Aus dem Biomedizinischen Centrum
der Ludwig-Maximilians-Universität München,
Lehrstuhl Molekularbiologie
Vorstand: Prof. Dr. rer. nat. Peter B. Becker

Establishment and Function of Nucleosome Organization at Eukaryotic Chromosome Replication Origins

Dissertation
zum Erwerb des Doktorgrades der Naturwissenschaften
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von
Erika Chacin

aus
La Guaira, Venezuela

2023

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Betreuer:	Prof. Dr. rer. nat. Philipp Korber
Zweitgutachter:	Prof. Dr. rer. nat. Aloys Schepers
Dekan:	Prof. Dr. med. Thomas Gudermann
Tag der mündlichen Prüfung:	08. Dezember 2023

Affidavit

Chacin, Erika

Surname, first name

I hereby declare, that the submitted thesis entitled

Establishment and Function of Nucleosome Organization at Eukaryotic Chromosome Replication Origins

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the dissertation presented here has not been submitted in the same or similar form to any other institution for the purpose of obtaining an academic degree.

Wuppertal, 18.03.2024

Place, Date

Erika Chacin

Signature doctoral candidate

Table of Contents

List of abbreviations

AAA$^+$ ATPases Associated with diverse cellular Activities

Abf1 ARS-Binding Factor 1

ACS ARS Consensus Sequence

ARS Autonomous Replicating Sequence

Asf1 Anti-Silencing Function 1

ATRX α-Thalassemia/mental Retardation X-linked

BAH Bromo-Adjacent Homology

CAF-1 Chromatin Assembly Factor 1

Cbf1 Centromere Binding Factor 1

Cdc6 Cell Division Cycle 6

Cdc45 Cell Division Cycle 45

Cdt1 Cdc10 Dependent Transcription 1

CDK Cyclin-Dependent Kinase

Chd1 Chromodomain, Helicase and DNA binding 1

CHD Chromodomain, Helicase and DNA binding

CMG Cdc45-MCM2-7-GINS

CMGE Cdc45-MCM2-7-GINS-Polymerase ϵ

Csm3/Tof1 Chromosome Segregation in Meiosis 3 / TOpoisomerase I-interacting Factor

CTCF CCCTC-binding Factor

CTD Carboxy-Terminal Domain

Ctf4 Chromosome Transmission Fidelity 4

DAXX Death domain-Associated protein

DDK Dbf4-Dependent Kinase

Dpb11 DNA Polymerase B (II)

DNaseI Deoxyribonuclease I

ERCE Early Replication Control Elements

FACT FAcilitates Chromatin Transcription/transactions

GAF GAGA Factor

GINS Go, Ichi, Ni and San complex

GRFs General Regulatory Factor

H3K27ac histone H3 lysine 27 mono-acetylated

H3K9me3 histone H3 lysine 9 tri-methylated

H3K27me3 histone H3 lysine 27 tri-methylated

H4K20me2 histone H4 lysine 20 di-methylated

HBO1 Human acetylase Binding to ORC1

HIR HIstone Regulatory

IDR Intrinsically Disordered Region

ISWI Imitation SWItch

ISW1a Imitation SWItch 1a

ISW2 Imitation SWItch 2

INO80 INOsitol requiring 80

MCM MiniChromosome Maintenance complex

Mcm1 MiniChromosome Maintenance 1

Mcm2-7 MiniChromosome Maintenance 2-7

Mcm10 MiniChromosome Maintenance 10

MCM-DH MCM double hexamers

MNase Micrococcal Nuclease

MNase-seq Micrococcal Nuclease digestion with deep Sequencing

Mrc1 Mediator of the Replication Checkpoint 1

Nap1 Nucleosome Assembly Protein 1

NDF Nucleosome-Displacing Factors

NFR Nucleosome Free Region

NRL Nucleosome Repeat Length

Nhp6a Non-Histone Protein 6a

NuA4 Nucleosome Acetyltransferase of H4

OCCM ORC-Cdc6-Cdt1-MCM

Oct4 Octamer-binding transcription factor 4

OGRE Origin G-rich Repeat Element

ORB Origin Recognition Boxes

ORC Origin Recognition Complex

Orc1 Origin Recognition Complex 1

Orc1/Cdc6 Origin Recognition Complex 1 / Cell Division Cycle 6

ORCA ORC-Associated

pre-IC Pre-Initiation Complex

pre-RC Pre-Replication Complex

PCNA Proliferating Cell Nuclear Antigen

Pob3 Pol1 Binding 3

PTMs Post-Translational Modifications

Rap1 Repressor/Activator site binding Protein 1

RFC Replication Factor C

Reb1 RNA polymerase I Enhancer Binding protein 1

RPA Replication Protein A

RSC Remodels the Structure of Chromatin

SAGA Spt-Ada-Gcn5-Acetyltransferase

SGD Salt-Gradient Dialysis

SF2 Super-Family 2

SIR Silent Information Regulator

Sir1 Silent Information Regulator 1

Sld2 Synthetically Lethal with Dpb11-1 2

Sld3/Sld7 Synthetically Lethal with Dpb11-1 3/7

SMARCA5 SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5

Sox2 SRY (sex determining region Y)-box 2

Spt16 SuPpressor of Ty's 16

SWI/SNF SWItch/Sucrose-Non-Fermenting

TF Transcription Factor

TFIIB-A Transcription Factor IIB domain A

TFIIB-B Transcription Factor IIB domain B

TopoI TOPOisomerase I

WHD Winged-Helix Domain

Yta7 Tat-binding homolog 7

List of publications

Paper I:

Chacin E., Reusswig K.U., Furtmeier J., Bansal P., Karl L., Pfander B., Straub T., Korber P., Kurat C.F. (2023). Establishment and Function of Chromatin Organization at Replication Origins. ***Nature***. 616, 836-842. https://doi.org/10.1038/s41586-023-05926-8

Paper II:

Chacin E.*, Bansal P.*, Reusswig K.U., Diaz-Santin L.M., Ortega P., Vizjak P., Gómez-González B., Müller-Planitz F., Aguilera A., Pfander B., Cheung A.C.M., Kurat C.F. (2021). A CDK-regulated chromatin segregase promoting chromosome replication. ***Nature Communications***. 12, 1-12. https://doi.org/10.1038/s41467-021-25424-7

Paper III:

Safaric B., **Chacin E.**, Scherr M.J., Rajappa L., Gebhardt C., Kurat C.F., Cordes T., Duderstadt K.E. (2022). The fork protection complex recruits FACT to reorganize nucleosomes during replication. ***Nucleic Acids Research***. 50, 1317-1334. https://doi.org/10.1093/nar/gkac005

Contribution to the publications

This thesis is comprised of three articles. The research was conducted from November 2018 to March 2023 in the lab of Dr. Christoph F. Kurat (Molecular Biology division, Biomedical Center Munich). My contributions to each publication are stated below.

Contribution to Paper I

Chacin E., Reusswig K.U., Furtmeier J., Bansal P., Karl L., Pfander B., Straub T., Korber P., Kurat C.F. (2023). Establishment and Function of Chromatin Organization at Replication Origins. ***Nature***. 616, 836-842. https://doi.org/10.1038/s41586-023-05926-8

1. Experimental work:

   (a) I designed, executed, and analyzed all the *in vitro* reconstitution experiments for Micrococcal Nuclease digestion with deep Sequencing (MNase-seq) shown in: Fig. 1c, d, f, h; Fig. 2a-d; Fig. 4a-b; ED Fig. 2-4; and ED Fig. 7. I prepared SGD chromatin, purified proteins and nucleic acid material, and all sequencing libraries for Illumina sequencing. Furthermore, I analyzed the sequencing data using bioinformatic tools and programming languages.

      i. I generated a smaller genomic yeast plasmid library collection that was used to prepare Salt-Gradient Dialysis (SGD) chromatin for all the *in vitro* reconstitution experiments.

      ii. I designed, prepared and characterized the DNA constructs/plasmids to generate the yeast expression strains for the protein purification of SWI/SNF and the Orc1-mutant ORC complexes: ΔBAH, ΔIDR, ΔBAH/ΔIDR and Walker B.

      iii. I purified the following recombinant proteins: histone octamers, ORC, Orc1-mutant ORCs (ΔBAH, ΔIDR, ΔBAH/ΔIDR and Walker B), INO80, ISW1a, ISW2, Chd1, SWI/SNF, RSC, and Cdc6.

      iv. I prepared all the Illumina sequencing libraries.

      v. I analyzed all the sequencing data and generated the composite plots to study nucleosome positioning.

   (b) I performed most of the *in vivo* MNase-seq experiments (Fig. 2e, ED Fig. 5b, and ED Fig. 6a-b) with the exception of the data shown in ED Fig. 5c, d and e. There, publicly available data from Kubik et al. (2019) was used as stated in the publication.

i. I designed and supervized the experiments carried by Jessica Furtmeier, who assisted with the lab work to generate the yeast strains containing Orc1 mutations (*orc1*-BAH, *orc1*-IDR, *orc1*-BAH-IDR, and *orc1*-Walker B).

ii. I prepared cell cultures, purified yeast nuclei and processed the samples for MNase-seq studies.

iii. I analyzed all the sequencing data and generated the composite plots to study nucleosome positioning.

(c) I prepared the SGD chromatin and *in vitro* reactions for the co-immunoprecipitation experiments (ED Fig. 8), while Christoph F. Kurat (CFK) performed the IP and western blots.

(d) I contributed to the establishment and optimization of the ORC binding and MCM loading to ARS1 DNA, as well as the *in vitro* replication assays with the chromatinized origin plasmid pool, by setting up initial experiments and replicates. The final gels shown in Fig. 3f, Fig. 4d and ED Fig. 9 were performed by CFK.

2. Manuscript preparation:

(a) I reviewed literature, planned and designed experiments, interpreted results and contributed to the manuscript content.

(b) I wrote the Methods section and figure legends.

(c) I designed all main and extended data figures.

(d) I contributed through the whole revision process.

Contribution to Paper II

Chacin E.*, Bansal P.*, Reusswig K.U., Diaz-Santin L.M., Ortega P., Vizjak P., Gómez-González B., Müller-Planitz F., Aguilera A., Pfander B., Cheung A.C.M., Kurat C.F. (2021). A CDK-regulated chromatin segregase promoting chromosome replication. ***Nature Communications***. 12, 1-12. https://doi.org/10.1038/s41467-021-25424-7

* These authors contributed equally with experimental work and manuscript preparation.

1. Experimental work:

(a) I contributed to protein purifications of Yta7 and the mutant complexes (Yta7 ATP-binding and Yta7 Phospho).

(b) I helped with the generation of mutant Yta7 yeast strains using standard molecular cloning techniques and tetrad dissection.

(c) I assisted with chromatin assembly and western blot experiments.

(d) I helped with the analysis of western blots (Figure 3b, c, e) and ATPase assays (Fig. 4d).

2. Manuscript preparation:

   (a) I contributed to the manuscript preparation and revisions.

   (b) I assisted with the design and assembly of all main and extended data figures.

