The Centenary Award presented by Professor Patrick Cramer

Structure determination of transient transcription complexes

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Abstract
The determination of detailed 3D structures of large and transient multicomponent complexes remains challenging. Here I describe the approaches that were used and developed by our laboratory to achieve structure solution of eukaryotic transcription complexes. I hope this collection serves as a resource for structural biologists seeking solutions for difficult structure determination projects.

Introduction
Our laboratory studies the mechanisms of gene transcription and its regulation in eukaryotic cells with a combination of *in vitro* and *in vivo* approaches. We use integrated structural biology to derive the molecular basis of gene transcription. We also use functional genomics and computational biology to investigate the principles of genome transcription and RNA metabolism. We have recently reviewed structural studies of transcription initiation by RNA polymerase (Pol) II [1], the structural basis for mRNA chain elongation [2], and structural studies of the transcriptional coactivator complex Mediator [3]. We also summarized structural results in movies that can be used for teaching the mechanisms of gene transcription [4,5], and we compared the Pol II machinery with the alternative transcription machineries of Pol I and Pol III [6].

In my award lecture I concentrate on the most recent work from the laboratory from both structural biology and functional genomics. Here I summarize the technical and methodological advances in structural biology that we made over the years and that were necessary to enable structure determination of large and transient multiprotein transcription complexes. It turns out that a different approach had to be taken for each new structure solution. I hope the collection of examples put together here will serve as a resource for structural biologists.

Key words: cryo-electron microscopy, eukaryotic RNA polymerase complex, integrated structural biology, multiprotein assembly, X-ray crystallography.
Abbreviations: cryo-EM, cryo-electron microscopy; NTP, nucleoside triphosphate; Pol, polymerase.
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Crystal shrinkage: core Pol II
As a postdoctoral fellow with Roger Kornberg at Stanford University, I was concerned with the crystal structure determination of the 10-subunit core Pol II enzyme from yeast [7–9]. A prerequisite for structure determination of the 10-subunit core Pol II enzyme was its availability in large quantities. To this end, the Kornberg laboratory used large-scale yeast fermentation and affinity purification of the endogenous enzyme based on an antibody from the Burgess laboratory [10]. Crystals of the core Pol II had been obtained in the mid-nineties but their diffraction was poorly reproducible and the resolution insufficient for high-resolution structure determination [10].

The trick to obtain better diffraction was to induce crystal shrinkage with a special crystal treatment and cryo protection protocol [7,8]. I had by accident come across the phenomenon of crystal shrinkage during my doctoral work on crystals of a transcription factor–DNA complex [11], and first thought that I had discovered something really new. However, I then found in the literature that shrinkage of haemoglobin crystals had been described by Max Perutz already in the fifties. Crystal shrinkage was the key to solve the core Pol II structure, but phasing also turned out to be difficult. In particular, it required a search for non-standard heavy metal derivatives such as water-soluble iridium and rhenium compounds [7]. Furthermore, model building was facilitated by incorporating selenomethionine into the endogenous yeast complex [12]. Crystal shrinkage dramatically improved diffraction, but only later we learned how. In the core Pol II enzyme, the so-called clamp domain is mobile, but is trapped by crystal contacts. Crystal shrinkage led to a further opening of the clamp and additional crystal contacts by the clamp, giving a more stable lattice.

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Combining samples: complete Pol II

When I started to set up our laboratory at the University of Munich (LMU), our first project was to arrive at the atomic structure of the complete Pol II enzyme comprising all 12 subunits. Pol II contains, in addition to its 10-subunit catalytic core, a subcomplex of two subunits called Rpb4 and Rpb7. For a long time, it was difficult to obtain Pol II with stoichiometric amounts of Rpb4/Rpb7. We therefore reconstituted the complete 12-subunit Pol II from the endogenous 10-subunit yeast core enzyme and a recombinant Rpb4/Rpb7 subcomplex that we obtained after co-expression of their subunits in *E. coli* [13]. The resulting complex was homogeneous and crystallized. However, the resolution was limited to approximately 4 Å (1 Å=0.1 nm), preventing model building. We therefore crystallized the recombinant Rpb4/Rpb7 subcomplex in isolation, and obtained a high-resolution structure [14]. This structure was combined with the structure of the core enzyme to arrive at an atomic model for the complete Pol II that could be refined [14]. This structure served as a reference for solving many different complexes of Pol II with nucleic acids and additional factors in the years afterwards.

