# True love: How transcription factors interact to create a heart

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Scientists at the Gladstone Institutes have discovered that three transcription factors—proteins that direct gene expression—interact with each other and the genome to influence how a heart forms in an embryo. Without these protein interactions, severe congenital heart defects can occur. By understanding how the transcription factors work together during heart development, researchers may discover new ways to treat heart disease.

Transcription factors dictate what genes are turned on or off in a cell during embryo formation, thereby controlling the type of organ the cell will form. The current study, published in *Cell*, tracked three transcription factors that are crucial for heart development—NKX2-5, TBX5, and GATA4—and revealed for the first time how they interact on a genomic and physical level. Mutations in the genes that regulate these proteins are implicated in congenital heart disease.

To test how transcription factors interact during development, the researchers created mouse embryos that were missing one or two of the proteins. A loss of one transcription factor resulted in known heart defects, while embryos missing two transcription factors had almost no heart, demonstrating the importance of both factors. Next, the scientists created cardiac cells missing the same transcription factors to investigate how their interactions affect gene expression. It turns out that transcription factors are a lot like teenagers in love; they exert a strong influence on one another, and if they are separated, they get themselves into trouble. The transcription factors often sat next to each other on the genome and required the presence of the other to bind to the DNA. If one of the proteins was absent, at times the other transcription factors would go rogue, binding to places on the genome where they were not supposed to be. This migration turned on genes that were supposed to remain off and silenced other genes when they should have been activated.

"Transcription factors have to stick together, or else the other one goes and gets into trouble," says first author Luis Luna-Zurita, PhD, a postdoctoral fellow at Gladstone. "Not only are these transcription factors vital for turning on certain genes, but their interaction is important to keep each other from going to the wrong place and turning on a set of genes that doesn't belong in a heart cell."

Where and when the transcription factors bound to the genome and how dependent they were on each other is written in the cell's DNA, which acts like an instruction manual by dictating the proteins' behavior. By learning the rules for these three proteins, the scientists say they can infer the rules of other transcription factors that are also important for heart development.

In a final step, the Gladstone scientists collaborated with researchers from the European Molecular Biology Laboratory (EMBL) to create a crystal structure, or 3D representation, of the transcription factors co-binding on the genome. The structure revealed that the proteins not only sat near each other on the genome, but they were also physically touching, like they were sitting on a bench and holding hands.

"The crystal structure critically shows the interaction between two of the transcription factors and how they influence one another's binding to a specific stretch of DNA," said Christoph Muller, PhD, a senior scientist at the EMBL. "Our detailed structural analysis revealed a direct physical connection between TBX5 and NKX2-5 and demonstrated that DNA plays an active role in mediating the interaction between the two proteins."

Senior author Benoit Bruneau, PhD, associate director of the Gladstone Institute of Cardiovascular Disease and a professor of pediatrics at the University of California, San Francisco, says these

findings have important implications for both congenital and adult heart disease. "Gene mutations that cause congenital heart disease lower the levels of these transcription factors by half, and we've shown that the dosage of these factors determines which genes are turned on or off in a cell. Other genetic variants that cause heart defects like arrhythmias also affect the function of these factors. Therefore, the better we understand these transcription factors, the closer we'll come to a treatment for heart disease. Our colleagues at Gladstone are using this knowledge to search for small molecules that can affect gene regulation and reverse some of the problems caused by the loss of these transcription factors."

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