Contribution to Paper III

Safaric B., **Chacin E.**, Scherr M.J., Rajappa L., Gebhardt C., Kurat C.F., Cordes T., Duderstadt K.E. (2022). The fork protection complex recruits FACT to reorganize nucleosomes during replication. ***Nucleic Acids Research***. 50, 1317-1334.
https://doi.org/10.1093/nar/gkac005

1. Experimental work:

   (a) I contributed to the experimental design of the *in vitro* replication assays and executed all experiments shown in Figure 5 and Supplementary Figure 5, including all replicates sent during the revision process.

2. Manuscript preparation:

   (a) I participated in the revision process and helped interpreting the results.

Introduction

DNA Replication

Initiation sites of DNA replication and their recognition

Chromosomes are duplicated once in each round of the cell cycle. The first essential molecular event of DNA replication is the binding of a protein (*initiator*) to a specific site in the genome (*replicator*) to mark and initiate replication (Jacob et al., 1963). In bacteria, archaea and some budding yeasts, the replicator corresponds to dA-dT-rich sequences that vary among different species (Leonard & Méchali, 2013), while in *Saccharomyces cerevisiae (S. cerevisiae)* a conserved motif is present (Broach et al., 1983). In bacteria, the initiator DnaA binds to AT-rich boxes clustered at the replicator oriC (Mackiewicz et al., 2004). In archaea, the initiator is the monomeric Orc1/Cdc6 (Origin Recognition Complex 1 / Cell Division Cycle 6), which binds to ORB (Origin Recognition Boxes) or mini-ORB sequences (Robinson et al., 2004). In *S. cerevisiae*, the hexameric ORC (Origin Recognition Complex) acts as the initiator, binds to the ACS (ARS Consensus Sequence) (Bell & Stillman, 1992) and then recruits Cdc6 as a separate complex (S. Donovan et al., 1997). The sequences found at origins in different *Schizosaccharomyces* species are more variable and encompass poly(A) tracks, AT-rich sequences and poly(G) motifs (J. Xu et al., 2012).

In metazoans, there is no sequence specificity and much less is known about the recruitment of ORC to origins, but it seems to rely more on DNA shape and the chromatin environment (Vashee et al., 2003; Remus et al., 2004). In mouse and humans, initiation sites and initiation regions have been defined instead of origins of replication. There, replication initiation seems to be influenced by an open chromatin environment, enrichment of OGRE (Origin G-rich Repeat Element) that can form G quadruplexes as well as specific acetylation and methylation histone marks (Cayrou et al., 2015; Ganier et al., 2019). Recently, cis-acting elements called ERCE (Early Replication Control Elements)s that are enriched for large stretches of histone H3K27ac (histone H3 lysine 27 mono-acetylated) and TF (Transcription Factor) binding sites like the Oct4 (Octamer-binding transcription factor 4), Nanog and Sox2 (SRY (sex determining region Y)-box 2), have been shown to promote earlier replication in mouse embryonic stem cells (Sima et al., 2019).

While a single origin is enough to replicate a prokaryotic genome (Cairns, 1963), larger eukaryotic genomes require multiple origins of replication (Taylor, 1968; Huberman & Riggs, 1968). The budding yeast *S. cerevisiae* uses approximately 400 origins of replication (Nieduszynski et al., 2006; Eaton et al., 2010; Liachko et al., 2013; Siow et al., 2012). In human cells, due to the size of their genome, approximately 30,000 origins have

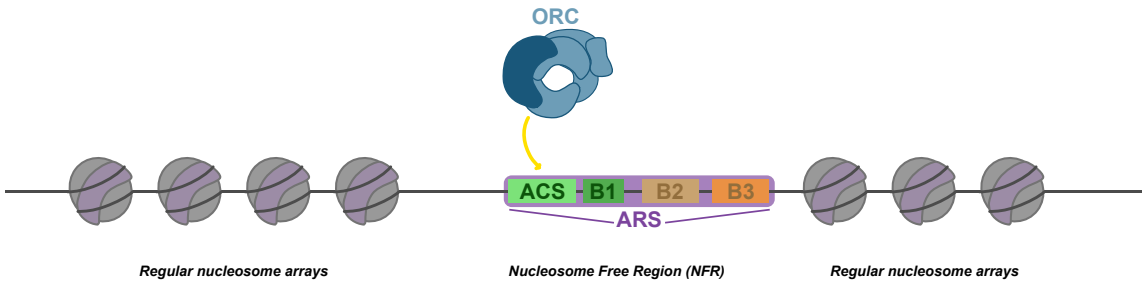

Figure 1: Scheme of an origin of replication in *S. cerevisiae*.

been predicted to be used (Akerman et al., 2020).

S. cerevisiae origins

In contrast to other species, origins of *S. cerevisiae* are well defined by a consensus sequence. The ARS (Autonomous Replicating Sequence)s (Fig. 1) are the replicators of *S. cerevisiae* and were first discovered as sequences that conferred plasmids the ability to replicate autonomously and showed high frequency transformation (Stinchcomb et al., 1979). ARSs are circa 200 bp long and contain an 11 bp AT-rich motif, the ACS (Broach et al., 1983), and a B1 element as the core elements (Rowley et al., 1995). Some origins contain also a B2 element that can stabilize the binding of the pre-RC (Pre-Replication Complex) proteins (ORC, Cdc6, and Cdt1 (Cdc10 Dependent Transcription 1)-Mcm2-7 (MiniChromosome Maintenance 2-7)) (Wilmes & Bell, 2002) and/or a B3 element that may serve as binding site for the Abf1 (ARS-Binding Factor 1) (Diffley & Stillman, 1988). This TF contributes to positioning nucleosomes next to the ARS1 origin (Lipford & Bell, 2001). ORC binds to the origin DNA at the ACS site as well as the B1 element, which was suggested to stabilize its binding (Rao & Stillman, 1995; Rowley et al., 1995; Li et al., 2018). Mutations in these conserved sequences can disrupt or eliminate origin function (Van Houten & Newlon, 1990; Marahrens & Stillman, 1992; Theis & Newlon, 1994).

It has been estimated that the number of potential ACS sites within the yeast genome is 6000-40,000 (Eaton et al., 2010), but only a subset of those ($\sim$ 400) function as real origins of replication (Nieduszynski et al., 2006; Eaton et al., 2010; Liachko et al., 2013; W. Xu et al., 2006; Siow et al., 2012). This indicates that the ACS is not enough to define where replication initiates and suggests that there are other components involved (Berbenetz et al., 2010).

S. cerevisiae origins are also classified as early- or late-firing, depending on when they are activated during the S phase (Fangman & Brewer, 1992). However, it is not clear whether there is an evolutionary advantage to replicate some genomic regions earlier, and/or if this is just a consequence of the nature of the sequences surrounding the origins. Related to that, it was suggested that rate-limiting firing factors could be involved in determining which origins fire first and that this might be due to differences in binding affinities among origins (Bell & Labib, 2016). What is clear, however, is that a malfunction of this replication program can lead to under-replicated DNA or stalled replication forks, resulting in DNA damage and replication stress, which are features observed in many cancer cells (Gaillard et al., 2015).

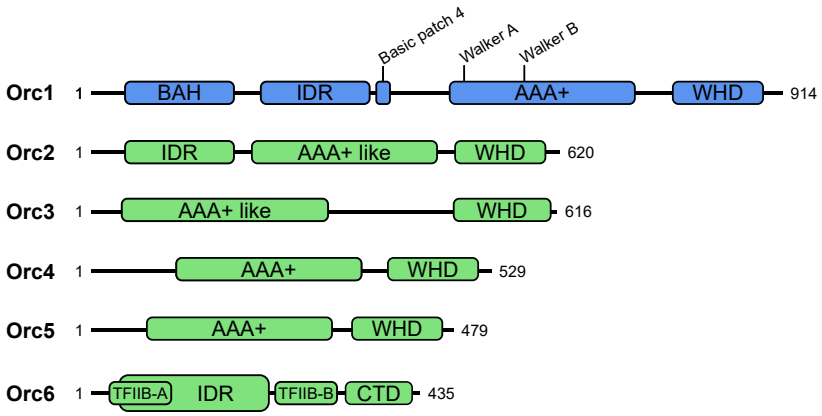

Figure 2: ORC subunits with annotated domains. The numbers indicate the amount of amino acid residues in each subunit. BAH (Bromo-Adjacent Homology), IDR (Intrinsically Disordered Region), AAA$^+$ (ATPases Associated with diverse cellular Activities)-ATPase domain, WHD (Winged-Helix Domain), TFIIB-A (Transcription Factor IIB domain A), TFIIB-B (Transcription Factor IIB domain B), CTD (Carboxy-Terminal Domain).

The Origin Recognition Complex

The canonical role of ORC is to function as the initiator of DNA replication in eukaryotes, specifically, as the loading factor of the replicative helicase, the MCM (MiniChromosome Maintenance complex). Moreover, ORC is also involved in the silencing of mating type genes and heterochromatin maintenance in yeast (Foss et al., 1993; Bell et al., 1993) but this thesis will focus on ORC's role in replication.

ORC (Fig. 2) is a hexamer composed of the subunits Orc1-6, from which all but Orc6 contain an AAA$^+$ (ATPases Associated with diverse cellular Activities)-ATPase domain (Li et al., 2018; Schmidt & Bleichert, 2020; Jaremko et al., 2020). This is a conserved feature of *initiators* across species that is also present in other replication-associated proteins like the loading factor Cdc6 and all subunits from the MCM complex (Mcm2-7). Interestingly, Yta7 (Tat-binding homolog 7), the chromatin segregase, which stimulates chromatin replication (see above) belongs to the family of AAA$^+$-ATPase-containing proteins. Proteins bearing AAA$^+$-ATPases tend to oligomerize into a ring-shaped conformation and require ATP binding and hydrolysis to exert their functions by sustaining conformational changes (Giraldo, 2003). A typical AAA$^+$-ATPase domain contains four conserved elements: a Walker A motif that is essential for binding the ATP molecule; a Walker B motif essential for ATP hydrolysis; as well as sensor-I and sensor-II elements required for ATP hydrolysis (Duderstadt & Berger, 2008).

The metazoan ORC subunits Orc1, Orc4 and Orc5 are capable of binding ATP while in yeast, Orc4 does not (Bleichert et al., 2015; Tocilj et al., 2017). Only ATP binding by ORC and not ATP hydrolysis, is essential for specific binding to the ACS and loading of the the MCM complex as a head-to-head double hexamer (Bell & Stillman, 1992; Klemm & Bell, 2001; Coster et al., 2014). ORC binding to DNA relies mainly on Orc1's basic patch, which is conserved among eukaryotes, but in yeast it also relies on interactions between Orc2 and Orc4 with the ACS sequence (Kawakami et al., 2015; Li

et al., 2018; Schmidt & Bleichert, 2020; Costa & Diffley, 2022). This basic patch in yeast is located close to the IDR, which is between the BAH domain and the AAA$^+$-ATPase domain (Li et al., 2018). *Drosophila* and human Orc1 also contain an IDR region but unlike yeast, they phase separate to generate condensates in the presence of DNA which may help clustering loading factors (Parker et al., 2019). IDRs have been described as flexible regions important for protein-protein interactions and in human cells, Orc1 and Cdc6 interact via their IDRs (Feng et al., 2021).

