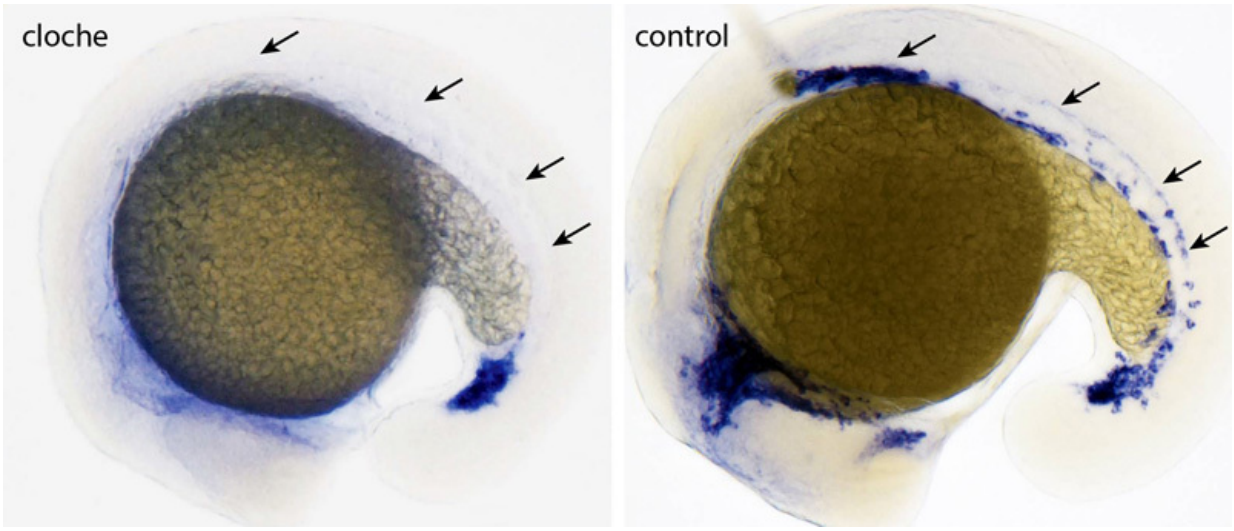
No blood vessels without cloche

July 15 2016



No blood vessel growth without cloche: While in the control embryo after 16 hours blood vessel cells can be detected (blue, arrows), in the cloche mutant neither blood nor vessel cells develop (arrows). The inner circle is the yolk. Credit: MPI for Heart and Lung Research, Bad Nauheim

The decade-long search by researchers worldwide for a gene, which is critical in controlling the formation of blood and blood vessels in the embryo, shows how fascinating science can be. It is more than 20 years since Didier Stainier, director at the Max Planck Institute for Heart and Lung Research in Bad Nauheim, discovered a zebrafish mutant named cloche. This mutant lacks development of both blood vessels and blood cells, and was, until now, a unique phenomenon. Now, his research group has succeeded in finding the gene responsible for it. It had quasi hidden

itself at the very end of chromosome 13 and was discovered using the latest molecular biological methods. The discovery of the gene is not only of scientific interest, but could also become important for regenerative medicine.

At a very early stage of embryonic development, blood vessels and blood cells form from common progenitor 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, Didier Stainier, Director of the Department of Developmental Genetics at the Max Planck Institute for Heart and Lung Research in Bad Nauheim, discovered in the model organism zebrafish, a mutant "possessing one of the most exciting developmental defects ever found in zebrafish", says 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 cannot develop. Stainier named the mutant "cloche" after another unique feature of the mutant, a cloche-like heart shape.

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.

Hidden in the 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. Now, with methods, which have only recently become available (for example, CRISPR/Cas9 and TALEN), do we have the tools to analyse these areas. "In addition, 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", says Reischauer.

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 Max Planck scientists showed how important Cloche is for the development of blood vessels and blood cells in the embryo: It transpired that all genes which were previously known to be involved in vessel formation, are only active after Cloche has been active. Accordingly, Cloche itself controls the activity of the entire program.

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 Stainier.

Cloche seems to be highly conserved in nature: The gene is present even in birds. In mammals there is a closely related gene that can take over the function of cloche in the zebrafish model. Therefore, the Bad

Nauheim scientists assume "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", Stainier says.

More information: Sven Reischauer et al. Cloche is a bHLH-PAS transcription factor that drives haemato-vascular specification, *Nature* (2016). DOI: 10.1038/nature18614

Provided by Max Planck Society