Crystal soaking: Pol II–TFIIS complex

The first structure of a Pol II complex with an additional protein factor could be obtained by soaking an entire recombinant factor, the elongation factor TFIIS, into preformed crystals of the complete Pol II [15]. We were encouraged to do this because the complete Pol II crystals showed a high solvent content, approximately 80% compared with only approximately 50% for core Pol II crystals. We reasoned that the solvent channels were large enough to allow for passage of an entire protein. However, we were lucky that the binding site of TFIIS on the Pol II surface was not involved in crystal contacts, allowing TFIIS to be accommodated in preformed crystals. Structure determination was enabled by difference Fourier analysis using model phases from Pol II. The resulting difference Fourier map clearly reveals TFIIS. Encouraged by these successes we tried to soak additional factors into preformed crystals, but were never lucky again. In the course of this work, we developed methods to follow protein binding in crystals using fluorescently labelled TFIIS [16]. Similar labelling approaches we used later to monitor the presence of nucleic acids in Pol II complexes. Indeed, nucleic acids tended to be lost from crystals unless present also in cryo-preserving solutions [15].

Covalent linkage: Pol II–TFIIB complex

The complex of Pol II with the initiation factor TFIIB was not stable in solution, preventing the formation of crystals that were suited for structure determination. To solve the structure of the Pol II–TFIIB complex, we prepared a recombinant Rpb4/Rpb7 variant in which the Rpb4 C-terminus was covalently fused to TFIIB. We then combined the fusion protein with endogenous core Pol II for crystallization [17]. The covalent, flexible linker ensured that TFIIB was recruited stoichiometrically near its binding site on the Pol II surface. To design this fusion protein, we made use of prior information. It had been shown that the N-terminal domain of TFIIB was located on the dock domain of the polymerase near the Rpb4/Rpb7 subcomplex [18,19]. Our resulting Pol II–TFIIB complex structure was important for obtaining models of the closed and open promoter complexes that first revealed the course of DNA over the polymerase surface during transcription initiation.

Later we were able to improve the resolution of the Pol II–TFIIB complex by adding a DNA scaffold and a short, 6-nucleotide RNA, mimicking an initially transcribing complex [20]. In this complex, a covalent linkage of TFIIB to Pol II was no longer required, likely because the presence of nucleic acids increased the affinity of TFIIB to Pol II. The resulting structure of a minimal initially transcribing complex together with functional data provided evidence for allosteric control of eukaryotic transcription. In particular, TFIIB bound on the polymerase surface far away from the catalytic site but allosterically changed the catalytic centre of Pol II such that RNA synthesis activity was stimulated [20].

Cross-linking: Pol II–TFIIF complex

Despite extensive trials, we could not prepare crystals of Pol II with another initiation factor, TFIIF. As we know now, TFIIF is a highly modular factor and some regions of this protein remain flexible even in the context of an initiation complex containing other initiation factors. To locate TFIIF on the Pol II surface, we used protein cross-linking and identification of the cross-linked lysine residues by mass spectrometry in collaboration with the laboratory of Juri Rappsilber [21]. At the time, this study presented the largest protein complex subjected successfully to such cross-linking analysis and provided a proof of principle that other large assemblies were amenable to this method.

We subsequently used the cross-linking-mass spectrometry approach in collaboration with Rudi Aebersold and Franz Herzog to derive the architecture of a core Pol II initiation complex [22], the middle module of the coactivator complex Mediator [23], RNA Pol I [24], and the Pol I–Rrn3 complex [25]. Our later crystal structure of Pol I provided independent proof of the correctness of the cross-linking-based structural approach [26]. Similarly, the cross-linking-based position of TFIIF on the polymerase surface was correct, as confirmed later by electron microscopy [27]. By now, cross-linking-based docking of protein structures is a widely used method to elucidate the architecture of protein assemblies.

