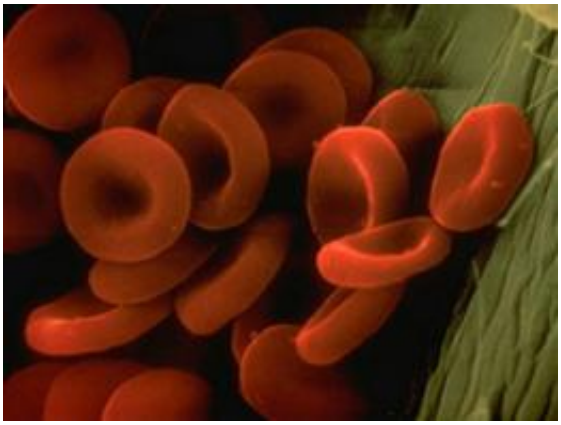
New research into the mechanisms of gene regulation

November 19 2009, by Pat Shipman



The team of scientists used red blood cells from a mouse to study the mechanisms of gene regulation. The research is expected to help in the development of new therapies to treat people who suffer from sickle-cell anemia and other blood disorders. Credit: Wikipedia Commons (Public Domain)

(PhysOrg.com) -- A team led by Penn State's Ross Hardison, T. Ming Chu Professor of Biochemistry and Molecular Biology, has taken a large step toward unraveling how regulatory proteins control the production of gene products during development and growth. Working with collaborators including Drs. Mitchell Weiss and Gerd Blobel at Children's Hospital of Philadelphia, they focused specifically on the complex process of producing red blood cells (erythrocytes). These cells contain large amounts of hemoglobin, a molecule essential for transporting oxygen throughout the body. Abnormalities in hemoglobin

figure in many serious diseases, such as sickle-cell disease, and abnormalities in producing blood cells can lead to leukemias. The work will be published in the December 2009 issue of the journal *Genome Research*.

As erythroid cells mature into red blood cells, the transcription factor, GATA-1, turns the genes responsible for making different proteins on and off. Hardison's team worked with a special strain of mouse erythroid cells that lack the gene gata-1. These cells could not mature into red blood cells unless the researchers added the protein GATA-1 experimentally. This procedure allows the investigators to monitor how the genes respond to GATA-1.

GATA-1 binds to special sites on the cell's DNA. The first step of the project was to locate the genes that are affected by GATA-1, so the researchers conducted a genome-wide search after adding GATA-1 to the cells. "Using microarrays developed by newer methods of manufacturing which allow for a much higher density of probes, the team examined 19,000 mouse genes using 45,000 probe sets, many more than previous researchers had been able to study."

They found that adding GATA-1 affected 2,616 genes significantly, which was defined as showing at least a twofold change in the amount of the gene's product. Substantially more genes (1,568) were turned down or off by GATA-1 than were turned on (1,048) by GATA-1. The rest showed little or no response to the addition of GATA-1.

Could the differences in gene response and expression be explained by the spatial patterning of GATA-1 binding? The team used two independent methods of mapping where the GATA-1 binding sites lay on the DNA, and how far those binding sites were from the responsive genes. The two methods yielded strikingly similar information. In all, they found 15,360 DNA segments occupied by GATA-1.

Genes responsive to GATA-1 tend to occur in clusters on the DNA, with each cluster being separated from the next by long regions without any responsive genes.

"We think each cluster of responsive genes may represent a regulatory domain," says Hardison. "This work also highlights the importance of local regulation by transcription factors."

One of the fascinating discoveries of this research is how the genes that are enhanced by GATA-1 differ from those that are repressed. Nearly all responsive genes (more than 88%) lie within 100 kilobase pairs (kb) of a GATA-1 site and more than 60% of those that are up-regulated are less than 5 kb away from the closest GATA-1 site. In contrast, 60% of repressed genes are up to 33 kb away from the closest DNA sites occupied by GATA-1. Enhanced genes also have more nearby GATA-1 sites than repressed genes do.

