# It takes ten to tango: the structure of transcription factor TFIIH

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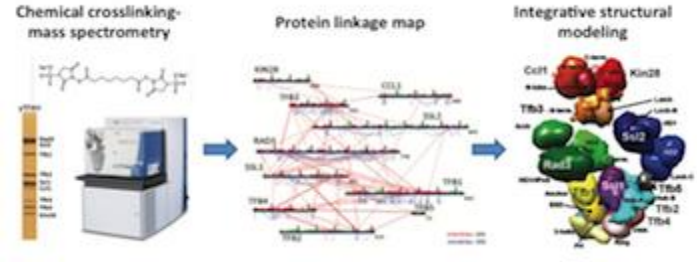


Workflow for determining the structure of the transcription factor TFIIH.

*Image provided by Dr. Jeff Ranish (Institute for Systems Biology, Seattle, WA).*

The maintenance and expression of genetic information stored in DNA depends on the function of many essential enzymes and proteins. The highly conserved general transcription factor TFIIH is required for transcription of DNA by RNA polymerase II (Pol II) as well as DNA repair. TFIIH consists of ten protein subunits, several of which have enzymatic activity. The XPB/Ssl2 and XPD/Rad3 (human/yeast) subunits are important for opening the DNA for transcription or repair, while the Cdk7/Kin28 kinase phosphorylates the C-terminal domain (CTD) of Pol II to initiate elongation.

The structure and function of TFIIH complex components have been studied extensively biochemically; however, an overall map of its architecture has only recently been attempted. Dr. Jeff Ranish (Institute for Systems Biology), along with collaborators including Dr. Steve Hahn (Fred Hutch, Basic Sciences Division), knew that a high-resolution structure would identify specific protein-protein interactions that are essential for TFIIH function.

Thus, researchers in the Ranish Lab at ISB performed cross-linking/mass spectrometry (CXMS) on purified TFIIH complex from budding yeast and human cells, prepared in collaboration with researchers at University of Colorado, Boulder. In order to further increase their resolution, authors in the Sali Lab at UCSF used an integrative modeling approach to build a structure of TFIIH. Integrative modeling finds solutions using all input data simultaneously and this often builds models that are more precise and complete. Using this method, researchers in the Sali Lab used all the data available to them, including the CXMS data from the Ranish Lab, published EM densities, X-ray crystallographic data, as well as other types of data, to find solutions that fit well to a comprehensive model of human or yeast TFIIH. Ultimately, they were able to identify the locations

and positions of each of the ten subunits of TFIIH, with the highest resolution for the protein XPD/Rad3 at 9.2Å and 17.7Å (human/yeast). They observed that the overall organization of the subunits was very similar between yeast and human TFIIH, however there was a difference in the overall shape of the complex. This could reflect differences in the preparation of the complexes for EM or may reflect real differences in structure.

The authors were very interested in mapping conserved interfaces between subunits of TFIIH because combining the data could reveal essential interactions. Interestingly, human disease mutations have been mapped to non-enzymatic components and therefore suggest that defects in the organization of the complex can have profound effects on the function of TFIIH. The authors mapped their crosslinks onto the aligned sequences of human and yeast TFIIH and found that many represented novel interactions between specific domains within and between subunits. Overall, the authors found four regional hubs of protein-protein interactions, naming them the Anchor, the Hub, the Lock and the Latch.

The Anchor consists of an unstructured region of p62/Tfb1 that contacts domains in XPD/Rad3, p44/Ssl1, and p34/Tfb4. The authors notice that there are not many crosslinks within p62/Tfb1 itself, suggesting that the unstructured Anchor region protrudes from the rest of protein. The Hub region is found in the C-terminus of the p52/Tfb2 protein and connects the XPB/Ssl2 subunit to the base of TFIIH, the p44/Ssl1-p34/Tfb4 dimer. The Lock region includes the N-terminal and C-terminal extensions of XPB/Ssl2 and shows a considerable number of interactions with other subunits as well as with each other. The authors suggest that these interactions may regulate the enzymatic activity of XPB/Ssl2. Finally, the authors found that the Latch region of MAT1/Tfb3 links Cdk7/Kin28-CyclinH/Ccl1, a Cdk-Activating Kinase (CAK) module, to core TFIIH through interactions with XPB/Ssl2, XPD/Rad3 and p44/Ssl1.

To further understand the function of the p62/Tfb1 protein, visiting Masters student Marine Dehecq (Université Paris) performed a deletion analysis of yeast Tfb1 while in the Hahn Lab (Basic Sciences Division) and found that a central part of the Anchor region is required for cell viability. This is particularly striking given that deletion of other known interaction domains in Tfb1 is not lethal but instead causes sensitivity to UV light. Furthermore, the authors showed that deletion of the Anchor domain in yeast Tfb1 and human p62 resulted in loss of co-IP with XPD/Rad3. These results show that the Anchor region is essential for TFIIH structural integrity.

Finally, the authors were able to map the position of disease-associated mutations in human patients with autosomal recessive diseases such as *Xeroderma pigmentosum* (XP) onto their model. Their

structural data suggests that some mutations associated with XP specifically disrupt the interaction of the Anchor protein p62/Tfb1 with XPD/Rad3, and not p44/Ssl1 as previously suggested. This work highlights the value of detailed structural information in the study of essential eukaryotic protein complexes.

Luo J*, Cimermancic P*, Viswanath S, Ebmeier CC, Kim B, Dehecq M, Raman V, Greenberg CH, Pellarin R, Sali A, Taatjes DJ, Hahn S, Ranish J. 2015. Architecture of the human and yeast general transcription and DNA repair factor TFIIH. Molecular Cell. 59:794-806.

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