CTCF, a Novel Regulator of Alternative Splicing

In a study published in the November 3, 2011, issue of *Nature*, Shalini Oberdoerffer, Ph.D., of CCR's Mouse Cancer Genetics Program, and Sanjeev Shukla, Ph.D., a Postdoctoral Fellow in her lab, investigated how exons with weak splicing signals are included using the *CD45* gene as a model system. At different stages of lymphocyte development, exons 4, 5, and 6 are specifically incorporated or excluded from the CD45 mRNA. Oberdoerffer previously found a protein, hnRNPLL, regulates exons 4 and 6, but the mechanism for regulating exon 5 was unclear.

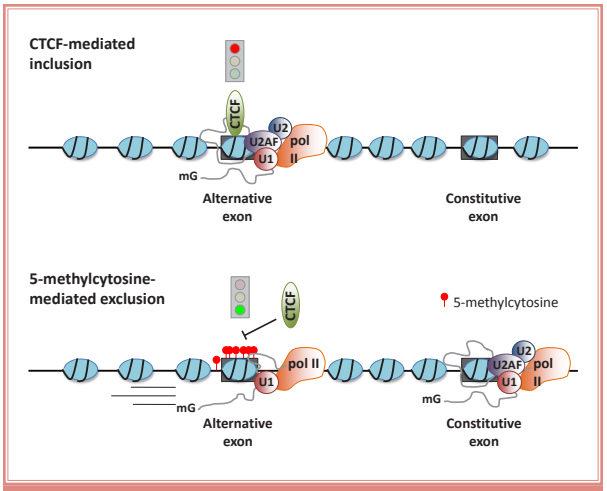
Analyzing previously published data, the researchers found that the DNA-binding protein CTCF, which is thought to shield inactive regions, had a strong interaction with *CD45* exon 5, even in cells expressing high levels of CD45 protein. This interaction was also observed in mouse immune cells. Contrary to previous studies, this data suggested that CTCF binding may play an important role in exon 5's inclusion in the CD45 mRNA transcript.

Next, by examining Burkitt lymphoma cell lines with variable exon 5 exclusion the researchers found that exon 5 was more likely to be included when CTCF was bound and that in cells where CTCF failed to bind CD45-5 expression was reduced. One way CTCF binding might help incorporate exon 5 is by affecting the activity of RNA polymerase II (pol II), the enzyme that produces mRNA transcripts. More active pol II was associated with exon 5 in cells expressing high levels of CD45 that incorporates exon 5 (CD45-5), which suggests that pol II spends more time, or pauses, at exon 5 in these cells. Depleting CTCF protein reduced pol II binding at exon 5. By slowing down pol II, CTCF could provide time for the splicing machinery to recognize the exon 5 splice site and incorporate exon 5 into the CD45 mRNA transcript.

The researchers then investigated how CTCF binding to exon 5 is regulated since CTCF is always expressed but exon 5 is only included in the CD45 transcript at certain stages of lymphocyte development. The addition of a methyl group to DNA nucleotides is known to interfere with CTCF binding, and in cell lines with methylated DNA at exon 5, CTCF failed to bind. To see whether this was also the case in normal lymphocytes, the investigators studied T cells that expressed higher or lower levels of CD45-5 and observed increased methylation and reduced CTCF binding in the T cells expressing less CD45-5. Inhibiting DNA methylation in the cells expressing lower CD45-5 increased CTCF binding and pol II pausing at exon 5. Importantly, these results are the first to link the processes of DNA methylation and alternative mRNA splicing.

Since CTCF binding sites are located in the exons of genes other than *CD45*, the researchers reduced CTCF levels in cell lines and then looked for RNA sequences that differed with the loss of CTCF. The researchers determined that exons with a CTCF binding site downstream were preferentially excluded when CTCF was depleted. Similar to CD45 exon 5, CTCF binding downstream of these exons induced pol II pausing.

These studies have revealed the importance of CTCF in the inclusion of alternative exons. Changes in CTCF function may play a critical role in diseases such as cancer where altered splicing and DNA methylation patterns have been observed.



(*Figure*: S. Oberdoerffer, CCR)

By binding downstream of some alternative exons, CTCF causes RNA polymerase II (pol II) to pause giving components of the splicing machinery time to incorporate the alternative exon. When the CTCF binding site is methylated, however, CTCF cannot bind, pol II does not pause, and the alternative exon is not incorporated into the transcript.

To learn more about Dr. Oberdoerffer's research, please visit her CCR Web site at http://ccr.cancer.gov/staff/staff.asp?Name=soberdoerffer.