ATP hydrolysis by ORC takes place in a single catalytic bipartite site via the interaction of Orc1's ATP binding site with Orc4's arginine finger (Bowers et al., 2004). Only upon ATP binding, Orc1's AAA$^+$-ATPase module gets closer to Orc4, and then after ATP hydrolysis, both subunits get further apart again – a process called ATPase autoinhibition. This has been observed only in metazoans (Jaremko et al., 2020; Schmidt & Bleichert, 2020). Orc1's Walker A/B motifs and Orc4's arginine finger motif impair ORC's function and/or are lethal (Klemm et al., 1997; Bowers et al., 2004). The DELD motif present in Orc1's Walker B motif is conserved in yeast and humans (Jaremko et al., 2020) and mutations in the motif also disrupt ATP hydrolysis in human ORC (Tocilj et al., 2017).

The structure of ORC resembles a crescent shape composed of Orc1-5 with Orc6 bound distal to the central channel, with ATP bound at the center of this cavity as observed in the structures of yeast, fruit fly and human ORC (Li et al., 2018; Schmidt & Bleichert, 2020; Jaremko et al., 2020). Interestingly, the active or open conformation of human ORC bound to origin DNA is similar to the yeast ORC conformation when part of the OCCM (ORC-Cdc6-Cdt1-MCM) complex, a loading intermediate (Sun et al., 2013; Li et al., 2018; Jaremko et al., 2020).

ORC exists as a stable hexameric complex in yeast that remains bound to origins throughout the cell cycle (Aparicio et al., 1997; Diffley et al., 1994; T. Tanaka et al., 1997). However, in mammalians, only ORC2-5 remains bound to DNA, whereas ORC1 binds during the G1 phase and is ubiquitylated for degradation during the S phase (Méndez et al., 2002; Ohta et al., 2003; Tocilj et al., 2017). Also, ORC6 is not essential in human cells for ORC1-5 assembly (Vashee et al., 2001; Siddiqui & Stillman, 2007). ORC is of clinical interest, as mutations in *ORC1*, *ORC4* and *ORC6* genes have been linked to the Meier-Gorlin syndrome, a form of primordial dwarfism (Bicknell, Bongers, et al., 2011). *ORC1* mutations cause the most extreme growth defects, both in humans and zebrafish, which, respectively, includes microcephalic dwarfism and a size decrease in all tissues. These malfunctions have been linked to problems in origin licensing and S-phase progression (Bicknell, Walker, et al., 2011; Bicknell, Bongers, et al., 2011).

Starting the process - the replication machinery

In eukaryotes, DNA replication is a two-step process. In the first step, origin licensing (Blow & Laskey, 1988), the MCM complex is loaded as a head-to-head double hexamer by ORC, Cdc6 and Cdt1 (Remus et al., 2009; Evrin et al., 2009), forming the loading intermediate OCCM complex (Sun et al., 2013). This happens during the G1 phase of the cell cycle and the MCM complexes are inactive at this stage. Then, in a sec-

ond step during S phase, the inactive MCM complexes are converted into active helicases to initiate replication (Bell & Labib, 2016).

As discussed above, it is imperative that DNA replication occurs only in S phase and only once per cell cycle to prevent events like re-replication. For example, re-replication might take place when licensing factors like Cdc6 and Cdt1 allow multiple licensing events at the same origin (Vaziri et al., 2003; Melixetian et al., 2004). Thus, is crucial for the cell to have different overlapping inhibitory mechanisms to ensure the genome is fully replicated at the right time (Nguyen et al., 2001).

To ensure this tight regulation, DNA replication is controlled by the cell cycle kinases S phase forms of CDK (Cyclin-Dependent Kinase) and DDK (Dbf4-Dependent Kinase) to trigger the cascade of molecular events leading to the initiation of DNA replication (Kelly & Brown, 2000). During most of G1, the activity of these kinases is very low, so that the loading factors can recruit the MCM complex. The kinases' activity-levels increase at the end of G1 phase, causing CDK to phosphorylate ORC and Cdc6, which prevents additional loading events outside G1 phase (Nguyen et al., 2001). This is followed by the formation of the pre-IC (Pre-Initiation Complex), formed by the pre-RC complex, Sld3/Sld7 (Synthetically Lethal with Dpb11-1 3/7), Cdc45 (Cell Division Cycle 45), Sld2, Dpb11 (DNA Polymerase B (II)), GINS (Go, Ichi, Ni and San complex), and Mcm10 (MiniChromosome Maintenance 10). In S phase, DDK phosphorylates the MCM-DH (MCM double hexamers), which then stabilizes the interactions of the firing factors Sld3/Sld7 and Cdc45 (Sheu & Stillman, 2010; S. Tanaka et al., 2011). In addition, S-CDK phosphorylates Sld3 and Sld2, which is essential for firing (Zegerman & Diffley, 2007; Masumoto et al., 2002).

Phospho-Sld2 and Phospho-Sld3 are recognized by another firing factor, Dpb11, which then recruits Sld2 to the MCM-DH, where Sld2 serves as a platform for the leading strand polymerase Polϵ and the replication factor GINS (Zegerman & Diffley, 2007; Masumoto et al., 2002; Muramatsu et al., 2010). Conformational changes take place here in which ADP is released and the MCM complex binds ATP resulting in two CMG (Cdc45-MCM2-7-GINS) complexes with processive helicase activity (Gambus et al., 2006; Moyer et al., 2006). Then, a stable CMGE (Cdc45-MCM2-7-GINS-Polymerase ϵ) complex (Langston et al., 2014) nucleates the double stranded DNA inside the MCM complex ring and Mcm10 was suggested to trigger the ejection of the lagging strand by ATP hydrolysis. This results in origin firing and escape of both CMGEs in a bidirectional manner to start replicating DNA (Douglas et al., 2018; Lewis et al., 2022).

Finally, the full replisome is composed of three DNA polymerases (α (primase), δ (lagging strand) and ϵ (leading strand), TopoI (TOPOisomerase I) and TopoII, the fork protection complex (Csm3/Tof1 (Chromosome Segregation in Meiosis 3 / TOpoisomerase I-interacting Factor) and Mrc1 (Mediator of the Replication Checkpoint 1)), the histone chaperone FACT (FAcilitates Chromatin Transcription/transactions)/Nhp6a (Non-Histone Protein 6a), Ctf4 (Chromosome Transmission Fidelity 4), the single-strand binding proteins RPA (Replication Protein A), RFC (Replication Factor C) and PCNA (Proliferating Cell Nuclear Antigen) (Gambus et al., 2006; Yeeles et al., 2015, 2017; Kurat et al., 2017; Devbhandari et al., 2017).

Chromatin

Not naked DNA but chromatin is the natural substrate of DNA replication and the replisome has to deal with chromatin as discussed later. Chromatin is composed of negatively charged DNA wrapped around positively charged histone proteins, to generate a denser compacted structure inside the cell nucleus. Nucleosomes are the basic units of chromatin and are composed of 147 bp of DNA wrapped around a histone octamer (Kornberg & Lorch, 1999). The histone octamer contains two H2A-H2B dimers and one H3-H4 tetramer (Luger et al., 1997). Chromatin is further compacted into higher order structures via the linker histone H1 and cohesin, and the distance between nucleosomes, the NRL (Nucleosome Repeat Length), seems to influence how the 30 nm chromatin fibers are formed (Chen et al., 2021). Recently, the existence of these fibers has been debated and new models have been proposed. Instead, higher order structures may be composed of tetranucleosome units (Schalch et al., 2005; Hsieh et al., 2015; Risca et al., 2017) or groups of 2 to 12 nucleosomes that phase-separate, and organized as shorter fibers (Krietenstein & Rando, 2020; Chen et al., 2021).

Histone variants, like H2A.Z, can replace canonical histones in the nucleosome and can be important for recruitment of specific factors, in particular in the context of DNA repair (Y. Xu et al., 2012). Histone tails can also contain specific chemical modifications, like acyl or methyl groups, which are deposited and removed by enzymes known as *writers* and *erasers*. These modifications are known as PTMs (Post-Translational Modifications) and to this date, more than twenty eight have been described (Millán-Zambrano et al., 2022). This has led to the hypothesis of a histone code, especially because some chromatin-associated proteins contain a *reader* domain that is able to recognize especific histone PTMs. For example, bromo- and chromo-domains can read acetylation and methylation marks, respectively (Allis & Jenuwein, 2016).

Nucleosome dynamics

The nucleosome structure is very stable and a hindrance for DNA template processes like replication or transcription (Kurat et al., 2017; Devbhandari et al., 2017; Lorch et al., 1987) and it is imperative for cells to overcome this nucleosomal barrier. Over the years, extensive research was carried out to define determinants and to characterize mechanisms that influence nucleosome dynamics. One invaluable tool on this endeavor was the endonuclease activity of an enzyme from *Staphylococcus aureus*, MNase (Micrococcal Nuclease). When compared to nucleosome-free DNA, the DNA wrapped around the nucleosome is better protected against MNase activity, which cleaves linker DNA with preference for AT-rich sequences (Noll, 1974; Cockell et al., 1983). The extent of the digest can be titrated to obtain different DNA fragments corresponding to mononucleosomes or polynucleosomes, and this has been exploited to study nucleosome positioning in the genome coupled with high-throughput sequencing for MNase-seq (Micrococcal Nuclease digestion with deep Sequencing) (Kent et al., 2011; Wal & Pugh, 2012; Chereji et al., 2019). Another endonuclease, DNaseI (Deoxyribonuclease I), can also be used to study nucleosome positioning and has been particularly useful for finding TF binding sites in the context of chromatin, defining them as DNAseI-hypersensitive sites (Noll, 1974; Gross & Garrard, 1988), rather than defining nucleosome positioning. Both approaches, however, revealed that the genome is vastly covered by nucleosomes and, depending on where they

are located, that they can facilitate or prevent the binding of TF to their binding sites, thus impacting gene transcription and/or chromatin organization (Gross & Garrard, 1988; Jiang & Pugh, 2009; Hesselberth et al., 2009).

Nucleosome positioning is mainly determined by the action of chromatin remodeling enzymes (discussed below) rather than the underlying DNA sequence. However, certain sequences are intrinsically rigid, like poly(dA:dT) sequences (Kaplan et al., 2009; Zhang et al., 2011) or poly(G) motifs (Tsankov et al., 2011; Fenouil et al., 2012). These sequences can affect the bendability of DNA required for nucleosome wrapping and can thus cause nucleosome depletion as observed in *in vitro* reconstituted chromatin by salt gradient dialysis (Krietenstein et al., 2016). However, the generation of an NFR (Nucleosome Free Region) *in vivo* is an active process regulated by chromatin remodelers like RSC (Remodels the Structure of Chromatin), which preferentially evicts nucleosomes from poly(dA:dT) tracts (Barnes & Korber, 2021).