Inhibitor trapping: Pol II translocation intermediate

After successful addition of a nucleotide to the growing RNA change, the polymerase must translocate to the next

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template position. Many Pol II elongation complex structures with DNA template strand and RNA transcript were solved. Among these was a structure that was pre-translocated, containing the newly added 3'-terminal nucleotide of the RNA in the nucleoside triphosphate (NTP)-binding site [28], and many structures that were post-translocated, containing a free NTP-binding site [2]. However, it remained unclear which structural changes occurred during translocation from the pre- to the post-translocated state.

When we soaked crystals of the complete Pol II elongation complex with the inhibitor α-amanitin, we could trap a novel conformation of Pol II that apparently represented a translocation intermediate [29]. These results were consistent with the idea that the polymerase is flexible and samples different conformational states, and that the inhibitor binds the translocation intermediate state, which is normally not observed because it is transient. Unfortunately there are no other well-characterized direct Pol II inhibitors that could be used to trap additional conformational states.

Serendipity: backtracked Pol II

Many Pol II-nucleic acid complexes could be resolved crystallographically by designing DNA–RNA scaffolds for co-crystallization. However, one complex we obtained only by serendipity, and not be design. We had tried to obtain a backtracked complex structure by designing nucleic acid scaffolds, but were unsuccessful. When we tried to prepare Pol II elongation complexes with mismatched nucleotides incorporated at the RNA 3'-end, we noted that incubation of the complexes with CTP led to misincorporation of several C residues. This was apparently followed by backtracking of the enzyme on DNA and RNA, extrusion of the backtracked RNA into a pore of the polymerase, and binding of the backtracked single-stranded RNA to a defined surface that we called the 'backtrack site' [30].

This study taught us that serendipity is much more than just luck. We were lucky, yes, but we also needed to be open-minded enough to realize that the unexpected, additional electron density we observed in the experiment was actually due to backtracked RNA. Indeed, obtaining new insights in science often requires one to reflect on surprises, and to follow unexpected observations. We often discard such information, believing that we look at failed experiments.

Crystal design: polymerase–Spt4/Spt5 complex

The heterodimer Spt4/Spt5 (or DSIF) is the only known polymerase-binding factor that is conserved in all three kingdoms of life. Because preparation of the Spt4/Spt5 elongation factor was initially difficult, we turned to the archaeal system to resolve its conserved complex with the archaeal polymerase. We could prepare a complex of the archaeal RNA polymerase with Spt4/Spt5, which however did not crystallize. We therefore aimed at crystallizing only the conserved dimer region of the factor with its conserved target domain on Pol II, the so-called clamp domain, which was identified in various species [31–34].

Based on the Pol II structure, we knew that the clamp domain comprises three protein regions. We used the structural information to design a recombinant fusion protein that combined these three protein regions in the right order. This enabled us to prepare a recombinant archaeal clamp domain, which readily bound Spt4/Spt5 [35]. We could indeed crystallize the clamp-Spt4/Spt5 complex. The complex structure revealed the relative position of Spt4/Spt5 and the clamp, and could be used to prepare models for Spt4/Spt5 bound to polymerases from all three kingdoms of life.

Identification of cores: Mediator subcomplexes

In 2002, we set out to determine the structure of Mediator, the central Pol II coactivator complex. Yeast Mediator comprises 25 subunits, is highly modular, flexible, instable and covalently modified, preventing structure determination of the endogenous complex. We therefore decided to take a long and winding road to eventually prepare a recombinant Mediator. To achieve this, we took a bottom-up approach and prepared individual subunits and their subcomplexes. Briefly, we coexpressed one Mediator subunit with a putative interacting subunit, and tried to obtain a complex after purification. Such subcomplexes we subjected to partial proteolysis in order to identify flexible regions in Mediator subunits. Subcloning to eliminate such flexible regions led to truncated subcomplexes with reduced flexibility. An iterative approach often led to crystallizable portions of Mediator and the determination of their structures [36–40]. We also used this approach of iterative identification and removal of flexible regions in protein complexes to crystallize the Pol I subcomplexes A14/A43 [41] and A34.5/A49 [42] and described our methods in detail [43]. Whereas proteins were generally truncated at their termini, it may also be required to predict and shorten a flexible internal loop, as exemplified by our crystal structure determination of the Pol III inhibitor Maf1 [44].

