Scientists Discover the Mystic Gene Controlling Vessel and Blood Cell Growth in Zebrafish Embryo

By **Irsyad Ramthan** - September 26, 2016

Gene that is critical in controlling the formation of blood and blood vessels in the embryo discovered

20 years ago, Prof Didier Stainier discovered a zebrafish mutant named '*cloche*' which lacks the development of both blood vessels and blood cells. Now, the Director of the Department of Developmental Genetics at the Max Planck Institute for Heart and Lung Research in Bad Nauheim has succeeded in finding the gene responsible for this mutation, which had quasi hidden itself at the very end of chromosome 13! The discovery of this gene is an important advancement for regenerative medicine.

At a very early stage of embryonic development, blood vessels and blood cells are formed via common progenitor cells, which are cells that are able to differentiate into other types of cells, but in a manner which is more specific as compared to stem cells. The timing and manner in which the blood and vessels form is regulated in a genetic program by multiple genes. This program is characterized by a cascade-like activity pattern.

In the mid-nineties, during his time in the United States, Prof Stainier discovered a zebrafish mutant which "[possessed] one of the most exciting developmental defects ever found in zebrafish", according to Sven Reischauer who, together with Oliver Stone and Alethia Villasenor, is one of the main authors of the study. Due to a genetic change in this fish, none of the genes involved in the genetic program for blood and blood vessel cells were activated. Consequently, these cells couldn't develop. Prof Stainier named the mutant "***cloche***" after another unique feature of the mutant, a cloche-like heart shape.

Cloche, which is French for bell, was chosen as the name of the mutation due to the zebrafish's heart resembling that of a cloche. Source: Wikimedia Commons

In the last two decades, various laboratories around the world took part in a real hunt for the gene behind the mutant.

> *"Identifying Cloche was, for all of us, like solving a decades-old criminal case of genetics. However, in this case, it was not the perpetrator who was unknown but the victim, the defective gene", says Reischauer. The Max Planck researchers in Bad Nauheim, together with international partners, have now successfully finished this hunt.*

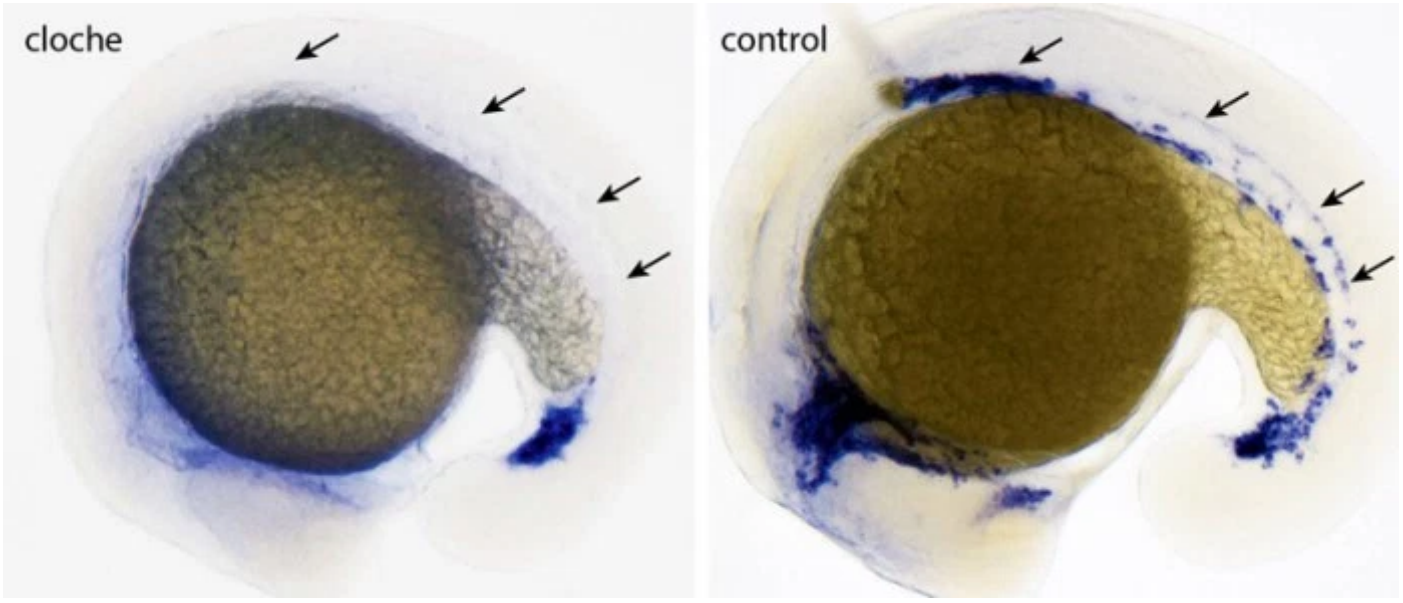
The Difficulty: Hidden in Chromosome End 'Caps'

"The search was made extremely complicated due to the fact that the cloche gene is located at the very end of chromosome 13, in a telomeric region", says Reischauer.

The advent of methods which have only recently become available, such as CRISPR/Cas9 and TALEN have enabled the scientists to analyse these areas in ways that were not previously possible. Reischauer further adds that "we had to assume that the gene is only active prior to the time at which the lack of vascular growth is evident. This made it much more difficult to identify the embryos".

First, the Bad Nauheim researchers examined the entire portion of the genome in which they suspected *Cloche* to be located. Analysis of data from 26,000 genes revealed 17 genes, which could be regarded as potential candidates. Then, they deactivated all of these candidate genes separately by producing knockout lines, and examined the blood vessel growth in these embryos. "Only in one case did we find the expected picture, namely that vessel growth failed to be induced. Then we were sure that we had found the cloche gene", says Reischauer.

In additional experiments, the importance of *Cloche* is for the development of blood vessels and blood cells in the embryo was demonstrated – All genes which were previously known to be involved in vessel formation were found only to be active after *Cloche* has been active. It could then be concluded that *Cloche* controls the activity of the entire program.



Left: The cloche mutant displayed neither blood vessel nor blood cell development (arrows, lacking blue areas). Right: Control embryo: Blood vessel growth was detected (arrows, pointing out clear blue areas) after 16 hours. The yellow area in both samples was the egg yolk. Credit: MPI for Heart and Lung Research, Bad Nauheim

This scenario was confirmed in so-called overexpression experiments in which the researchers injected pure cloche mRNA into embryos. This approach enabled them to start the program for vascular and blood cell formation at a time during embryo development at which it is not normally active. "We could, therefore, propose we had found the gene responsible for controlling the developmental program", says Prof Stainier.

The Significance: *Cloche* in Other Organisms

The *Cloche* gene seems to be present in birds. Although *Cloche* itself is not found in mammals, there is a closely related gene that can take over the function of *Cloche*. Therefore, Prof Stainier hopes that "with the identification of the gene and its function, there will be great opportunities to develop new applications in the context of personalized stem cell therapy".

The original publication can be accessed here.

Press release by the Max Plank Institute can be found here.

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Irsyad is currently pursuing his undergraduate degree in Biomedical Engineering. He is deeply interested in the open source concept, and how it can be applied to the biotech and medtech fields.