Chromatin remodelers are molecular motors that use the energy of ATP hydrolysis to assemble, evict, slide, or modify nucleosomes (by exchanging histone variants or evicting dimers). They are part of the helicase SF2 (Super-Family 2) superfamily and further classified into four sub-families based on the sequence similarity of their main ATPase motor subunit: (1) SWI/SNF (SWItch/Sucrose-Non-Fermenting), (2) ISWI (Imitation SWItch), (3) INO80 (INOsitol requiring 80) and (4) CHD (Chromodomain, Helicase and DNA binding). At least one remodeler from each family is present in most eukaryotes (Flaus et al., 2006; Clapier & Cairns, 2009; Narlikar et al., 2013). SWI/SNF-type remodelers can evict nucleosomes, while ISWI-, CHD- and INO80- type remodelers are the only known spacers. INO80 remodelers can also exchange histone variants. Chromatin remodelers have greater affinity for nucleosomes than naked DNA, because they recognize specific features in histones and PTMs as well as multiple domains that allow for interaction with histone chaperones, transcription factors and extranucleosomal DNA (Clapier, 2021). The ATPase active site of all chromatin remodelers resides between two RecA-like lobes, that function as a DNA translocase. In our current understanding, the cycling between ATP binding and ATP hydrolysis, causes the catalytic subunit to open and close, allowing the remodeler to progress along the DNA tracking strand following an inchworming mechanism. Each ATP cycle allows for movement at a 1 bp rate. Interestingly, the way the ATP molecule is processed by the RecA-like lobes resembles the Orc1-Orc4 ATP hydrolysis interaction, characteristic of AAA^{+}-ATPase containing proteins (Bowers et al., 2004): the Lobe1 from the RecA-like module harbors Walker A and B motifs while Lobe2 contains an arginine finger (Clapier, 2021).

In addition to chromatin remodelers, another class of proteins without catalytic activity known as histone chaperones, are involved in the removal, deposition and recycling of histones. Different histone chaperones can select for specific histone dimers and histone variants, making them an important component of different processes in the cell such as gene transcription, DNA replication and DNA repair (Hammond et al., 2017). Histone chaperones are classified into two groups based on their preference to interact with either H2A-H2B or H3-H4 dimers. However, some chaperones like Nap1 (Nucleosome Assembly Protein 1) and FACT/Nhp6a can bind with similar affinities to both (A. Bowman et al., 2011; McCullough et al., 2015; Chen et al., 2018). Some histone chaperones also function as histone donors for other chaperones, like Asf1 (Anti-Silencing

Function 1) does with the CAF-1 (Chromatin Assembly Factor 1) and HIR (HIstone Regulatory) complexes. Others may assist chromatin remodelers, like the histone chaperone DAXX (Death domain-Associated protein) that interacts with the SWI/SNF-type remodeler ATRX (α-Thalassemia/mental Retardation X-linked) at telomeres in mammalian cells (Gurard-Levin et al., 2014).

In vivo, some TFs can displace nucleosomes by binding to specific motifs with different affinities, in a manner that is probably facilitated by the activity of chromatin remodelers. In yeast, there are six strong NDF (Nucleosome-Displacing Factors) that have been shown to be able to decrease nucleosome occupancy by binding with high affinity at specific sites: Abf1, Reb1 (RNA polymerase I Enhancer Binding protein 1), Rap1 (Repressor/Activator site binding Protein 1), Cbf1 (Centromere Binding Factor 1), Mcm1 (MiniChromosome Maintenance 1) and Orc1 (Yan et al., 2018). All but Cbf1 are essential for cell viability in *S. cerevisiae* and have roles in either DNA transcription and Orc1 in DNA replication (Fourel et al., 2002, 1999). These factors are more likely to bind at the entry/exit site of nucleosomes by taking advantage of nucleosome dynamics in a similar manner as human pioneer factors. For Reb1 and Cbf1, this mechanism has been referred as dissociation rate compensation and is characterized by a long residence time at the binding sites once a binding event occurs (B. T. Donovan et al., 2019). Abf1, Reb1 and Rap1 are also known as GRFs (General Regulatory Factor) with an insulator function that may contribute to higher-order chromatin organization (Fourel et al., 2002). Furthermore, the association and dissociation events take place in seconds and may contribute to the binding of chromatin remodelers or histone chaperones, which in turn can also affect the binding rates of these TFs (G. D. Bowman & McKnight, 2017; Yan et al., 2018; B. T. Donovan et al., 2019; Ahmad et al., 2022). Similar TFs are also present in other organisms, like GAF (GAGA Factor) and Phaser in *Drosophila* and CTCF (CCCTC-binding Factor) in vertebrates (Tsukiyama et al., 1994; Fu et al., 2008; Baldi, Jain, et al., 2018).

Nucleosome phasing

The molecular mechanisms that regulate nucleosome positioning have been studied extensively at gene promoters (Krietenstein et al., 2016; Kubik et al., 2018, 2019; Oberbeckmann, Niebauer, et al., 2021). Active transcribed genes are characterized by an NFR and a well-positioned +1 nucleosome in the downstream region of the gene. Nucleosomes upstream and downstream of the NFR are regularly spaced as arrays with similar or equal linker lengths. Moreover, nucleosomes are phased with respect to the +1 nucleosome or a barrier-like factor. Active promoters have binding sites for GRFs like Abf1, Reb1 and Rap1 that can be used as a reference point by chromatin remodelers to space nucleosomes and generate a chromatin organization permissive for transcription. On top, the SWI/SNF-family remodeler RSC can work together with GRFs to generate an NFR (Hartley & Madhani, 2009; Krietenstein et al., 2016; Kubik et al., 2018).

RSC and SWI/SNF function as *pushers* by evicting and sliding nucleosomes and expanding the NFR at promoters; while spacing remodelers like ISW1a (Imitation SWItch 1a), ISW2 (Imitation SWItch 2), INO80 and Chd1 (Chromodomain, Helicase and DNA binding 1) act as *pullers* by sliding nucleosomes towards the NFR, counteracting the actions of the *pushers* (Krietenstein et al., 2016; Kubik et al., 2019). Spacing remodelers con-

tain a *protein ruler* to stablish the linker DNA length by measuring the distance between two nucleosomes (Yamada et al., 2011; Oberbeckmann, Niebauer, et al., 2021). These remodelers can generate different spacing by a mechanism that may involve the remodeler adopting specific conformations depending on the surrounding nucleosome density, DNA shape mechanics and via alignment to GRFs or double-strand breaks (Oberbeckmann, Niebauer, et al., 2021; Oberbeckmann, Krietenstein, et al., 2021).

The cooperation between TF and chromatin remodelers to generate phased nucleosomal arrays, is conserved in human cells. In mammalian cells the most predominant GRF-equivalent is CTCF and it has been reported to interact with chromatin remodelers like SNF2H (SMARCA5), SNF2L (SMARCA1) and Chd4, and its binding may be modulated by the remodeler activity (Wiechens et al., 2016; Clarkson et al., 2019; Bomber et al., 2023). High affinity binding sites of CTCF are enriched in promoters regions and correlated to shorter linker lengths, which seems to be a key feature of open/active chromatin regions in different organisms (Valouev et al., 2011; Baldi, Krebs, et al., 2018; Chereji et al., 2018). Moreover, CTCF and cohesin are involved in the organization of chromatin loops, that also seem to depend on nucleosome positioning and NRL, and symmetrical regular nucleosomal arrays have been observed around CTCF-cohesin interaction sites (Clarkson et al., 2019; Alpsoy et al., 2021). In particular, early replication initiation zones show an enrichment in CTCF-cohesin sites (Emerson et al., 2022).

The influence of chromatin on replication

As mentioned above, chromatin is a natural barrier for DNA template processes like transcription and replication. The replisome travels through nucleosomes with the assistance of chromatin remodelers, like INO80 or ISW1a, and histone chaperones like FACT/Nhp6a (Kurat et al., 2017; Devbhandari et al., 2017). The histone chaperone FACT/Nhp6a travels together with the replisome and does so without requiring direct contact with histones like other chaperones do. FACT/Nhp6a seems to work together with Mcm2, a subunit of the MCM complex, for the retention of the parental histones, by transferring them from the parental strand to the daughter strand (Foltman et al., 2013). FACT/Nhp6a also has been shown to greatly enhance replication rates *in vitro* (Kurat et al., 2017). Chromatin might influence when in the S phase an origin is fired, e.g., euchromatic regions tend to replicate earlier compared to heterochromatin (Fangman & Brewer, 1992). Histone acetylation has been shown to influence origin timing *in vivo* by stimulating origins to fire earlier (Vogelauer et al., 2002; Goren et al., 2008). In support of this, histone acetylation *in vitro*, catalyzed by NuA4 (Nucleosome Acetyltransferase of H4) and SAGA (Spt-Ada-Gcn5-Acetyltransferase), can enhance replication efficiency (Kurat et al., 2017).

Interestingly and similar to gene promoters, yeast origins show a stereotypical chromatin landscape characterized by an asymmetric NFR flanked by phased nucleosome arrays that is maintained throughout the cell cycle (Eaton et al., 2010; Berbenetz et al., 2010; Lai & Pugh, 2017). Both origins and promoters show a modular organization in which the origin's ACS motif could be compared to the TATA box required for gene transcription. The replication and transcription activity is also influenced by other elements that are proximal or distal to the ACS/TATA box, that through binding of specific pro-

teins (ORC, Cdc6 / TFIID) activate their function (Marahrens & Stillman, 1992; Lewin, 1990; Ptashne & Gann, 1990).

Both, active promoters and origins, contain very well positioned -1 and +1 nucleosomes around the NFR, that are followed by phased nucleosomal arrays (Berbenetz et al., 2010; Eaton et al., 2010; Lai & Pugh, 2017) (Fig. 3). The phased array in the downstream direction of promoters seems to display a higher nucleosome occupancy than in the upstream direction, however, this is an effect of the alignment procedure and not a true asymmetry in occupancy as it has been previously shown (Oberbeckmann et al., 2019). Further, origins show an asymmetrical pattern from the alignment point at the ACS, in which the downstream NFR is larger than the upstream. This asymmetry may be explained by a high composition of A-rich islands downstream the ACS. Moreover, both origins and active promoters show an A-rich/T-rich polarity that may promote DNA unwinding (Maicas & Friesen, 1990; Eaton et al., 2010).

Studies on single origins have shown that the flanking nucleosomes are important for origin function. If the nucleosome is positioned closer or further to the ACS, plasmid stability upon transformation decreases and affects replication initiation (Simpson, 1990; Lipford & Bell, 2001). Thus, precise nucleosome positioning at origins was suggested to be important.

ORC and chromatin

Remarkably, ORC influences nucleosome positioning at origins. *In vivo* studies have shown that after preventing ORC interaction with DNA by the use of two different temperature-sensitive alleles, *orc2-1* and *orc1-161*, nucleosome occupancy increases at the ACS (Berbenetz et al., 2010) and nucleosome phasing is disrupted (Eaton et al., 2010).

The Orc1 subunit or ORC in particular is associated with chromatin: Orc1 harbors a BAH domain, a non-essential chromatin binding module, that enhances ORC's association to a subset of origins in yeast and that promotes ORC binding to chromatin in human cells (Müller et al., 2010; Noguchi et al., 2006; De Ioannes et al., 2019). Human ORC1-BAH shows high affinity to histone H4K20me2 (histone H4 lysine 20 di-methylated) and mutations in this domain have been reported in patients with Meier-Gorlin Syndrome and other growth-related diseases (Bicknell, Walker, et al., 2011; Kuo et al., 2012). The depletion of ORC1-BAH's ability to recognize H4K20me2 causes reduced body size in mice (Kuo et al., 2012). Orc1 may also have pioneer factor functions as reported in yeast and human cells (Yan et al., 2018; Kara et al., 2015).

