Keeping Up with Live Cell DNA Looping

POSTED JUNE 25, 2013

The reality TV era has made voyeurs of us all. So it makes sense that we'd want to take a peek inside live cells too. Now comes a report of a new method that shows, for the first time, DNA getting loopy inside live E. coli cells.

Johns Hopkins University Med School researchers say that several in vitro studies suggest that DNA loops around to bring two distant sites together, which can affect transcription. But no one had ever directly seen this happen in live bacterial cells. Until now.

The talented JHU crew developed a high-res imaging method with two different fluorescent protein tags. The scientists looked at the genetic switch of bacteriophage λ, in which the CI repressor binds to operator sequences that are 2.3 kb apart, bringing them together in a loop inside E. coli cells. The method has a precision of a few tens of nanometers.

Here's what lead author Jie Xiao, assistant prof at JHU, had to say in a short interview with EpiGenie:

Keep in the DNA Loop with Direct Imaging

“This is the first work visualizing DNA looping dynamics in live cells on such a short length scale. DNA looping mediated by transcription factors has long been speculated to occur in live cells, and all very clever in vivo and in vitro experiments do point to its presence. The first work suggested its existence is by Bob Schleif and his colleagues about 30 years ago. We are very pleased that now we were able to prove this in the classic system with the direct imaging method. Perhaps most importantly, we were able to measure both the looping frequency and the corresponding gene expression level independently, and hence provide the direct evidence of how DNA looping regulates gene expression. In previous studies the looping frequency is always inferred from gene expression levels of different DNA mutants based on a priori assumption of DNA looping states.”

Chromosomal DNA Runs a Tight Ship

“One very surprising thing we discovered from this study is that the chromosomal DNA is even more compact than we expected. We know that chromosomal DNA is highly compact, but not to the level we measured. If we model the DNA as a non-interacting worm-chain, based on our measurement of the end-to-end separation of the 2.3 kb DNA, the apparent persistent length would be only ~ 5 nm, which is physically impossible. This highlights the importance of DNA-organizing proteins and also possibly negative supercoiling in organizing the chromosome.”

Stay Tuned for Precise DNA Visualization

“This method seems simple and straightforward, but in practice it needs very careful tuning. The
critical factor in determining the position of DNA in live cells with high precision is to minimize the fluorescence background caused by the unbound fluorescent protein molecules, while at the same time ensuring the DNA binding sites are bound most of the time. This requires careful optimization of the expression levels of the fluorescent fusion proteins. This project would not be possible if it were not for the very talented students I have, especially the lead author of the work Zach Hensel."

Find out how to get DNA loops camera-ready at PLOS Biology, June 2013.

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