New study finds that one key mechanism in development involves 'paused' RNA polymerase

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For a tiny embryo to grow into an entire fruit fly, mouse or human, the correct genes in each cell must turn on and off in precisely the right sequence. This intricate molecular dance produces the many parts of the whole creature, from muscles and skin to nerves and blood.

So what are the underlying principles of how those genes are controlled and regulated?

At the most basic level, scientists know, genes are turned on when an enzyme called RNA polymerase binds to the DNA at the beginning of a gene. The RNA polymerase copies the DNA of the gene into a complementary strand of messenger RNA, which then instructs the cell to make the protein coded for by the gene.

But several years ago, Julia Zeitlinger, Ph.D., now an assistant investigator at the Stowers Institute, made a surprising discovery. The RNA polymerase doesn't just attach to DNA and start copying. Instead, it binds and then pauses, waiting for another signal before it goes to work. In many cases, therefore, the key regulatory step isn't getting the polymerase to the gene, it is re-starting the paused enzyme.

"The dogma was that the recruitment of polymerase is the rate-limiting step," Zeitlinger explains. "Suddenly it was clear that this isn't always true." For many genes, the presence of paused polymerase indicates

whether a gene is poised and ready for transcription.

Now, new research by Zeitlinger's lab, described in the December 27, 2012, issue of *Cell Reports*, has revealed far more about the role of paused RNA polymerase in embryonic development—and turned up another surprise. The Stowers scientists looked for genes with poised polymerase at five separate developmental stages in fruit fly muscle cells , from the very early embryo (the mesoderm) to fully differentiated muscle cells. They also compared the development of muscle cells to that of nerve cells.

Such work hadn't been done before because it requires examining a large number of cells and developing new software programs and methods of analysis. Graduate student Bjoern Gaertner did most of the lab work, while bioinformatician Jeff Johnston performed most of the analysis.

The question was whether genes could switch between having a poised polymerase and having no polymerase at all. The general expectation was that such differences would be found between different cell types. After all, sets of specific genes have to be activated to make muscle or nerve, and thus it might be wise to turn the wrong genes off when the cell type is fully developed.

However, the team found that the pattern of genes with poised polymerase varied depending on the stage of development. "It was surprising that the poised state was regulated over time, rather than by tissue type," says Zeitlinger.

So how then can a single cell give rise to all cell types without turning on the wrong genes? The answer, the new research suggests, is that there are other regulatory mechanisms at work that keep the poised polymerase in check. The team found that this can be accomplished by a family of proteins called the Polycomb group, which has previously been

implicated in repressing the poised polymerase.

The Stowers team found that the action of these proteins varies by tissue type and thus can prevent the wrong poised genes from being turned on. Together, these two mechanisms explain how genes during the development of both muscle and nerves can be first poised to be expressed at the right time by paused polymerase, but then only actually activated in the right tissue type.

That's not the whole story, though. Zeitlinger's team also found that some genes don't actually need paused polymerase at all to be turned on. These genes have a distinctive DNA sequence—TATA—in their promoter regions. "What we found is that these promoters work in a fundamentally different way," Zeitlinger says. "It's very exciting."

Given the vast number of data and the complexity of the analysis, "this research took us a long time," says Zeitlinger. There are also many additional questions to be answered, such as what are the biochemical signals that bring the poised polymerase to genes over time.

But the work is bringing unprecedented clarity and detail to the complicated story of gene regulation. And because the researchers were able to show that the same mechanisms are at work in human cells too, the findings could eventually lead to a better understanding of disease. "The bigger vision is being able to understand the biochemical changes in development and map how development actually works," explains Zeitlinger. "And if we understand the cell better, we may be able to better predict what is going to happen in a diseased or cancer cell."

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