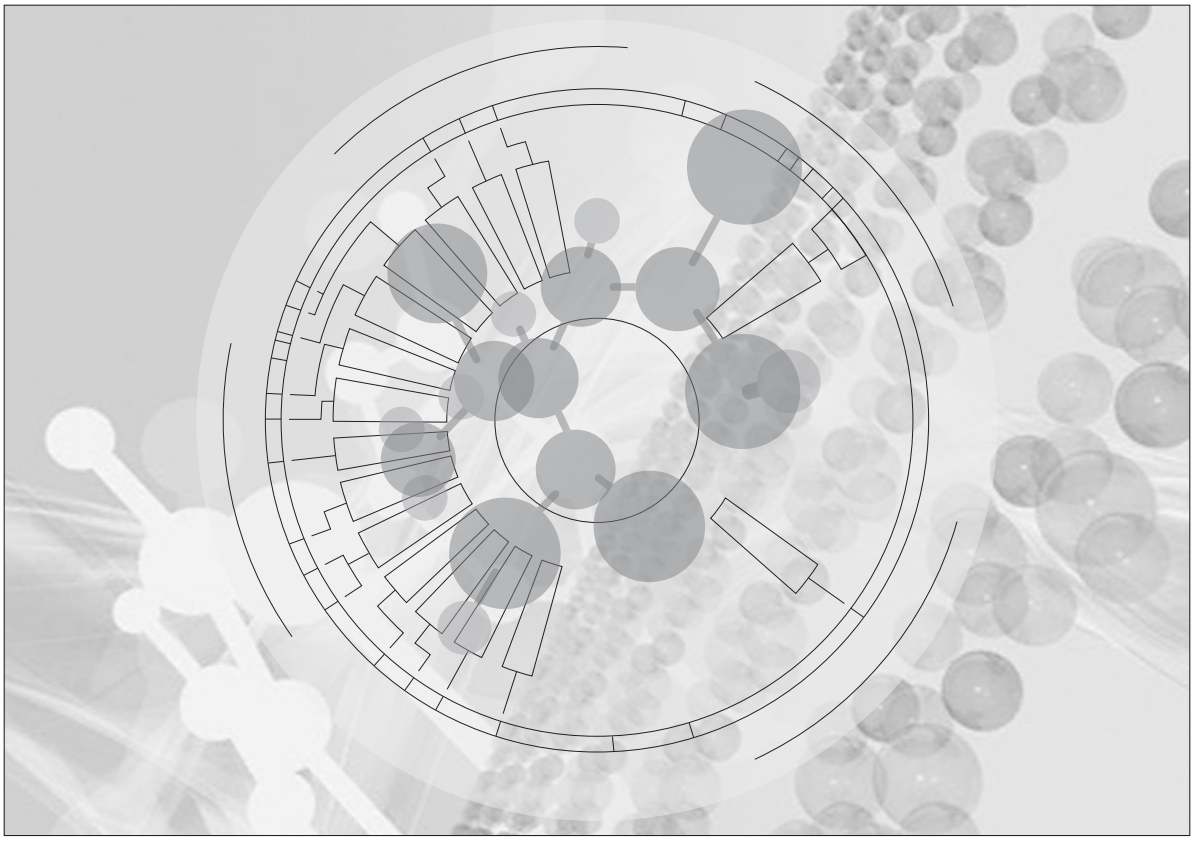

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Multitude of Genetic Regions Key to Embryonic Stem Cell Development Identified

More than 2,000 genetic regions involved in early human development have been identified by researchers at the Stanford University School of Medicine. The regions, called enhancers, are responsible for triggering the expression of distant genes when embryonic stem cells begin to divide to form the many tissues of a growing embryo.

"This is going to be an enormous resource for researchers interested in tracking cells involved in early human development," said Joanna Wysocka, PhD, Assistant Professor of Developmental Biology and of Chemical and Systems Biology. "It will be very interesting to learn how these enhancers affect gene expression in each cell type."

Wysocka is the senior author of the research, which will be published online Dec. 15 in Nature. Postdoctoral scholar Alvaro Rada-Iglesias, PhD, is the first author of the study.