Switching species: Mediator head module

Proteins often fail to crystallize, but homologues from a different species may nevertheless form crystals. This approach of 'switching species' was used to solve the structure of the TATA box-binding protein TBP over two decades ago. In the case of the Mediator head module, which comprises seven subunits, switching species was required to obtain a high-resolution structure. We could prepare and crystallize the Mediator head module of the budding yeast *S. cerevisiae*, but the crystals were limited in resolution. When we made the module from the fission yeast *S. pombe*, the structure could be resolved and an atomic model was refined [45] that enabled completion and adaptation of an earlier, lower-resolution model obtained from the *S. cerevisiae* protein [46]. Switching species can be beneficial for crystallogenesis because homologous proteins often differ

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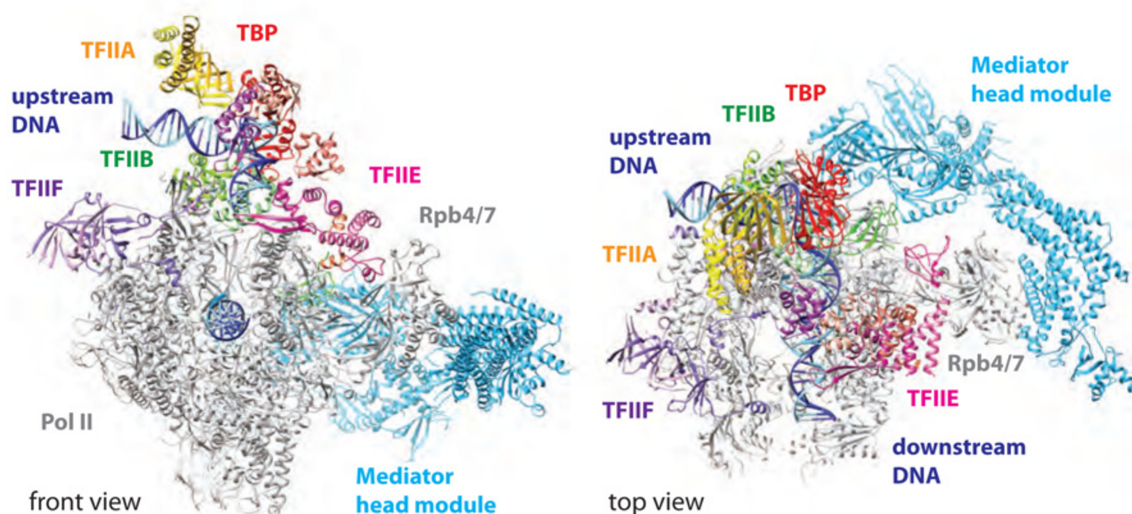
Figure 1 | Current model of the yeast RNA polymerase II initiation complex bound by the Mediator head module

The model is obtained by superposition of the cryo-EM structures of an open promoter initiation complex [48] and the cryo-EM structure of an initially transcribing Pol II complex with bound core Mediator [27]. The cryo-EM models relied on prior crystal structure determinations of several subcomplexes. For details compare text.



in their surface properties and the nature of their flexible regions.