Orc1 interacts with proteins that can alter chromatin like Sir1 (Silent Information Regulator 1), which recruits the SIR complex to promote chromatin silencing at mating-type loci in yeast (Foss et al., 1993; Gardner et al., 1999). Yeast Orc1 and Sir3, another component of the SIR complex, are close homologs. However, while Sir3 can recognize and bind to deacetylated H4 tails, Orc1 has been suggested to interact with H4 tails regardless of their acetylation status (De Ioannes et al., 2019). In human cells, ORC1 interacts with the acetyltransferase HBO1 (Human acetylase Binding to ORC1) (Iizuka & Stillman, 1999) and is recruited to heterochromatic H3K9me3 and H3K27me3 (histone H3 lysine 9/27 tri-methylated)-containing regions by ORCA (ORC-Associated) (Shen et al., 2010; Bartke et al., 2010).

However, although all this knowledge was accumulated over the last decades, ORC´s precise function as a chromatin modulator, especially at origins, remained unknown.

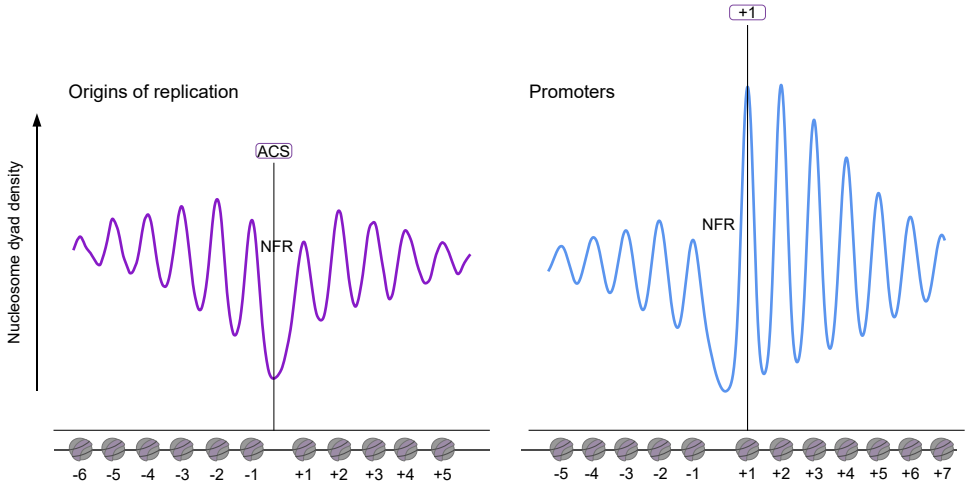

Figure 3: Representative comparison of the chromatin landscape between origins of replication and active gene promoters in asynchronous yeast cells.

Aims of the Thesis

In this thesis I mainly studied how chromatin architecture around origins of replication is established and whether this is important for the cell, which has been a long standing question in the field. In addition, I investigated how the chromatin factors Yta7 and FACT are recruited to active sites of replication and, in the case of Yta7, are regulated to assist the replisome go through the nucleosome barrier.

To uncover which factors are involved in generating nucleosomal arrays at origins, I used an *in vitro* reconstitution assay developed in the Korber lab that involves the use of chromatin generated by SGD, purified factors and high-throughput sequencing (Krietenstein et al., 2016; Oberbeckmann, Niebauer, et al., 2021) to screen several protein candidates. ORC was of especial interest since its binding site is present in all origins and was implicated to influence nucleosome positioning (Eaton et al., 2010; Berbenetz et al., 2010). To further determine whether nucleosome arrays were required for chromatin replication, we used the *in vitro* replication assay developed in the Diffley lab (Yeeles et al., 2015, 2017; Kurat et al., 2017) coupled with the *in vitro* reconstitution assay described above. This allowed us to perform for the first time, the in vitro replication of ~300 origins through a chromatinized template. Previous *in vitro* studies in the replication field have used just one or a couple of origins.

Yta7 is a AAA$^+$-ATPase-containing hexamer involved in genomic silencing by functioning as a barrier protein against heterochromatin spreading that also modulates histone gene transcription by binding all histone loci (Kurat et al., 2011). Because AAA$^+$-ATPase-containing complexes generally have catalytic functions related to the disassembly

and unfolding of proteins (Hanson & Whiteheart, 2005), we hypothesized that Yta7 might have a similar function. To dissect Yta7's function, we generated different mutants; and determined whether there was an effect related to chromatin or DNA replication by using a combination of *in vivo* and *in vitro* approaches.

In a collaborative paper with the Duderstadt lab, we aimed to find out how the histone chaperone FACT, a component of the replisome (Foltman et al., 2013), is able to enhance replication rates as previously reported *in vitro* (Kurat et al., 2017). We aimed to determine how FACT is able to interact with the replisome and nucleosomes by testing different FACT mutants using diverse biochemical approaches, including the *in vitro* replication assay through chromatin.

Summary

The whole genome has to be replicated only once per cell cycle and only during S phase. DNA replication initiates at specific sequences within the genome referred as origins of replication, and the number of origins correlates with the size of the genome. Problems with replication are associated with genomic instability and replication stress, both hallmarks of cancer and/or growth defects.

The budding yeast *S. cerevisiae* has been the preferred model organism to study DNA replication in detail because (a) its origins are well defined, (b) decades of genetics have painted a pretty clear picture of the process, and importantly, (c) replication has been reconstituted with purified components.

Replication origins contain an ARS that harbors an AT-rich conserved motif, known as the ACS, which is only present in yeast. ORC binds specifically to this motif and, together with other loading factors, recruits and loads the replication helicase, the MCM or Mcm2-7/Cdt1, as a double-hexamer. The chromatin structure at yeast origins is characterized by an NFR with flanking nucleosomal arrays of regular spacing. ORC has been shown to influence nucleosome positioning at origins of replication. However, prior to our work, the precise mechanism was unknown. Further, it was unclear if this stereotypical chromatin structure is functionally important for replication.

By screening ORC and seventeen purified chromatin factors via genome-scale *in vitro* reconstitution, we were able to determine the factors that establish this chromatin structure at origins. We found that ORC works together with the spacing remodelers INO80, ISW1a, ISW2 and Chd1 to generate nucleosome arrays at origins. Moreover, by testing different mutations of the Orc1 subunit of ORC, we were able to dissect ORC's chromatin function at origins of replication by uncoupling it from its canonical function as the MCM loader. We found that nucleosome array generation depends on Orc1's ability to hydrolyze ATP and on the BAH and IDR domains. These mutations were lethal *in vivo* and lost their arrays *in vitro*. Most importantly, this hindered DNA replication in *in vivo* and *in vitro*.

To replicate chromatinized DNA, the replisome requires the assistance of chromatin factors. It is known that some chromatin remodelers and histone chaperones enhance replication rates. In this context, we characterized Yta7 as a new type of chromatin remodeler namely chromatin segregase, that is different from the classical SF2 chromatin remodelers. One major difference is that its motor subunit belongs to the AAA^+-ATPase superfamily. We found that Yta7 is activated during the S phase by S-CDK, which phosphorylates it in close proximity of the ATPase domain. Interestingly, phosphorylation causes stimulation of the ATPase activity, activation of its chromatin segregase function

and strongly facilitated chromatin replication *in vitro*.

Finally, we collaborated with the Duderstadt lab at the MPI Biochemistry, to study how FACT, a two-subunit complex composed of Spt16 (SuPpressor of Ty's 16) and Pob3 (Pol1 Binding 3), engages with the replisome to enhance replication as previously reported. We found that Spt16's N-terminus is required for FACT's direct interaction with the replication machinery while the C-terminus of both Spt16 and Pob3 is required for nucleosome interaction ahead of the replication fork.

Zusammenfassung

Das gesamte Genom darf nur einmal pro Zellzyklus während der S-Phase repliziert werden. Die DNA-Replikation beginnt an bestimmten Sequenzen innerhalb des Genoms, den so genannten Replikationsursprüngen, wobei die Anzahl der Replikationsursprünge mit der Größe des Genoms korreliert. Probleme mit der Replikation werden mit genomischer Instabilität und Replikationsstress in Verbindung gebracht, beides Kennzeichen von Krebs und/oder Wachstumsstörungen.

Die Hefe *S. cerevisiae (Saccharomyces cerevisiae)* ist der bevorzugte Modellorganismus, um die DNA-Replikation im Detail zu studieren, weil (a) ihre Ursprünge gut definiert sind, (b) jahrzehntelange genetische und molekularbiologische Forschung ein ziemlich klares Bild des Prozesses gezeichnet hat und, was besonders wichtig ist, (c) die Replikation mit gereinigten Komponenten rekonstruiert werden konnte.

Replikationsursprünge enthalten eine ARS (Autonom Replizierende Sequenz), die ein Hefe-spezifisches AT-reiches konserviertes Motiv, die sogenannte ACS (ARS Konsensus-Sequenz), enthält. ORC (Origin-Erkennungskomplex) bindet spezifisch an dieses Motiv und lädt zusammen mit anderen Ladefaktoren die Replikations-Helikase Mcm2-7/Cdt1 oder MCM (Minichromosomen-Erhaltungsprotein) als Doppelhexamer. Die Chromatinstruktur an Replikationsursprüngen in Hefe ist durch eine NFR (Nukleosomenfreie Region) mit flankierenden Anordnung von Nukleosomen mit regelmäßigen Abständen gekennzeichnet. Es hat sich gezeigt, dass ORC die Positionierung der Nukleosomen an den Replikationsursprüngen beeinflusst. Vor unserer Arbeit war der genaue Mechanismus jedoch unbekannt. Außerdem war unklar, ob diese stereotype Chromatinstruktur für die Replikation funktionell wichtig ist.

Durch das Screening von ORC und siebzehn gereinigten Chromatinfaktoren mittels biochemischer *in vitro*-Rekonstitution im Genommaßstab konnten wir die Faktoren bestimmen, die die Chromatinstruktur an den Ursprüngen aufbauen. Wir fanden heraus, dass ORC mit den Spacing-Remodelern INO80 (Inositolbedarf 80), ISW1a (SWItch-Imitat 1a), ISW2 (SWItch-Imitat 2) und Chd1 (Chromodomäne, Helikase und DNA-Bindung 1) zusammenarbeitet, um Nukleosomen-Arrays an den Ursprüngen aufzubauen. Darüber hinaus konnten wir durch das Testen verschiedener Mutationen der Orc1-Untereinheit von ORC die Chromatinfunktion von ORC an den Replikationsursprüngen aufschlüsseln, indem wir sie von seiner kanonischen Funktion als MCM-Ladungsfaktor abkoppelten. Wir fanden heraus, dass ORC´s Chromatin-Funktion von der Fähigkeit von Orc1 ATP zu hydrolysieren abhängt. Weiteres sind die BAH (Bromo-Adjacent-Homologie) Domäne, sowie eine IDR (intrinsisch ungeordnetes Region) beteiligt. Diese Mutationen waren letal *in vivo* und einen konnten *in vitro* keine Arrays mehr aufbauen, welches auch die DNA-Replikation negativ beeinflusste.

