Patrick Cramer - Research Summary

Patrick Cramer has given outstanding contributions to our understanding of gene transcription at the molecular and cellular level. He established the structural basis of eukaryotic transcription initiation and elongation, and developed new approaches to investigate transcription regulation genome-wide in living cells. In the following, key findings and discoveries are briefly summarized.

Structure of RNA polymerase II

Patrick Cramer got trained in macromolecular X-ray crystallography with Alan Fersht at Cambridge, England, and with Christoph Müller at the European Molecular Biology Laboratory in Grenoble, France. As a postdoctoral fellow with Roger Kornberg at Stanford University, Cramer determined the first crystal structure of a eukaryotic RNA polymerase, the Pol II core enzyme from yeast (Cramer et al. *Science* 2000, 2001). The Pol II structure was amongst the first atomic structures of large assemblies and transformed the transcription field, enabling mechanistic studies of DNA-dependent synthesis of messenger-RNA from protein-coding genes. Together with a structure of Pol II in complex with DNA and RNA (Gnatt, Cramer et al. *Science* 2001), this work was cited by the Nobel committee in their justification for giving the 2006 Nobel Prize in Chemistry to Roger Kornberg '*…for his studies of the molecular basis of eukaryotic transcription*'.

A molecular movie of transcription elongation

In 2001, Patrick Cramer set up a laboratory at the Ludwig-Maximilians-Universität in Munich, Germany, which moved to the Max-Planck-Institute for Biophysical Chemistry in Göttingen in 2014. Over the first decade, the Cramer laboratory solved many crystal structures of Pol II complexes with nucleic acids and protein factors that could be combined into the first molecular movie of transcription (Cheung and Cramer, *Cell* 2012). The movie is based mainly on structure-function studies of transcription elongation, which revealed the nucleotide addition cycle during RNA chain elongation and the mechanisms of transcription arrest, backtracking, and reactivation (reviewed in Martinez-Rucobo et al., *BBA* 2012).

Structural basis of eukaryotic transcription initiation

The Cramer laboratory has recently provided the structural basis of transcription initiation in eukaryotic cells. A key step towards this goal was the crystal structure of Pol II in complex with the general transcription factor TFIIB, which led to a model of a minimal Pol II initiation complex (Kostrewa et al. *Nature* 2009). After several medium-resolution studies of the initiation complex architecture (reviewed in Sainsbury et al., *Nat.Rev.Mol.Cell.Biol.* 2015) the Cramer group reported the cryo-EM structure at 3.6 Angstroms resolution of the core Pol II initiation complex including transcription factors TFIIA, TFIIB, TFIIE, TFIIF, and the TATA box binding protein TBP (Plaschka et al., *Nature* 2016). The laboratory has recently extended this structure to the complete Pol II pre-initiation complex comprising also TFIIH at 4.7 Angstroms resolution (Schilbach et al., *Nature*, 2017). This work illustrates how the general transcription factors cooperate with Pol II to bind and open promoter DNA and start RNA synthesis. Related work was conducted in the human system by the laboratory of Eva Nogales at Berkeley University.

Mediator structure and initiation regulation

In a long-term effort, the Cramer laboratory also wishes to determine the structural basis for gene regulation. To this end, they elucidated the structure of Mediator, the central coactivator that enables regulated Pol II initiation. In 2002-2012, the group identified Mediator subcomplexes and resolved their X-ray structures (reviewed in Lariviere et al., *Curr. Op. Cell Biol*. 2012). They then arrived at the structure of the 7-subunit Mediator head module (Lariviere et al., *Nature* 2012). After several more years, the group located the 15-subunit core Mediator on the core Pol II initiation complex (Plaschka et al., *Nature* 2015). The laboratory recently resolved the crystal structure of the Mediator core (Nozawa et al., *Nature* 2017). These studies now culminated with the formation of the Pol II pre-initiation complex with bound core Mediator, an assembly comprising a total of 46 proteins with a molecular weight of ~2 MegaDalton (Schilbach et al., *Nature*, 2017). This structure was determined by cryo-electron microscopy at 5.8 Angstroms resolution, comprises all initiation factors that are essential for yeast growth, and provides a framework to investigate the molecular mechanisms of Mediator-regulated Pol II transcription. X-ray crystallography was the key to obtaining these insights, in particular to obtain the correct Mediator structure and the correct location of Mediator on the Pol II pre-initiation complex.

Structural basis of elongation regulation

The laboratory has recently used a combination of biochemistry and structural biology to provide a mechanistic framework to understand transcription regulation during early RNA chain elongation (Vos, Farnung et al., *Nature* 2018a; Vos et al., *Nature* 2018b). In higher cells, RNA polymerase II often pauses in the promoter-proximal region. Regulatory signal can then release paused Pol II into active RNA chain elongation. This regulatory mechanism is widespread and often used for rapid and coordinated gene transcription regulation during cell differentiation and organism development. The Cramer lab resolved the structures of the mammalian RNA polymerase II elongation complex in two states, in the paused state stabilized by the factors DSIF and NELF, and in the released and activated state, stabilized by the PAF complex and SPT6. The paused elongation complex reveals a tilted DNA-RNA hybrid that prevents RNA chain elongation, and shows how the NELF factor stabilizes this off-line state. The released and activated elongation complex reveals that the PAF complex displaces NELF from the polymerase funnel region and provided insights into how the kinase P-TEFb causes stable binding of SPT6 and PAF. Together these studies suggest the molecular mechanism of gene regulation at the step of RNA polymerase II promoter-proximal pausing.