Changing crystal packing: Pol I

In 2005, our group was able to grow crystals of a second eukaryotic RNA polymerase, Pol I from *S. cerevisiae*, but we only reported the crystal structure in 2013 [26]. The reason why this structure determination took so long was that the initial crystals were difficult to reproduce and were diffracting poorly [47]. After many unsuccessful rounds of optimizing initial crystals, we switched species and prepared Pol I from *S. pombe*. This was a major effort because we had to work out a purification protocol for the endogenous protein complex, without the benefit of overexpression. However, the *S. pombe* enzyme did not yield good crystals. Eventually, we returned to the *S. cerevisiae* enzyme and tried to obtain a different crystal form, i.e. to crystallize the protein in a different crystal packing. We eventually succeeded by changing parameters at essentially all steps from yeast cell fermentation to protein purification and crystallization [26]. The crystals with the alternative packing led to better and more reproducible diffraction that enabled experimental phasing and structure determination.

Advent of cryo-EM: initiation complexes

Our crystallographic studies used a different approach for each new complex. For most projects, many things had to be tried before the desired structures could be resolved crystallographically. And there were generally no signs which route would be the fastest. Many higher-order complexes also failed to crystallize despite extensive trials. It is thus a great relief that cryo-electron microscopy (cryo-EM) has recently advanced to a level where it can be viewed as an alternative method to resolve detailed structures of large protein complexes. This became possible with the recent development of direct electron detectors and improved image processing software.

We could indeed use cryo-EM and single particle reconstruction not only to confirm the locations of TFIIB and TFIIF on Pol II, but also to locate TFIIA, TFIIE and TBP on initiation complexes [27,48]. We additionally positioned the Mediator core comprising the head and middle modules on the Pol II surface [27]. These studies provided electron densities that were sufficiently detailed to unambiguously place crystal structures of Pol II and domains of the initiation factors. The structures of these pieces of the 3D puzzle were instrumental for structure determination of the entire assembly. Cryo-EM also revealed that binary Pol II-factor complexes resolved from crystals generally agreed well with the larger assemblies obtained in solution after cryo-preservation. As a result from these efforts, a model for the yeast Pol II initiation complex with bound Mediator head module has been obtained (Figure 1).

Conclusions

As can be seen from this summary, there is no general recipe for structure determination of large assemblies. This is true even for very closely related complexes of Pol II with additional factors and nucleic acids. Instead each structure determination project turned out to be different. The conceptual challenge was however always the same, namely to stabilize the transient nature of a complex, to

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arrive at a uniform state with a preferred conformation, and to reduce flexibilities and remove mobile regions. We generally used X-ray crystallography, because it was until recently the only method that provided detailed information on the 3D structure of large protein assemblies, and thus mechanistic insights. The examples provided here emphasize the many different approaches we developed and used to arrive at samples that enabled formation of such crystals. My hope is that this summary serves as a resource of ideas for structural biologists who are desperate to tackle similar technical challenges.

In the future, cryo-EM will become increasingly important, as it removes a frequent bottleneck for X-ray analysis, namely the formation of diffraction-quality, ordered crystals. Cryo-EM can also be carried out with less protein material, and can resolve heterogeneity in the sample by compuational sorting of the particle images. Cryo-EM also holds the promise of determining structures of several subcomplexes in a single experiment, and of providing information about structural dynamics. Thus, future structural studies of large and transient transcription complexes can rely on two complementary techniques, X-ray crystallography and cryo-EM, and these can be further complemented by other biophysical methods such as cross-linking-mass spectrometry, small angle X-ray scattering and nuclear magnetic resonance. Such structural biology hybrid methods ultimately characterize the entire transcription cycle as a process of transitions between multicomponent complexes that are formed temporarily at a specific stage of the cycle.