Damit das Replisom chromatinisierte DNA replizieren kann, benötigt es die Unterstützung von Chromatinfaktoren, Chromatin Remodeler und Histone Chaperone. Es ist bekannt, dass einige dieser Faktoren die Replikationsraten erhöhen, aber die genaue Wechselwirkung zwischen diesen Proteinen ist noch nicht klar. In diesem Zusammenhang haben wir Yta7 (Tat-bindungshomolog 7) als einen neuen Typ von Chromatinremodelern charakterisiert, nämlich als „Chromatin-Segregase“, die sich von den klassischen SF2 (Super Familie)-Chromatin-Remodelern unterscheidet. Ein wesentlicher Unterschied besteht darin, dass seine motorische Untereinheit zur AAA^+-ATPase (Assoziiert ATPasen mit verschiedenen zellulären Aktivitäten)-Superfamilie gehört. Wir fanden heraus, dass Yta7 während der S-Phase durch S- Phase CDK (Cyclin-abhängige Kinase) aktiviert wird, welches Yta7 in unmittelbarer Nähe der ATPase-Domäne phosphoryliert. Interessanterweise bewirkt diese Phosphorylierung eine Stimulierung der ATPase-Aktivität, welche zur Folge hat, dass Yta7´s Chromatin-Segregase Funktion stimuliert wird. Dies stimuliert dann die Replikation durch Chromatin.

Schließlich untersuchten wir in Zusammenarbeit mit dem Duderstadt-Labor am MPI für Biochemie, wie FACT (FAcilitates Chromatin Transcription/transactions), ein Proteinkomplex bestehend aus den Untereinheiten Spt16 (SuPpressor of Ty's) und Pob3 (POl1 Binding), mit dem Replisom interagiert. Wir fanden heraus, dass der N-Terminus von Spt16 für die direkte Interaktion von FACT mit der Replikationsmaschinerie, während der C-Terminus sowohl von Spt16 als auch von Pob3 für die Nukleosomeninteraktion vor der Replikationsgabel erforderlich sind.

Paper I: Establishment and Function of Chromatin Organization at Replication Origins

Erika Chacin[1], Karl-Uwe Reusswig[2], Jessica Furtmeier[1], Priyanka Bansal[1], Leonhard Karl[2], Boris Pfander[2,3,4], Tobias Straub[5], Philipp Korber[1] and Christoph F. Kurat[1]

[1] Biomedical Center Munich (BMC), Division of Molecular Biology, Faculty of Medicine, Ludwig-Maximilians-Universität in Munich, Martinsried, Germany; [2] Max Planck Institute of Biochemistry, DNA Replication and Genome Integrity, Martinsried, Germany; [3] Genome Maintenance Mechanisms in Health and Disease, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany; [4] Institute of Genome Stability in Aging and Disease, CECAD, University of Cologne, Medical Faculty, Cologne, Germany; [5] Core Facility Bioinformatics, BMC, Faculty of Medicine, Ludwig-Maximilians-Universität in Munich, Martinsried, Germany

This article was published in *Nature* **616**, Issue Number: 7958, 836-842.

DOI: https://doi.org/10.1038/s41586-023-05926-8

Author contributions:

I performed all the *in vitro* reconstitution reactions on SGD chromatin, purified most proteins, prepared all the nuclei samples, sequencing libraries for MNase-seq, designed all the Orc1 mutant constructs for *in vivo* and *in vitro* studies, molecular cloning of the Orc1 mutant strains for protein purification, analyzed the sequencing data, prepared all the figures except the heatmaps, and wrote the methods. K-U.R. performed flow cytometry experiments and analyses; J.F. performed the molecular cloning of the Orc1 mutant strains for *in vivo* studies; P.B. generated the yeast expression strain and purified Spt6; L.K. purified and characterized Fun30; T.S. generated the heatmaps; C.F.K. performed the *in vitro* replication assays; C.F.K. and I performed the Co-IP experiments; C.F.K. designed the story and wrote most of the paper with input from P.K. and B.P. Supervision and funding was secured by C.F.K., P.K. and B.P. All authors were involved in editing.

Paper II: A CDK-regulated chromatin segregase promoting chromosome replication

Erika Chacin[1,8], Priyanka Bansal[1,8], Karl-Uwe Reusswig[2], Luis M. Diaz-Santin[3,4], Pedro Ortega[5], Petra Vizjak[1], Belen Gómez-González [5], Felix Müller-Planitz[1,6], Andrés Aguilera[5], Boris Pfander [2], Alan C. M. Cheung[3,4,7] and Christoph F. Kurat[1]

[1] Molecular Biology Division, Biomedical Center Munich, Ludwig-Maximilians-Universität, Munich, Planegg-Martinsried, Germany. [2] Max Planck Institute of Biochemistry, DNA Replication and Genome Integrity, Planegg-Martinsried, Germany. [3] Department of Structural and Molecular Biology, Institute of Structural and Molecular Biology, University College London, London, UK. [4] Institute of Structural and Molecular Biology, Biological Sciences, Birkbeck College, London, UK. [5] Andalusian Molecular Biology and Regenerative Medicine Centre-CABIMER, University of Seville-CSIC, Seville, Spain. [6] Present address: Institute of Physiological Chemistry, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany. [7] Present address: School of Biochemistry, University of Bristol, Bristol, UK. [8] These authors contributed equally: Erika Chacin, Priyanka Bansal.

This article was published in *Nature Communications* **12**, Article number: 5224 (2021).

DOI: https://doi.org/10.1038/s41467-021-25424-7

Author contributions:

P.B. and I performed *in vitro* experiments, protein purifications and prepared figures; L.M.D-S. and A.C.M.C. performed electron microscopy; A.C.M.C. aligned AAA+-ATPase sequences. K-U.R. performed flow cytometry experiments; P.O. perfomed DNA repair analyses; P.V. performed ATPase reactions. B.G-G., F. M-P., A.A., A.C.M.C., B.P. and C.F.K. analyzed experiments and data. C.F.K. conceived the study; C.F.K., F. M-P., A.A., A.C.M.C. and B.P. supervised the study and provided funding. C.F.K. wrote the paper and all authors were involved in the editing.

Paper III: The fork protection complex recruits FACT to reorganize nucleosomes during replication

Barbara Safaric[1], Erika Chacin[2], Matthias J. Scherr[1], Lional Rajappa[1], Christian Gebhardt[3], Christoph F. Kurat[2], Thorben Cordes[3] and Karl E. Duderstadt[1,4]

[1]Structure and Dynamics of Molecular Machines, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany, [2]Biomedical Center (BMC), Division of Molecular Biology, Faculty of Medicine, Ludwig-Maximilians-Universität München, Großhaderner Str. 9, 82152 Planegg, Germany, [3]Physical and Synthetic Biology, Faculty of Biology, Ludwig-Maximilians-Universität München, Großhadernerstr. 2-4, 82152 Planegg-Martinsried, Germany and [4]Physics Department, Technische Universität München, James-Franck-Straße 1, 85748 Garching, Germany

This article was published in *Nucleic Acids Research* **50**, Issue: 3 (2022), pages 1317-1334.

DOI: https://doi.org/10.1093/nar/gkac005

Author contributions:

I performed the *in vitro* chromatin replication assays; K.E.D. and B.S. designed and performed most experiments and interpreted data; M.J.S. designed Tof1 truncations; L.R. assisted with protein purifications; C.G. assisted with ALEX experiments. T.C., C.F.K. and K.E.D. supervised research. B.S. and K.E.D. wrote the manuscript with input from all authors.

References

Ahmad, K., Henikoff, S., & Ramachandran, S. (2022). Managing the Steady State Chromatin Landscape by Nucleosome Dynamics. *Ann. Rev. Biochem.*, *91*, 183–195. doi: 10.1146/annurev-biochem-032620-104508

Akerman, I., Kasaai, B., Bazarova, A., Sang, P. B., Peiffer, I., Artufel, M., ... Méchali, M. (2020). A predictable conserved DNA base composition signature defines human core DNA replication origins. *Nature Communications*, *11*(4826). doi: 10.1038/s41467-020-18527-0

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*, *17*(8), 487–500. doi: 10.1038/nrg.2016.59

Alpsoy, A., Sood, S., & Dykhuizen, E. C. (2021). At the crossroad of gene regulation and genome organization: Potential roles for ATP-dependent chromatin remodelers in the regulation of CTCF-mediated 3D architecture. *Biology*, *10*(272), 1–15. doi: 10.3390/biology10040272

Aparicio, O. M., Weinstein, D. M., & Bell, S. P. (1997). Components and dynamics of DNA replication complexes in S. cerevisiae: Redistribution of MCM proteins and Cdc45p during S phase. *Cell*, *91*, 59–69. doi: 10.1016/S0092-8674(01)80009-X

Baldi, S., Jain, D. S., Harpprecht, L., Zabel, A., Scheibe, M., Butter, F., ... Becker, P. B. (2018). Genome-wide Rules of Nucleosome Phasing in Drosophila. *Molecular Cell*, *72*, 661–672. doi: 10.1016/j.molcel.2018.09.032

Baldi, S., Krebs, S., Blum, H., & Becker, P. B. (2018). Genome-wide measurement of local nucleosome array regularity and spacing by nanopore sequencing. *Nature Structural and Molecular Biology*, *25*, 894–901. doi: 10.1038/s41594-018-0110-0

Barnes, T., & Korber, P. (2021). The active mechanism of nucleosome depletion by Poly(da:dt) tracts in vivo. *International Journal of Molecular Sciences*, *22*(8233), 1–20. doi: 10.3390/ijms22158233

Bartke, T., Vermeulen, M., Xhemalce, B., Robson, S. C., Mann, M., & Kouzarides, T. (2010). Nucleosome-interacting proteins regulated by DNA and histone methylation. *Cell*, *143*(3), 470–484. doi: 10.1016/j.cell.2010.10.012

Bell, S. P., Kobayashi, R., & Stillman, B. (1993). Yeast origin recognition complex functions in transcription silencing and DNA replication. *Science*, *262*, 1844–1849. doi: 10.1126/science.8266072

Bell, S. P., & Labib, K. (2016). Chromosome duplication in Saccharomyces cerevisiae. *Genetics*, *203*, 1027–1067. doi: 10.1534/genetics.115.186452

Bell, S. P., & Stillman, B. (1992). ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. *Nature*, *357*, 128–134. doi: 10.1038/357128a0

Berbenetz, N. M., Nislow, C., & Brown, G. W. (2010). Diversity of Eukaryotic DNA replication origins revealed by Genome-wide analysis of chromatin structure. *PLoS Genet*, *6*(9), e1001092. doi: 10.1371/journal.pgen.1001092

Bicknell, L. S., Bongers, E. M., Leitch, A., Brown, S., Schoots, J., Harley, M. E., ... Jackson, A. P. (2011). Mutations in the pre-replication complex cause Meier-Gorlin syndrome. *Nature Genetics*, *43*(4), 356–360. doi: 10.1038/ng.775

Bicknell, L. S., Walker, S., Klingseisen, A., Stiff, T., Leitch, A., Kerzendorfer, C., ... Jeggo, P. A. (2011). Mutations in ORC1, encoding the largest subunit of the origin recognition complex, cause microcephalic primordial dwarfism resembling Meier-Gorlin syndrome. *Nature Genetics*, *43*(4), 350–356. doi: 10.1038/ng.776

Bleichert, F., Botchan, M. R., & Berger, J. M. (2015). Crystal structure of the eukaryotic origin recognition complex. *Nature*, *519*(7543), 321–326. doi: 10.1038/nature14239

Blow, J. J., & Laskey, R. A. (1988). A role for the nuclear envelope in controlling DNA replication within the cell cycle. *Nature*, *332*, 546–548.

