Researchers learn from nature

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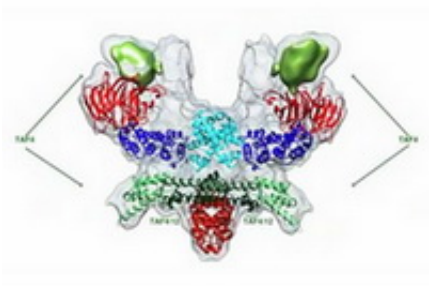

Credit: Gabor Papai-IGBMC

A research team of scientists from the European Molecular Biology Laboratory of Grenoble and the Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) in Strasbourg have now, for the first time, succeeded in describing in molecular detail the architecture of the central scaffold of human TFIID, the protein complex essential for transcription from DNA to mRNA. The Human TFIID is a megadalton-sized multiprotein complex composed of TATA-box-binding protein (TBP) and 13 TBP-associated factors (TAFs). Despite its crucial role, the detailed architecture and assembly mechanism of TFIID remain elusive.

This was done by applying innovative methods. Scientists coaxed insect cell cultures which they infected with a custom designed baculovirus to

produce their TFIID complex in the quality and quantity required for detailed studies. This has opened new perspectives in the study of transcription and of the structure and mechanism of other large multi-protein assemblies involved in gene regulation.

'Some time ago, we had developed our MultiBac system which uses a custom engineered recombinant baculovirus to express protein complexes, and many researchers all around the world applied it highly successfully to produce complicated biological samples that they could not make before.' says Imre Berger, who lead the study at the EMBL Grenoble. 'However, for our human TFIID complexes, even this successful method was just not good enough.'

The scientists realised the insect cell cultures infected with recombinant baculovirus, were simply unable to produce the TAFs in a balanced fashion. Instead, some were produced in high amounts, and some in very low amounts, resulting in the complex not forming properly. The solution to this bottleneck came from studying the strategy certain viruses such as Coronavirus use to make their proteins. When Coronavirus replicates, it produces very large polyproteins that are then cut apart by a highly specific protease into the individual enzymes and protein factors that Coronavirus needs.

'There were numerous theories, based on scant data, trying to rationalize how this essential complex is held together' explains Christoph Bieniossek, first author of the study. 'Our analyses show that basically none of these theories were correct. The way how the TAFs assemble tuned out to be much more complex than previously assumed.'

The authors further describe how this TFIID core complex, which they found to have two-fold symmetry, becomes asymmetric when it grows by accreting further TAFs, on its way to the complete holo-TFIID. Two subunits, separately imported into the nucleus, bind exactly at the two-

fold axis present in TFIID core, leading to large structural rearrangements but only in one half of the complex. By this simple yet elegant mechanism, the two halves of the complex which were previously identical adopt different shapes, and the resulting asymmetry is then propagated until functional holo-TFIID complex is formed.

'This work is the result of many years of intense effort, and it was only possible in this fruitful and exciting collaboration with our colleagues at the IGBMC who are leaders in human transcription factor research.' concludes Imre Berger. 'We know now in some detail how the core of TFIID looks like, and what happens when further TAFs are bound. We believe that we have opened the door to working out the architecture of the entire human TFIID complex in the near future, and likewise the other large multiprotein assemblies involved in gene regulation, and explain their roles in catalyzing biological function.'

The study is published today in the journal *Nature*.

More information: ComplexINC project complexinc.eu/innovation/

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