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References

1 Sainsbury, S., Bernecky, C. and Cramer, P. (2015) Structural basis of transcription initiation by RNA polymerase II. Nat. Rev. Mol. Cell Biol. **16**, 129–143 CrossRef PubMed
2 Martinez-Rucobo, F.W. and Cramer, P. (2013) Structural basis of transcription elongation. Biochim. Biophys. Acta **1829**, 9–19 CrossRef PubMed
3 Lariviere, L., Seizl, M. and Cramer, P. (2012) A structural perspective on Mediator function. Curr. Opin. Cell Biol. **24**, 305–313 CrossRef PubMed
4 Brueckner, F., Ortiz, J. and Cramer, P. (2009) A movie of the RNA polymerase nucleotide addition cycle. Curr. Opin. Struct. Biol. **19**, 294–299 CrossRef PubMed
5 Cheung, A.C. and Cramer, P. (2012) A movie of RNA polymerase II transcription. Cell **149**, 1431–1437 CrossRef PubMed
6 Vannini, A. and Cramer, P. (2012) Conservation between the RNA polymerase I, II, and III transcription initiation machineries. Mol. Cell **45**, 439–446 CrossRef PubMed
7 Cramer, P., Bushnell, D.A., Fu, J., Gnatt, A.L., Maier-Davis, B., Thompson, N.E., Burgess, R.R., Edwards, A.M., David, P.R. and Kornberg, R.D. (2000) Architecture of RNA polymerase II and implications for the transcription mechanism. Science **288**, 640–649 CrossRef PubMed
8 Cramer, P., Bushnell, D.A. and Kornberg, R.D. (2001) Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. Science **292**, 1863–1876 CrossRef PubMed
9 Cramer, P. (2006) Deciphering the RNA polymerase II structure: a personal perspective. Nat. Struct. Mol. Biol. **13**, 1042–1044 CrossRef PubMed
10 Fu, J., Gnatt, A.L., Bushnell, D.A., Jensen, G.J., Thompson, N.E., Burgess, R.R., David, P.R. and Kornberg, R.D. (1999) Yeast RNA polymerase II at 5 Å resolution. Cell **98**, 799–810 CrossRef PubMed
11 Cramer, P., Larson, C.J., Verdine, G.L. and Muller, C.W. (1997) Structure of the human NF-kappaB p52 homodimer-DNA complex at 2.1 Å resolution. EMBO J. **16**, 7078–7090 CrossRef PubMed
12 Bushnell, D.A., Cramer, P. and Kornberg, R.D. (2001) Selenomethionine incorporation in Saccharomyces cerevisiae RNA polymerase II. Structure **9**, R11–R14 CrossRef PubMed
13 Armache, K.J., Kettenberger, H. and Cramer, P. (2003) Architecture of initiation-competent 12-subunit RNA polymerase II. Proc. Natl. Acad. Sci. U.S.A. **100**, 6964–6968 CrossRef PubMed
14 Armache, K.J., Mitterweger, S., Meinhart, A. and Cramer, P. (2005) Structures of complete RNA polymerase II and its subcomplex, Rpb4/7. J. Biol. Chem. **280**, 7131–7134 CrossRef PubMed
15 Kettenberger, H., Armache, K.J. and Cramer, P. (2003) Architecture of the RNA polymerase II-TFIIS complex and implications for mRNA cleavage. Cell **114**, 347–357 CrossRef PubMed
16 Kettenberger, H. and Cramer, P. (2006) Fluorescence detection of nucleic acids and proteins in multi-component crystals. Acta Crystallogr. D Biol. Crystallogr. **62**, 146–150 CrossRef PubMed
17 Kostrewa, D., Zeller, M.E., Armache, K.J., Seizl, M., Leike, K., Thomm, M. and Cramer, P. (2009) RNA polymerase II-TFIIB structure and mechanism of transcription initiation. Nature **462**, 323–330 CrossRef PubMed
18 Chen, H.T. and Hahn, S. (2003) Binding of TFIIB to RNA polymerase II: mapping the binding site for the TFIIB zinc ribbon domain within the preinitiation complex. Mol. Cell **12**, 437–447 CrossRef PubMed
19 Bushnell, D.A., Westover, K.D., Davis, R.E. and Kornberg, R.D. (2004) Structural basis of transcription: an RNA polymerase II-TFIIB cocrystal at 4.5 Angstroms. Science **303**, 983–988 CrossRef PubMed
20 Sainsbury, S., Niesser, J. and Cramer, P. (2013) Structure and function of the initially transcribing RNA polymerase II-TFIIB complex. Nature **493**, 437–440 CrossRef PubMed
21 Chen, Z.A., Jawhari, A., Fischer, L., Buchen, C., Tahir, S., Kamenski, T., Rasmussen, M., Lariviere, L., Bukowski-Wills, J.C., Nilges, M. et al. (2010) Architecture of the RNA polymerase II-TFIIF complex revealed by cross-linking and mass spectrometry. EMBO J. **29**, 717–726 CrossRef PubMed
22 Muhlbacher, W., Sainsbury, S., Hemann, M., Hantsche, M., Neyer, S., Herzog, F. and Cramer, P. (2014) Conserved architecture of the core RNA polymerase II initiation complex. Nat. Commun. **5**, 4310 CrossRef PubMed
23 Lariviere, L., Plaschka, C., Seizl, M., Petrotchenko, E.V., Wenzeck, L., Borchers, C.H. and Cramer, P. (2013) Model of the Mediator middle module based on protein cross-linking. Nucleic Acids Res. **41**, 9266–9273 CrossRef PubMed