Bomber, M. L., Wang, J., Liu, Q., Barnett, K. R., Layden, H. M., Hodges, E., ... Hiebert, S. W. (2023). Article Human SMARCA5 is continuously required to maintain nucleosome spacing Article Human SMARCA5 is continuously required to maintain nucleosome spacing. *Molecular Cell*, *83*, 1–16. doi: 10.1016/j.molcel.2022.12.018

Bowers, J. L., Randell, J. C., Chen, S., & Bell, S. P. (2004). ATP hydrolysis by ORC catalyzes reiterative Mcm2-7 assembly at a defined origin of replication. *Molecular Cell*, *16*, 967–978. doi: 10.1016/j.molcel.2004.11.038

Bowman, A., Ward, R., Wiechens, N., Singh, V., El-Mkami, H., Norman, D. G., & Owen-Hughes, T. (2011). The Histone Chaperones Nap1 and Vps75 Bind Histones H3 and H4 in a Tetrameric Conformation. *Molecular Cell*, *41*, 398–408. doi: 10.1016/j.molcel.2011.01.025

Bowman, G. D., & McKnight, J. N. (2017). Sequence-specific targeting of chromatin remodelers organizes precisely positioned nucleosomes throughout the genome. *BioEssays*, *39*(1), 1–8. doi: 10.1002/bies.201600183

Broach, J., Li, Y.-Y., Feldman, J., Jayaram, M., Abraham, J., Nasmyth, K., & Hicks, J. (1983). Localization and sequence analysis of yeast origins of dna replication. *Cold Spring Harbor Symposia on Quantitative Biology*, *47*, 1165-1173. doi: 10.1101/SQB.1983.047.01.132

Cairns, J. (1963). The bacterial chromosome and its manner of replication as seen by autoradiography. *Journal of Molecular Biology*, *6*(3), 208-213. doi: 10.1016/S0022-2836(63)80070-4

Cayrou, C., Ballester, B., Peiffer, I., Fenouil, R., Coulombe, P., Andrau, J.-C., ... Méchali, M. (2015). The chromatin environment shapes dna replication origin organization and defines origin classes. *Genome Research*, *25*(12), 1873-1885. doi: 10.1101/gr.192799.115

Chen, P., Dong, L., Hu, M., Wang, Y. Z., Xiao, X., Zhao, Z., ... Li, G. (2018). Functions of FACT in Breaking the Nucleosome and Maintaining Its Integrity at the Single-Nucleosome Level. *Molecular Cell*, *71*(2), 284–293.e4. doi: 10.1016/j.molcel.2018.06.020

Chen, P., Li, W., & Li, G. (2021). Structures and Functions of Chromatin Fibers. *Annual Review of Biophysics*, *50*, 95–116. doi: 10.1146/annurev-biophys-062920-063639

Chereji, R. V., Bryson, T. D., & Henikoff, S. (2019). Quantitative MNase-seq accurately maps nucleosome occupancy levels. *Genome biology*, *20*(198), 1–18. doi: 10.1186/s13059-019-1815-z

Chereji, R. V., Ramachandran, S., Bryson, T. D., & Henikoff, S. (2018). Precise genome-wide mapping of single nucleosomes and linkers in vivo. *Genome Biology*, *19*(19), 1–20. doi: 10.1186/s13059-018-1398-0

Clapier, C. R. (2021). Sophisticated conversations between chromatin and chromatin remodelers, and dissonances in cancer. *Int. J. Mol. Sci.*, *22*(5578). doi: 10.3390/ijms22115578

Clapier, C. R., & Cairns, B. R. (2009). The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.*, *78*, 273–304. doi: 10.1146/annurev.biochem.77.062706.153223

Clarkson, C. T., Deeks, E. A., Samarista, R., Mamayusupova, H., Zhurkin, V. B., & Teif, V. B. (2019). CTCF-dependent chromatin boundaries formed by asymmetric nucleosome arrays with decreased linker length. *Nucleic acids research*, *47*(21), 11181–11196. doi: 10.1093/nar/gkz908

Cockell, M., Rhodes, D., & Klug, A. (1983). Location of the primary sites of micrococcal nuclease cleavage on the nucleosome core. *J. Mol. Biol.*, *170*, 423–446. doi: 10.1016/S0022-2836(83)80156-9

Costa, A., & Diffley, J. F. (2022). The Initiation of Eukaryotic DNA Replication. *Annual Review of Biochemistry*, *91*, 107–131. doi: 10.1146/annurev-biochem-072321-110228

Coster, G., Frigola, J., Beuron, F., Morris, E. P., & Diffley, J. F. (2014). Origin Licensing Requires ATP Binding and Hydrolysis by the MCM Replicative Helicase. *Molecular Cell*, *55*, 666–677. doi: 10.1016/j.molcel.2014.06.034

De Ioannes, P., Leon, V. A., Kuang, Z., Wang, M., Boeke, J. D., Hochwagen, A., & Armache, K. J. (2019). Structure and function of the Orc1 BAH-nucleosome complex. *Nature Communications*, *10*(2894), 1–11. doi: 10.1038/s41467-019-10609-y

Devbhandari, S., Jiang, J., Kumar, C., Whitehouse, I., & Remus, D. (2017). Chromatin Constrains the Initiation and Elongation of DNA Replication. *Molecular Cell*, *65*, 131–141. doi: 10.1016/j.molcel.2016.10.035

Diffley, J. F., Cocker, J. H., Dowell, S. J., & Rowley, A. (1994). Two steps in the assembly of complexes at yeast replication origins in vivo. *Cell*, *78*, 303–316. doi: 10.1016/0092-8674(94)90299-2

Diffley, J. F., & Stillman, B. (1988). Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. *Proc Natl Acad Sci USA*, *85*, 2120–2124. doi: 10.1073/pnas.85.7.2120

Donovan, B. T., Chen, H., Jipa, C., Bai, L., & Poirier, M. G. (2019). Dissociation rate compensation mechanism for budding yeast pioneer transcription factors. *eLife*, *8*(e43008), 1–24. doi: 10.7554/eLife.43008

Donovan, S., Harwood, J., Drury, L. S., & Diffley, J. F. (1997). Cdc6p-dependent loading of Mcm proteins onto pre-replicative chromatin in budding yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(11), 5611–5616. doi: 10.1073/pnas.94.11.5611

Douglas, M. E., Ali, F. A., Costa, A., & Diffley, J. F. (2018). The mechanism of eukaryotic CMG helicase activation. *Nature*, *555*(7695), 265–268. doi: 10.1038/nature25787

Duderstadt, K. E., & Berger, J. M. (2008). AAA+ ATPases in the initiation of DNA replication. *Critical Reviews in Biochemistry and Molecular Biology*, *43*, 163–187. doi: 10.1080/10409230802058296

Eaton, M. L., Galani, K., Kang, S., Bell, S. P., & MacAlpine, D. M. (2010). Conserved nucleosome positioning defines replication origins. *Genes and Development*, *24*(8), 748–753. doi: 10.1101/gad.1913210

Emerson, D. J., Zhao, P. A., Cook, A. L., Barnett, R. J., Klein, K. N., Saulebekova, D., ... Phillips-Cremins, J. E. (2022). Cohesin-mediated loop anchors confine the locations of human replication origins. *Nature*, *606*, 812–819. doi: 10.1038/s41586-022-04803-0

Evrin, C., Clarke, P., Zech, J., Lurz, R., Sun, J., Uhle, S., ... Speck, C. (2009). A double-hexameric MCM2-7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication. *Proc Natl Acad Sci USA*, *106*(48), 20240–20245. doi: 10.1073/pnas.0911500106

Fangman, W. L., & Brewer, B. J. (1992). A question of time: Replication origins of eukaryotic chromosomes. *Cell*, *71*, 363–366. doi: 10.1016/0092-8674(92)90505-7

Feng, X., Noguchi, Y., Barbon, M., Stillman, B., Speck, C., & Li, H. (2021). The structure of ORC–Cdc6 on an origin DNA reveals the mechanism of ORC activation by the replication initiator Cdc6. *Nature Communications*, *12*(3883), 1–12. doi: 10.1038/s41467-021-24199-1

Fenouil, R., Cauchy, P., Koch, F., Descostes, N., Cabeza, J. Z., Innocenti, C., ... Andrau, J. C. (2012). CpG islands and GC content dictate nucleosome depletion in a transcription-independent manner at mammalian promoters. *Genome Research*, *22*(12), 2399–2408. doi: 10.1101/gr.138776.112

Flaus, A., Martin, D. M., Barton, G. J., & Owen-Hughes, T. (2006). Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Research*, *34*(10), 2887–2905. doi: 10.1093/nar/gkl295

Foltman, M., Evrin, C., De Piccoli, G., Jones, R. C., Edmondson, R. D., Katou, Y., ... Labib, K. (2013). Eukaryotic replisome components cooperate to process histones during chromosome replication. *Cell Reports*, *3*, 892–904. doi: 10.1016/j.celrep.2013.02.028

Foss, M., Mcnally, F. J., Laurenson, P., & Rine, J. (1993). Origin Recognition Complex and in Transcriptional DNA Replication in S . cerevisiae. *Science*, *262*, 1838–1844.

