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**MS7-O2 Recombinant multiprotein complex production: identification of a novel building block of the general transcription factor TFIID**

Simon Trowitzsch¹

1. Institute of Biochemistry, Goethe Universität Frankfurt

email: trowitzsch@biochem.uni-frankfurt.de

Multiprotein complexes are a cornerstone of biological activity, as many proteins appear to participate stably or transiently in large multisubunit assemblies. Analysis of the architecture of these assemblies and their manifold interactions is imperative for understanding their function at the molecular level. Powerful recombinant protein production technologies, e.g. MultiBac - a baculoviral expression vector system for multiprotein complex production, are constantly being developed to produce material in great enough quantities for structural and functional studies.

Here, I will present our recent work¹ on the general transcription factor IID (TFIID), which plays a key role in RNA polymerase II transcription initiation in eukaryotic cells. Human TFIID is a megadalton-sized multiprotein complex composed of the TATA-binding protein (TBP) and 13 TBP-associated factors (TAFs). How these individual proteins assemble into a functional transcription factor is poorly understood to date. We identified a heterotrimeric TFIID subcomplex consisting of the TAF2, TAF8 and TAF10 proteins, which assemble in the cytoplasm. This heterotrimeric TAF complex was produced recombinantly in insect cells using the MultiBac system. By means of native mass spectrometry, we defined the interactions between the TAFs and uncovered a central role for TAF8 in nucleating the complex. X-ray crystallography reveals a non-canonical arrangement of the TAF8-TAF10 histone fold domains. Binding assays including peptide arrays and surface plasmon resonance experiments showed that TAF2 binds to multiple motifs within the TAF8 C-terminal region. These interactions direct the incorporation of TAF2 into a core-TFIID complex that exists in the nucleus. Our results provide evidence for a stepwise assembly pathway of nuclear holo-TFIID, regulated by nuclear import of preformed cytoplasmic submodules.


Keywords: multiprotein complexes, transcription, TFIID, BEVS, MultiBac