Researchers demonstrate double-lock protection mechanism in crucial cellular switches

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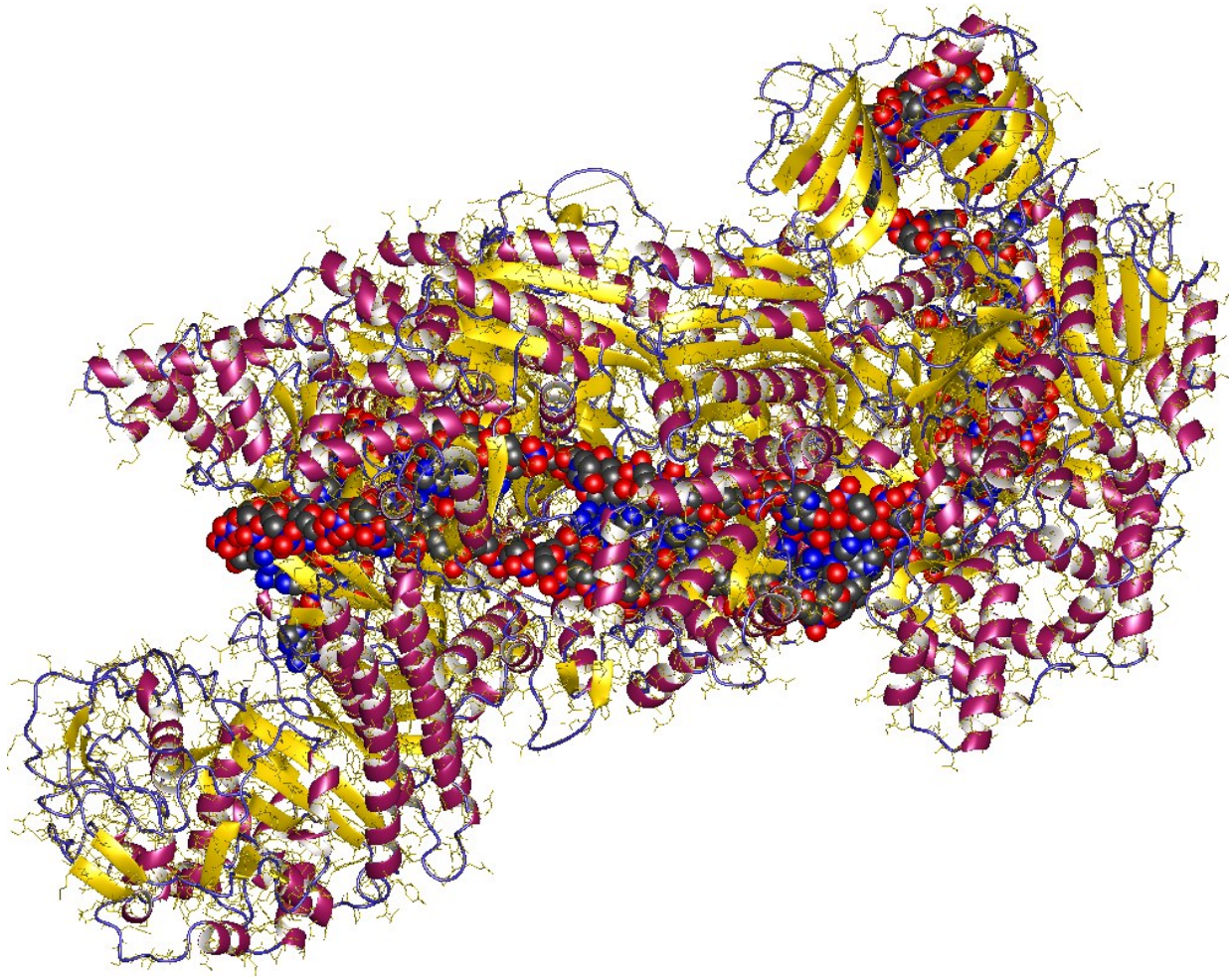

CRISPR (= Clustered Regularly Interspaced Short Palindromic Repeats) + DNA fragment, E.Coli. Credit: Mulepati, S., Bailey, S.; Astrojan/Wikipedia/ CC BY 3.0

Ludwig-Maximilians-Universitaet (LMU) in Munich researchers have used CRISPR technology to probe the mechanisms that guide the developmental trajectories of stem cells in the brain. The results show that crucial cellular switches are doubly protected against unintended activation.

Higher eukaryotic organisms are made up of a wide variety of specialized cell types. The precise identity and function of each individual cell is established by molecular processes that control gene expression during the course of its development. Proteins known as master transcription factors play a vital role in this context. They are the key switching elements that initiate the specific genetic programs which determine cell identity. The genes that code for these proteins must therefore be tightly controlled, as their improper activation could endanger the integrity of the entire organism by effectively overriding the previously established cell state. Researchers led by Dr. Stefan Stricker of the Munich Center for Neurosciences in LMU's Biomedical Center and the Helmholtz Zentrum München have now shown that the progenitors of nerve cells use a double-lock mechanism to avoid untimely production of master transcription factors. The study appears in the online journal *Nature Communications*.

The project actually set out to address a different question. "We initially wanted to investigate how genes can be turned on and off with the help of the CRISPR-Cas9 technique," Stricker says. In order to test technical aspects of the methodology in a system that is directly relevant for the differentiation of cell types, he and his colleagues chose the mouse Sox1 gene as their target. This gene codes for a master transcription factor that is known to be active in neural stem cells (NSCs). In all other classes of neural cells, including the somewhat more mature neuronal progenitor cells (NPCs), Sox1 is repressed. In the absence of the Sox1 protein,

NSCs progressively lose the capacity to differentiate into neurons that transmit electrical signals.

Using the CRISPR/Cas9 approach, the researchers guided a trans-activating protein specifically to the Sox1 gene in NPCs in which the gene is normally inactive. Many other genes have already been shown to be activated by this method, but Sox1 responded very poorly. This finding suggested that reactivation of Sox1 in NPCs might be prevented by some special mechanism. To test this hypothesis, the LMU team then focused on the methylation pattern around the Sox1 gene in NPCs. Methylation—the addition of methyl (CH_3) groups to certain nucleotide bases in the genomic DNA—can play a significant role in the epigenetic inactivation of genes. "To explore the role of methylation, we again made use of CRISPR-Cas9, this time to remove methyl groups from the DNA around the Sox1 gene," Stricker explains. "And indeed, the combination of targeted demethylation and transactivation allowed us to reactivate Sox1. This is essentially equivalent to the rejuvenating the cells, as revealed by the fact that they recovered their stem-cell properties and were able to differentiate into neurons."

The authors of the new study believe that, in addition to inhibiting expression, cells impose a specific repressive chromatin layer on genes that control cell identity, which protects them from inadvertent activation. Together, these measures act as a double lock to prevent reactivation of such genes after they have done their job. Nevertheless, the ability to reactivate repressed genes might also make it possible to cause neuronal progenitor cells revert to the stem-cell state. Such rejuvenated stem cells would have great therapeutic potential. "But the route from our basic research to the application of reactivated stem cells is a very long one," Stricker cautions.

More information: Valentin Baumann et al, Targeted removal of epigenetic barriers during transcriptional reprogramming, *Nature*

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