Structural basis of Pol I transcription

The Cramer lab also pioneered structural studies of alternative RNA polymerases, in particular Pol I, the enzyme that synthesizes ribosomal RNA. The group described the first model and later the crystal structure of the 14-subunit Pol I enzyme, which is very distinct from Pol II (Kuhn et al. *Cell* 2007, Engel et al. *Nature* 2013). This work has recently culminated in the structure of actively transcribing Pol I (Neyer et al., *Nature* 2017) and the Pol I initiation complex, which revealed a possible basis for promoter specificity (Engel et al., *Cell* 2017). The Pol I studies also suggested the the concept of allosteric control of RNA polymerases (Sainsbury et al. *Nature* 2012; Engel et al. *Nature* 2013). The laboratory further obtained the first model of Pol III,

which synthesizes transfer-RNA (Jasiak et al. *Mol. Cell* 2006; Vannini et al. *Cell* 2010). They also solved the first structure of a mitochondrial RNA polymerase (Ringel et al. *Nature* 2011) and recently provided the structures of human mitochondrial RNA polymerase bound to its two initiation factors (Hillen et al., Cell, in press) and in complex with its elongation factor (Hillen et al., *Cell,* 2017). These studies provided the structural basis of Pol I transcription and insights into differences between promoter-specific transcription systems.

Development of integrated structural biology

In the course of their studies, the Cramer laboratory developed methods for the structure determination of large and transient macromolecular complexes (reviewed in Cramer, *Biochem. Soc. Trans.* 2016). Examples for technically pioneering structure determinations include the first structure of Pol II in complex with a transcription factor (Kettenberger et al. *Cell* 2003), the structure of Pol II in an off-line state with backtracked RNA (Cheung et al. *Nature* 2011), and the structure of an active complex of transcribing bacterial RNA polymerase with a translating ribosome, termed 'expressome' (Kohler et al., *Science* 2017). The latter study visualizes, for the first time, the central dogma of molecular biology within one three-dimensional structure. The Cramer laboratory also set the ground for detailed structural studies of the mammalian transcription system, reporting the first atomic model of a mammalian RNA polymerase (Bernecky et al., *Nature* 2016), and recently solved the first structure of a complete chromatin-remodeling enzyme, Chd1, bound to its natural substrate, the nucleosome (Farnung et al., *Nature*, 2017).

New transcriptomics tools reveal mRNA buffering and map enhancer landscapes

The Cramer laboratory also transformed transcriptomics, which traditionally measure only mRNA levels. They established methods that use metabolic RNA labeling to estimate mRNA synthesis and degradation rates *in vivo* (Miller et al. *Mol. Sys. Biol.* 2011). This led to the discovery that cells buffer mRNA levels by compensating between RNA synthesis and degradation (Sun et al. *Genome Research* 2012, *Mol. Cell* 2013). Based on this work in yeast, the Cramer laboratory developed 'TT-seq', a method that monitors genome activity and can map short-lived newly synthesized RNA onto the human genome (Schwalb et al., *Science* 2016). TT-seq is now widely used because it can monitor genome activity and changes in enhancer landscapes with high temporal resolution (Michel et al., *Mol. Sys. Biol.* 2017). In a collaboration with the Sandberg lab, Cramer has recently developed a protocol to take their RNA labeling and sequencing of newly synthesized RNAs to the level of single cells (Hendriks, Jung et al., *Nat. Comm.* 2019).

Development of methods to investigate global transcription regulation

The Cramer laboratory also developed other functional genomics approaches to study the regulation of gene transcription and mRNA metabolism in cells. They pioneered the correlation analysis between molecular structure and genome-wide expression (Lariviere et al. *Genes Dev.* 2008), and elucidated the transcription cycle with occupancy profiling (Mayer et al. *NSMB* 2010; Lidschreiber et al., *MCB* 2013; Baejen et al., *Mol. Cell* 2016). They monitored transcriptome surveillance in the yeast cell nucleus and found how aberrant non-coding RNAs are recognized (Schulz et al., *Cell* 2013). They also mapped protein binding to pre-mRNA in cells (Baejen et al., *Mol. Cell* 2014), and discovered widespread binding of transcription factors to nascent RNA *in vivo* (Battaglia, *eLife* 2017). Recently, the Cramer laboratory developed a

multi-omics approach coupled to kinetic modeling in order to demonstrate that the duration of RNA polymerase II pausing downstream of gene promoters controls the frequency of transcription initiation (Gressel, Schwalb et al., *eLife* 2017 & *Nat. Comm.* 2019). Through these efforts the Cramer laboratory has provided new insights into how transcription of the eukaryotic genome is regulated and controlled in living cells.