The researchers also learned something interesting about human embryonic stem cells: They're not too shabby at planning ahead. The cells prepare for the demands of future embryonic development by priming a subset of enhancers for activation with proteins and chemical tags. These "poised" enhancers are simultaneously kept in check by other modifications that keep them inactive. When the modifications (also known as epigenetic changes) are removed, the enhancers can quickly trigger the expression of genes needed to toggle from a mere stem cell to a developing embryo.

The identification of the previously unknown enhancers, and the discovery of how they're kept quiet until needed, represent a moment of research serendipity. Wysocka and Rada-Iglesias didn't start out trying to identify enhancers involved in development. Instead, they were looking for regions that activated genes involved in the maintenance of the embryonic stem cell state.

"We are interested in understanding how genomic information is integrated with epigenetic changes to produce cell-type-specific regulation -- in this case in the embryonic stem cells," said Wysocka. "Often this regulation is accomplished via gene activation mediated by a distant enhancer."

But enhancers can be difficult to identify because they trigger the activation of genes tens to hundreds of kilobases away; it's rarely clear if and where enhancers of that gene might lie without conducting laborious genetic studies. Recent studies identifying specific protein and DNA modifications associated with active enhancers are making the process easier, though.

Rada-Iglesias mixed antibodies that specifically recognize epigenetic changes associated with active enhancers with extracts from human embryonic stem cells. He then used a technique called chromatin immunoprecipitation to remove the antibodies from the solution and catalogued the snippets of DNA that were included in the antibody complexes.

As expected, he found about 5,000 candidate active enhancers, which he termed class-1 elements. But 2,200 additional regions were more perplexing. These had two types of modifications -- both activating and inactivating. Some of these dually tagged DNAs, he noticed, were near genes known to be involved in early embryonic development. Rada-Iglesias called these regions class-2 elements.

Wysocka and Rada-Iglesias used a software program called GREAT (for Genomic Regions Enrichment of Annotation Tools) developed in the laboratory of Stanford researcher Gill Bejerano, PhD, Assistant Professor of Developmental Biology and of Computer Science, to analyze the categories of genes the two classes of enhancers might be controlling. They confirmed that the class-1 enhancers regulate genes active in embryonic stem cells, while the class-2 enhancers are associated with genes involved in processes such as gastrulation and the formation of germ layers -- events that occur very early in development.

"When we compared the expression of the genes controlled by class-1 and class-2 elements, we found that the class-1 genes were active, as you would expect in human embryonic stem cells, and the class-2 genes were inactive," said Wysocka.

When the researchers triggered the differentiation of the embryonic stem cells into a cell type called neuroectoderm, however, about 200 of the class-2 enhancers began to display a class-1, or activated, signature, and their associated genes were turned on. The researchers expect that specific sets of class-2 elements are activated during the development of various tissue types during embryogenesis.

"These class-2 elements are clearly poised to orchestrate development in a cell-type-specific manner," said Wysocka.

Finally, the researchers attached individual enhancers to a reporter gene that would glow green when expressed. When they introduced the enhancer-reporter constructs into one-celled zebrafish embryos and allowed the embryos to develop, they saw a pattern of expression that was developmentally specific in location and timing and mimicked the expression of nearby developmental genes involved in normal fish embryogenesis.

"It's clear that these enhancers are becoming active at specific times during development," said Wysocka. "Now we have over 2,000 elements that can be used to study development and isolate transient cell populations."

In addition, Wysocka and Rada-Iglesias and their colleagues are now interested in identifying the mechanism that triggers the switch of the class-2 elements from an inactive to an active state, as well as how the poised state is initially set up at these elements in the embryonic stem cells.

In addition to Rada-Iglesias and Wysocka, other Stanford researchers involved in the work include Postdoctoral Scholars Ruchi Bajpai, PhD, and Samantha Brugmann, PhD; Senior Research Scientist Tomek Swigut, PhD; and Medical Student Ryan Flynn.

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Alvaro Rada-Iglesias, Ruchi Bajpai, Tomek Swigut, Samantha A. Brugmann, Ryan A. Flynn, Joanna Wysocka. A unique chromatin signature uncovers early developmental enhancers in humans. Nature, 15 December 2010 DOI: 10.1038/nature09692