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24 Jennebach, S., Herzog, F., Aebersold, R. and Cramer, P. (2012) Crosslinking-MS analysis reveals RNA polymerase I domain architecture and basis of rRNA cleavage. Nucleic Acids Res. **40**, 5591–5601 CrossRef PubMed
25 Blattner, C., Jennebach, S., Herzog, F., Mayer, A., Cheung, A.C., Witte, G., Lorenzen, K., Hopfner, K.P., Heck, A.J., Aebersold, R. and Cramer, P. (2011) Molecular basis of Rrn3-regulated RNA polymerase I initiation and cell growth. Genes Dev. **25**, 2093–2105 CrossRef PubMed
26 Engel, C., Sainsbury, S., Cheung, A.C., Kostrewa, D. and Cramer, P. (2013) RNA polymerase I structure and transcription regulation. Nature **502**, 650–655 CrossRef PubMed
27 Plaschka, C., Lariviere, L., Wenzeck, L., Seizl, M., Hemann, M., Tegunov, D., Petrotchenko, E.V., Borchers, C.H., Baumeister, W., Herzog, F. et al. (2015) Architecture of the RNA polymerase II-Mediator core initiation complex. Nature **518**, 376–380 CrossRef PubMed
28 Gnatt, A.L., Cramer, P., Fu, J., Bushnell, D.A. and Kornberg, R.D. (2001) Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 A resolution. Science **292**, 1876–1882 CrossRef PubMed
29 Brueckner, F. and Cramer, P. (2008) Structural basis of transcription inhibition by alpha-amanitin and implications for RNA polymerase II translocation. Nat. Struct. Mol. Biol. **15**, 811–818 CrossRef PubMed
30 Cheung, A.C. and Cramer, P. (2011) Structural basis of RNA polymerase II backtracking, arrest and reactivation. Nature **471**, 249–253 CrossRef PubMed
31 Sevostyanova, A., Svetlov, V., Vassylyev, D.G. and Artsimovitch, I. (2008) The elongation factor RfaH and the initiation factor sigma bind to the same site on the transcription elongation complex. Proc. Natl. Acad. Sci. U.S.A. **105**, 865–870 CrossRef PubMed
32 Mooney, R.A., Schweimer, K., Rosch, P., Gottesman, M. and Landick, R. (2009) Two structurally independent domains of E. coli NusG create regulatory plasticity via distinct interactions with RNA polymerase and regulators. J. Mol. Biol. **391**, 341–358 CrossRef PubMed
33 Hirtreiter, A., Damsma, G.E., Cheung, A.C., Klose, D., Grohmann, D., Vojnic, E., Martin, A.C., Cramer, P. and Werner, F. (2010) Spt4/5 stimulates transcription elongation through the RNA polymerase clamp coiled-coil motif. Nucleic Acids Res. **38**, 4040–4051 CrossRef PubMed
34 Sevostyanova, A. and Artsimovitch, I. (2010) Functional analysis of Thermus thermophilus transcription factor NusG. Nucleic Acids Res. **38**, 7432–7445 CrossRef PubMed
35 Martinez-Rucobo, F.W., Sainsbury, S., Cheung, A.C. and Cramer, P. (2011) Architecture of the RNA polymerase-Spt4/5 complex and basis of universal transcription processivity. EMBO J. **30**, 1302–1310 CrossRef PubMed
