Designer of protein factories exposed

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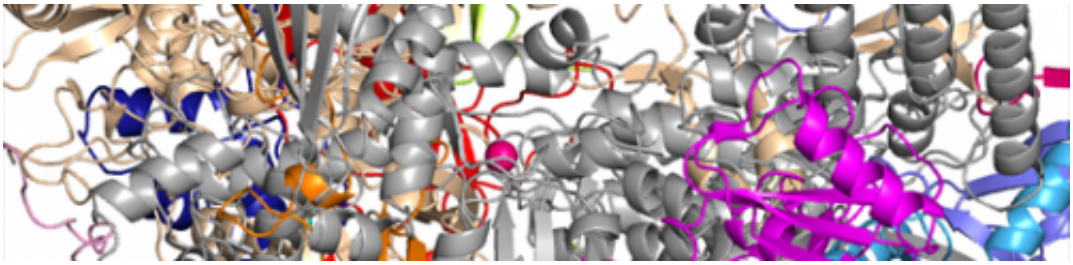

For 10 years, Patrick Cramer and his colleagues at Ludwig-Maximilians-Universitaet (LMU) in Munich have probed the structure of RNA polymerase I, a crucial cog in the machinery of all cells. Now they unveil the full three-dimensional conformation of the enzyme – at atomic resolution.

Actively growing cells must synthesize large amounts of protein, which in turn requires huge numbers of protein production plants, the so-called ribosomes. Ribosomes themselves consist of proteins mounted on a framework of ribosomal RNA. The latter accounts for as much as 60% of the RNA in cells, and is synthesized by the enzyme RNA polymerase I (Pol I). In the absence of ribosomal RNA, no protein synthesis can take place. Pol I is therefore a central pacemaker for cell growth, and aberrant hyperactivation of ribosome biogenesis is a hallmark of many types of cancer.

In spite of its pivotal role in the cell, the structure of the enzyme has remained poorly understood. As a very large and highly complex molecular machine, Pol I has so far proven resistant to conventional high-resolution structural analyses. But in a technical tour-de-force, Professor Patrick Cramer – the Director of LMU's Gene Center – and his team have now revealed its detailed architecture. In an article in the latest issue of *Nature*, the researchers describe the complete three-dimensional structure of Pol I – at a resolution that not only allows them to localize all of its 14 subunits, but also to define the positions of its approximately 35,000 (non-hydrogen) atoms. The resulting model provides detailed insights into the enzyme's mode of action.

X-rays elucidate crystal structure

"The decisive breakthrough was the result of 10 years of hard work, which taught us how to grow high-quality crystals of the enzyme that were amenable to high-resolution structural analysis by X-ray diffraction. The size and complexity of Pol I made this a very difficult task," as Cramer explains. The crystals are composed of many copies of the Pol I complex, symmetrically arranged in a lattice structure. When such a crystal is exposed to an X-ray beam, part of the radiation is deflected by the atoms in the regularly arrayed protein molecules. The scattered rays may reinforce or interfere with each other, giving rise to characteristic diffraction pattern that can be captured on film. Mathematical analysis of the pattern and intensity of the diffraction spots then permits the spatial disposition of the atoms in the protein complex to be reconstructed.

The results of the analysis revealed some interesting structural differences between Pol I and the related RNA polymerase II (Pol II), which is responsible for the synthesis of the messenger RNAs that act as the immediate blueprints for protein synthesis. Cramer had determined the structure of Pol II in the year 2000, while he was a postdoc at

Stanford University in California.

An open-and-shut case

Among other things, Pol I differs from Pol II in having several extra elements in its active center, which are involved in the regulation of the enzyme. By modulating the form of the entrance to the active site (which lies in a deep cleft) they enable it to adopt an "open" or a "closed" conformation. It turns out that the enzyme is inactive in the "open" state. The researchers suggest that this phenomenon allows Pol I activity to be inhibited, thus preventing uncontrolled cell growth and proliferation. It is conceivable that this mechanism might serve as a target for the development of new drugs that could retard the growth of tumors. Thus the new structure could point the way to novel agents for the treatment of cancer.

"With this conformational switch between inactive and active states, we appear to have stumbled on a general mechanism that regulates the expression of genetic information in the cell," Cramer says. He and his associates will now turn their attention to the problem of how the polymerases recognize their respective target genes, with a view to understanding how related polymerases have become specialized for the production of functionally distinct classes of RNA. Their ultimate goal is to depict the complete sequence of events that leads to the activation of a gene only when its product is required in the cell concerned.

More information: www.nature.com/nature/journal/ … ull/nature12712.html

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