LS19-029 - Imaging of DNA folding by cohesin through time resolved single molecule light, atomic force and cryo-electron microscopy

Abstract

Almost every cell in our body stores the complete human genetic information in the form of 46 DNA molecules. These DNA molecules have a total length of two meters but must fit into a microscopically small cell nucleus. To achieve this, the DNA molecules are packaged into chromosomes, reducing their length approximately two hundred thousand-fold. This dramatic shortening is achieved through several mechanisms. One of these involves folding the DNA into loops. This occurs with the help of the protein cohesin, which acts like a tiny motor actively pumping DNA into loops. The resulting DNA loops contribute to DNA packaging but are also used for other important processes. Some of these DNA loops help to activate genes. When mutations occur in cohesin, this can impair the formation of DNA loops and thus gene regulation. Such cohesin mutations can contribute to the development of rare genetic diseases and certain cancers. In immune system cells, the formation of DNA loops is additionally used to create a large number of new gene variants, according to whose blueprints antibodies are produced. These play a central role in defending against pathogenic viruses and bacteria. Understanding how cohesin molecules form DNA loops is therefore an important goal of basic biomedical research. Despite the importance of these DNA loops, it was largely unknown at the beginning of this project how cohesin functions as a motor. To answer this question, the project team used methods that allowed visualization of how cohesin molecules form DNA loops. The basic idea of this approach was to understand through direct observation how the cohesin motor works, similar to how much could be learned about the function of a human-made motor simply by watching how it runs. In the case of the cohesin motor, the major challenge with this approach was that cohesin molecules are tiny. Therefore, the project team used high-resolution microscopy techniques that can visualize individual proteins, namely fluorescence microscopy, atomic force microscopy, and electron microscopy. By combining these methods, the project team succeeded for the first time in gaining insights into how the cohesin motor works. These approaches were complemented by computer simulations. The results of these investigations showed that individual cohesin molecules bind to DNA, "hold" it with one part of cohesin, and use other mobile parts of the cohesin motor to pump it into loops at high speed from one side. This process uses the universal "fuel" of cells, ATP, which releases energy when enzymatically cleaved by cohesin. These results make an important contribution to understanding biological processes at the molecular level. The application of high-resolution microscopy methods and modern computer simulations has also advanced basic research technically, as similar approaches can be used in the future to investigate other biological processes. For medicine, the results obtained in this project do not yet have any immediate applications. However, since medical applications can usually only be developed once the underlying biological processes are understood at the molecular level, the insights gained in this project may represent an important building block for such medical applications in the future.

Scientific disciplines:
Biochemistry (30%) | Biophysics (37%) | Structural biology (33%)

Keywords:
cohesin, cryo EM, single molecule FRET, high speed AFM

Principal Investigator:	Jan-Michael Peters
Institution:	IMP - Research Institute of Molecular Pathology
Co-Principal Investigator(s):	David Haselbach (IMP - Research Institute of Molecular Pathology) Peter Hinterdorfer (Johannes Kepler Universität Linz)

Status: Completed (01.05.2020 - 31.07.2024)
GrantID: 10.47379/LS19029

Further links to the persons involved and to the project can be found under
https://www.gmbh.wwtf.at/funding/programmes/ls/LS19-029/