely that the mesoderm-inducing signal had to be in the vegetal half.

What was the signal? Evidence pointing at Vg-1 kept piling up. By determining Vg-1's amino acid sequence in 1987, Melton's team showed that it belongs to the transforming growth factor-beta (TGF-β) superfamily of proteins, many of which induce undifferentiated cells to develop into various types of tissue. And work in several labs established that the Vg-1 messenger RNA produces Vg-1 protein precisely at the time that mesoderm structures first appear in the frog embryo.

Yet nobody could catch Vg-1 in a transforming role. All the methods devised by biologists to pin down a protein's effect on an embryo drew a blank. For instance, researchers injected extra Vg-1 messenger RNA into embryos—a technique that usually produces tissue abnormalities when performed with mRNA for developmental proteins—but Vg-1 had no effect whatsoever.

Then in 1992, Melton and Thomsen, a postdoc in Melton's lab at the time, decided to attack the problem from a different angle. Many proteins in the TGF-β family, they knew, don't become active until enzymes slice them into a shorter form and connect them to another, identical protein. Perhaps the extra Vg-1 mRNA they were injecting into embryos produced massive quantities of protein, but some unknown embryonic cutoff mechanism ensured that only a tiny amount was sliced and made into the active form. If that were the case, scientists could add all the extra Vg-1 they wanted, but it would not affect development.

To get around this bottleneck, Melton and Thomsen created a molecular disguise. They started with a Vg-1 molecule that had been sliced but not joined to another one. They then attached it to a "command" section of another TGF-β family member—a section that tells the cell to process the protein into final form. Melton and Thomsen hoped that the attached TGF-β commands would fool the embryo into treating the Vg-1 section like a different protein. Thus it would avoid the embryo's Vg-1 cutoff mechanism and become processed into additional active protein.

The disguise worked: The hybrid Vg-1 did produce deformities when the researchers injected it into embryos. Then Melton and Thomsen tried two experiments that have become a gold standard for the study of mesoderm induction. The first of these is the "animal cap" experiment, in which the animal hemisphere of a fertilized egg is cut away from the vegetal half. The separated animal hemisphere normally develops into a ball of ectoderm, with no mesoderm. But when the researchers injected the animal caps with the Vg-1 hybrid, the caps developed into mesoderm-like structures.

The second experiment involved intact fertilized *Xenopus* eggs. Melton and Thomsen irradiated the eggs with ultraviolet (UV) light before the mesoderm began to appear. Ordinarily when that is done, mesoderm never appears and the embryo loses the proper head-to-tail arrangement of its remaining organs. But when Thomsen injected hybrid Vg-1 mRNA into the UV-treated eggs, they formed normal-looking embryos, with heads, eyes, and other organs all in their proper places.

Other proteins, such as activin and members of the Wnt family, can also "rescue" UV-treated embryos. But Vg-1 outperforms many of the others, producing embryos that are more normal in appearance. Melton and Thomsen point out that the full story of head formation will no doubt include lots of proteins interacting with each other. And in a talk at the Society for Developmental Biology meeting this June, Melton noted that Vg-1 has yet to pass the final test, in which the Vg-1 gene will be obliterated from a normal embryo to test how the gene's absence affects development. But at this point, it's clear that Vg-1 is no longer lurking in the shadows of development research but has emerged into the spotlight as a key player.

–Traci Watson

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