The team also found evidence that the specific motif on the DNA sites to which the GATA-1 binds when it enhances genes is strongly conserved evolutionarily, while the binding motifs for repressed genes are not so strongly conserved across different species. This suggests that the selective pressure to increase gene expression is stronger than that to decrease gene expression.

Another issue is whether or not GATA-1 forms a complex with TAL1 (T-cell acute lymphocytic leukemia protein 1) at the binding sites. Almost all (at least 83%) of the activated genes have one or more complexes of GATA-1 and TAL1 nearby. A substantial fraction (38%) of the repressed genes lack or have decreased amounts of TAL1 after GATA-1 is added. Thus for many genes, the presence of TAL1 at the GATA-1 binding sites distinguishes activation from repression. However, another class of repressed genes, comprising 62% of the total, have complexes of GATA-1 and TAL1 in their vicinity.

"It is not a simple relationship," explains Hardison. "Genes that are activated almost invariably have complexes of GATA-1 and TAL1 at the binding sites, and genes lacking TAL1 at the GATA1-binding sites are almost always down-regulated. This suggests a model in which the GATA-1/TAL1 complex is involved in gene activation. However, we also see a large number of repressed genes with both proteins at the binding sites. These data indicate that there must be at least two mechanisms for repressing genes in these red blood cells, and only one of those mechanisms involves a loss of TAL1," concludes Hardison.

Another factor involved in regulating the expression of genes responsive to GATA-1 is the modification of histone H3, a protein that coils and compresses DNA inside the nucleus. Overall, Hardison's team found that histone modification is common in the large regions of the DNA that contain the GATA-1 responsive genes and the GATA-1 binding sites, but histone modification does not occur in the adjacent "dead zones" that lack genes responsive to GATA-1.

One particular histone modification, the trimethylation of amino acid lysine at position 27 of histone H3 -- or H3K27me3 -- is well-known to be associated with repression of some genes. By analyzing the location and amount of H3K27me3 around GATA1-responsive genes, the team gained new insights into how this modification influences the expression of genes. As expected, genes enhanced by GATA-1 have very little H3K27me3. However, the two classes of repressed genes differ in the level of H3K27me3. Like enhanced genes, the repressed genes that retain TAL1 after the addition of GATA-1 have low levels of H3K27me3. Those genes that lose some or all of their TAL1 after adding GATA-1 have relatively high levels of H3K27me3.

One class of down-regulated genes, perhaps the one with low TAL1 and increased H3K27me3, may be directly repressed by GATA-1. Possibly GATA-1 recruits other proteins that help reduce the level of expression.

This repressed state appears to be indicated by the presence of H3K27me3.

In addition, the team hypothesizes that there is another, indirect mechanism for repressing genes. They suggest that the capacity for a cell to transcribe genes and manufacture their products is limited. In effect, if the cell has a series of transcription factories -- each of which has particular favored "customers" (genes) -- then placing a big order for one set of customers (the genes promoted by GATA-1) will indirectly cause the gene products for other customers (those not being promoted by GATA-1) to be diminished. This scenario implies that all the GATA-1 responsive genes are served by a limited number of local transcription factories with limited capacities.

"Though we developed this hypothesis about gene regulation by studying erythrocytes," comments Hardison, "it could obviously apply to the more general function of many different types of gene regulation. Since almost every disease is related in some way to gene expression, this could provide a powerful new model for thinking about many different diseases and their treatment.

"For example," Hardison continues, "sickle-cell disease is a devastating illness affecting more than 70,000 Americans1. It is caused by mutations in hemoglobin genes. All adults still produce some amount of fetal hemoglobin, and that amount differs among individuals. Some sickle-cell patients produce relatively high levels of it, and these patients have much milder symptoms. If we could learn how to repress the genes that produce the defective hemoglobin and promote those that produce the fetal hemoglobin, we may be able to develop an important new therapy to combat this disease."

Source: Pennsylvania State University (news : web)