36 Baumli, S., Hoeppner, S. and Cramer, P. (2005) A conserved mediator hinge revealed in the structure of the MED7.MED21 (Med7.Srb7) heterodimer. J. Biol. Chem. **280**, 18171–18178 CrossRef PubMed
37 Hoeppner, S., Baumli, S. and Cramer, P. (2005) Structure of the mediator subunit cyclin C and its implications for CDK8 function. J. Mol. Biol. **350**, 833–842 CrossRef PubMed
38 Lariviere, L., Geiger, S., Hoeppner, S., Rother, S., Strasser, K. and Cramer, P. (2006) Structure and TBP binding of the Mediator head subcomplex Med8-Med18-Med20. Nat. Struct. Mol. Biol. **13**, 895–901 CrossRef PubMed
39 Koschubs, T., Seizl, M., Lariviere, L., Kurth, F., Baumli, S., Martin, D.E. and Cramer, P. (2009) Identification, structure, and functional requirement of the Mediator submodule Med7N/31. EMBO J. **28**, 69–80 CrossRef PubMed
40 Seizl, M., Lariviere, L., Pfaffeneder, T., Wenzeck, L. and Cramer, P. (2011) Mediator head subcomplex Med11/22 contains a common helix bundle building block with a specific function in transcription initiation complex stabilization. Nucleic Acids Res. **39**, 6291–6304 CrossRef PubMed
41 Kuhn, C.D., Geiger, S.R., Baumli, S., Gartmann, M., Gerber, J., Jennebach, S., Mielke, T., Tschochner, H., Beckmann, R. and Cramer, P. (2007) Functional architecture of RNA polymerase I. Cell **131**, 1260–1272 CrossRef PubMed
42 Geiger, S.R., Lorenzen, K., Schreieck, A., Hanecker, P., Kostrewa, D., Heck, A.J. and Cramer, P. (2010) RNA polymerase I contains a TFIIF-related DNA-binding subcomplex. Mol. Cell **39**, 583–594 CrossRef PubMed
43 Geiger, S.R., Kuhn, C.D., Leidig, C., Renkawitz, J. and Cramer, P. (2008) Crystallization of RNA polymerase I subcomplex A14/A43 by iterative prediction, probing and removal of flexible regions. Acta Crystallogr. Sect. F Struct Biol. Cryst. Commun. **64**, 413–418 CrossRef PubMed
44 Vannini, A., Ringel, R., Kusser, A.G., Berninghausen, O., Kassavetis, G.A. and Cramer, P. (2010) Molecular basis of RNA polymerase III transcription repression by Maf1. Cell **143**, 59–70 CrossRef PubMed
45 Lariviere, L., Plaschka, C., Seizl, M., Wenzeck, L., Kurth, F. and Cramer, P. (2012) Structure of the Mediator head module. Nature **492**, 448–451 CrossRef PubMed
46 Imasaki, T., Calero, G., Cai, G., Tsai, K.L., Yamada, K., Cardelli, F., Erdjument-Bromage, H., Tempst, P., Berger, I., Kornberg, G.L. et al. (2011) Architecture of the Mediator head module. Nature **475**, 240–243 CrossRef PubMed
47 Kostrewa, D., Kuhn, C.D., Engel, C. and Cramer, P. (2015) An alternative RNA polymerase I structure reveals a dimer hinge. Acta Crystallogr. D Biol. Crystallogr. **71**, 1850–1855 CrossRef PubMed
48 Plaschka1, C., Hantsche, M., Dienemann, C., Burzinski, C., Plitzko, J. and Cramer, P. (2016) Transcription initiation complex structures elucidate DNA opening. Nature **533**, 353–358 CrossRef PubMed

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