Fourel, G., Miyake, T., Defossez, P. A., Li, R., & Gilson, É. (2002). General Regulatory Factors (GRFs) as genome partitioners. *Journal of Biological Chemistry*, *277*(44), 41736–41743. doi: 10.1074/jbc.M202578200

Fourel, G., Revardel, E., Koering, C. E., & Gilson, E. (1999). Cohabitation of insulators and silencing elements in yeast subtelomeric regions. *EMBO Journal*, *18*(9), 2522–2537. doi: 10.1093/emboj/18.9.2522

Fu, Y., Sinha, M., Peterson, C. L., & Weng, Z. (2008). The insulator binding protein CTCF positions 20 nucleosomes around its binding sites across the human genome. *PLoS Genetics*, *4*(7), e1000138. doi: 10.1371/journal.pgen.1000138

Gaillard, H., García-Muse, T., & Aguilera, A. (2015). Replication stress and cancer. *Nature Reviews Cancer*, *15*, 276–280. doi: 10.1038/nrc3916

Gambus, A., Jones, R. C., Sanchez-Diaz, A., Kanemaki, M., van Deursen, F., Edmondson, R. D., & Labib, K. (2006). GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nature Cell Biology*, *8*(4), 358–366. doi: 10.1038/ncb1382

Ganier, O., Prorok, P., Akerman, I., & Méchali, M. (2019). Metazoan dna replication origins. *Current Opinion in Cell Biology*, *58*, 134-141. (Cell Nucleus) doi: 10.1016/j.ceb.2019.03.003

Gardner, K. A., Rine, J., & Fox, C. A. (1999). A region of the Sir1 protein dedicated to recognition of a silencer and required for interaction with the Orc1 protein in Saccharomyces cerevisiae. *Genetics*, *151*, 31–44. doi: 10.1093/genetics/151.1.31

Giraldo, R. (2003). Common domains in the initiators of DNA replication in Bacteria, Archaea and Eukarya: Combined structural, functional and phylogenetic perspectives. *FEMS Microbiology Reviews*, *26*, 533–554. doi: 10.1016/S0168-6445(02)00145-6

Goren, A., Tabib, A., Hecht, M., & Cedar, H. (2008). DNA replication timing of the human β-globin domain is controlled by histone modification at the origin. *Genes and Development*, *22*, 1319–1324. doi: 10.1101/gad.468308

Gross, D. S., & Garrard, W. T. (1988). Nuclease hypersensitive sites in chromatin. *Ann. Rev. Biochem.*, *57*, 159–197. doi: 10.1146/annurev.bi.57.070188.001111

Gurard-Levin, Z. A., Quivy, J. P., & Almouzni, G. (2014). Histone chaperones: Assisting histone traffic and nucleosome dynamics. *Annual Review of Biochemistry*, *83*, 487–517. doi: 10.1146/annurev-biochem-060713-035536

Hammond, C. M., Strømme, C. B., Huang, H., Patel, D. J., & Groth, A. (2017). Histone chaperone networks shaping chromatin function. *Nature Reviews Molecular Cell Biology*, *18*(3), 141–158. doi: 10.1038/nrm.2016.159

Hanson, P. I., & Whiteheart, S. W. (2005). AAA+ proteins: Have engine, will work. *Nature Reviews Molecular Cell Biology*, *6*, 519–529. doi: 10.1038/nrm1684

Hartley, P. D., & Madhani, H. D. (2009). Mechanisms that specify promoter nucleosome location and identity. *Cell*, *137*(3), 445–458. doi: 10.1016/j.cell.2009.02.043

Hesselberth, J. R., Chen, X., Zhang, Z., Sabo, P. J., Sandstrom, R., Reynolds, A. P., ... Stamatoyannopoulos, J. A. (2009). Global mapping of protein-DNA interactions in vivo by digital genomic footprinting. *Nature Methods*, *6*(4), 283–289. doi: 10.1038/nmeth.1313

Hsieh, T. H. S., Weiner, A., Lajoie, B., Dekker, J., Friedman, N., & Rando, O. J. (2015). Mapping Nucleosome Resolution Chromosome Folding in Yeast by Micro-C. *Cell*, *162*(1), 108–119. doi: 10.1016/j.cell.2015.05.048

Huberman, J. A., & Riggs, A. D. (1968). On the Mechanism in Mammalian of DNA Replication Chromosomes. *J. Mol. Biol.*, *32*(2), 327–341. doi: 10.1016/0022-2836(68)90013-2

Iizuka, M., & Stillman, B. (1999). Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. *Journal of Biological Chemistry*, *274*(33), 23027–23034. doi: 10.1074/jbc.274.33.23027

Jacob, F., Brenner, S., & Cuzin, F. (1963). On the regulation of dna replication in bacteria. *Cold Spring Harbor Symposia on Quantitative Biology*, *28*, 329-348. doi: 10.1101/SQB.1963.028.01.048

Jaremko, M. J., On, K. F., Thomas, D. R., Stillman, B., & Joshua-Tor, L. (2020). The dynamic nature of the human origin recognition complex revealed through five cryoem structures. *eLife*, *9*(e58622), 1–19. doi: 10.7554/eLife.58622

Jiang, C., & Pugh, B. F. (2009). Nucleosome positioning and gene regulation: Advances through genomics. *Nature Reviews Genetics*, *10*, 161–172. doi: 10.1038/nrg2522

Kaplan, N., Moore, I. K., Fondufe-Mittendorf, Y., Gossett, A. J., Tillo, D., Field, Y., ... Segal, E. (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*, *458*, 362–366. doi: 10.1038/nature07667

Kara, N., Hossain, M., Prasanth, S. G., & Stillman, B. (2015). Orc1 binding to mitotic chromosomes precedes spatial patterning during G1 phase and assembly of the origin recognition complex in human cells. *Journal of Biological Chemistry*, *290*(19), 12355–12369. doi: 10.1074/jbc.M114.625012

Kawakami, H., Ohashi, E., Kanamoto, S., Tsurimoto, T., & Katayama, T. (2015). Specific binding of eukaryotic ORC to DNA replication origins depends on highly conserved basic residues. *Scientific Reports*, *5*(14929), 1–14. doi: 10.1038/srep14929

Kelly, T. J., & Brown, G. W. (2000). Regulation of Chromosome Replication. *Annu. Rev. Biochem.*, *69*, 829–880.

Kent, N. A., Adams, S., Moorhouse, A., & Paszkiewicz, K. (2011). Chromatin particle spectrum analysis: A method for comparative chromatin structure analysis using paired-end mode next-generation DNA sequencing. *Nucleic Acids Research*, *39*(5), 1–12. doi: 10.1093/nar/gkq1183

Klemm, R. D., Austin, R. J., & Bell, S. P. (1997). Coordinate binding of ATP and origin DNA regulates the ATPase activity of the origin recognition complex. *Cell*, *88*, 493–502. doi: 10.1016/S0092-8674(00)81889-9

Klemm, R. D., & Bell, S. P. (2001). ATP bound to the origin recognition complex is important for preRC formation. *Proc Natl Acad Sci USA*, *98*(15), 8361–8367. doi: 10.1073/pnas.131006898

Kornberg, R. D., & Lorch, Y. (1999). Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell*, *98*, 285–294. doi: 10.1016/S0092-8674(00)81958-3

Krietenstein, N., & Rando, O. J. (2020). Mesoscale organization of the chromatin fiber. *Current Opinion in Genetics and Development*, *61*, 32–36. doi: 10.1016/j.gde.2020.02.022

Krietenstein, N., Wal, M., Watanabe, S., Park, B., Peterson, C. L., Pugh, B. F., & Korber, P. (2016). Genomic Nucleosome Organization Reconstituted with Pure Proteins. *Cell*, *167*, 709–721. doi: 10.1016/j.cell.2016.09.045

Kubik, S., Bruzzone, M. J., Challal, D., Dreos, R., Mattarocci, S., Bucher, P., ... Shore, D. (2019). Opposing chromatin remodelers control transcription initiation frequency and start site selection. *Nature Structural & Molecular Biology*, *26*, 744–754. doi: 10.1038/s41594-019-0273-3

Kubik, S., O'Duibhir, E., de Jonge, W. J., Mattarocci, S., Albert, B., Falcone, J. L., ... Shore, D. (2018). Sequence-Directed Action of RSC Remodeler and General Regulatory Factors Modulates +1 Nucleosome Position to Facilitate Transcription. *Molecular Cell*, *71*, 89–102. doi: 10.1016/j.molcel.2018.05.030

Kuo, A. J., Song, J., Cheung, P., Ishibe-Murakami, S., Yamazoe, S., Chen, J. K., ... Gozani, O. (2012). The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier-Gorlin syndrome. *Nature*, *484*, 115–119. doi: 10.1038/nature10956

Kurat, C. F., Lambert, J. P., van Dyk, D., Tsui, K., van Bakel, H., Kaluarachchi, S., ... Andrews, B. J. (2011). Restriction of histone gene transcription to S phase by phosphorylation of a chromatin boundary protein. *Genes and Development*, *25*, 2489–2501. doi: 10.1101/gad.173427.111

Kurat, C. F., Yeeles, J. T., Patel, H., Early, A., & Diffley, J. F. (2017). Chromatin Controls DNA Replication Origin Selection, Lagging-Strand Synthesis, and Replication Fork Rates. *Molecular Cell*, *65*(1), 117–130. doi: 10.1016/j.molcel.2016.11.016

Lai, W. K., & Pugh, B. F. (2017). Understanding nucleosome dynamics and their links to gene expression and DNA replication. *Nature Reviews Molecular Cell Biology*, *18*, 548–562. doi: 10.1038/nrm.2017.47

Langston, L. D., Zhang, D., Yurieva, O., Georgescu, R. E., Finkelstein, J., Yao, N. Y., ... O'Donnell, M. E. (2014). CMG helicase and DNA polymerase ϵ form a functional 15-subunit holoenzyme for eukaryotic leading-strand DNA replication. *Proc Natl Acad Sci USA*, *111*(43), 15390–15395. doi: 10.1073/pnas.1418334111

Leonard, A. C., & Méchali, M. (2013). Dna replication origins. *Cold Spring Harbor Perspectives in Biology*, *5*(10). doi: 10.1101/cshperspect.a010116

Lewin, B. (1990). Commitment and activation at pol II promoters: A tail of protein-protein interactions. *Cell*, *61*, 1161–1164. doi: 10.1016/0092-8674(90)90675-5

Lewis, J. S., Gross, M. H., Sousa, J., Henrikus, S. S., Greiwe, J. F., Nans, A., ... Costa, A. (2022). Mechanism of replication origin melting nucleated by CMG helicase assembly. *Nature*, *606*, 1007–1014. doi: 10.1038/s41586-022-04829-4

Li, N., Lam, W. H., Zhai, Y., Cheng, J., Cheng, E., Zhao, Y., ... Tye, B. K. (2018). Structure of the origin recognition complex bound to DNA replication origin. *Nature*, *559*, 217–222. doi: 10.1038/s41586-018-0293-x

Liachko, I., Youngblood, R. A., Keich, U., & Dunham, M. J. (2013). High-resolution mapping, characterization, and optimization of autonomously replicating sequences in yeast. *Genome Research*, *23*(4), 698–704. doi: 10.1101/gr.144659.112

Lipford, J. R., & Bell, S. P. (2001). Nucleosomes Positioned by ORC Facilitate the Initiation of DNA Replication. *Molecular Cell*, *7*, 21–30. doi: 10.1016/S1097-2765(01)00151-4

Lorch, Y., LaPointe, J. W., & Kornberg, R. D. (1987). Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell*, *49*, 203–210. doi: 10.1016/0092-8674(87)90561-7

Luger, K., Ma, A. W., Richmond, R. K., Sargent, D. F., & Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, *389*, 251–260.

Mackiewicz, P., Zakrzewska-Czerwińska, J., Zawilak, A., Dudek, M. R., & Cebrat, S. (2004, 01). Where does bacterial replication start? Rules for predicting the oriC region. *Nucleic Acids Research*, *32*(13), 3781-3791. doi: 10.1093/nar/gkh699

Maicas, E., & Friesen, J. D. (1990). A sequence pattern that occurs at the transcription initiation region of yeast RNA polymerase II promoters. *Nucleic Acids Research*, *18*(11), 3387–3393. doi: 10.1093/nar/18.11.3387

Marahrens, Y., & Stillman, B. (1992). A yeast chromosomal origin of DNA replication defined by multiple functional elements. *Science*, *255*(5046), 817–823. doi: 10.1126/science.1536007

Masumoto, H., Muramatsu, S., Kamimura, Y., & Araki, H. (2002). S-Cdk-dependent phosphorylation of Sld2 essential for chromosomal DNA replication in budding yeast. *Nature*, *415*(6872), 651–655. doi: 10.1038/nature713

McCullough, L., Connell, Z., Petersen, C., & Formosa, T. (2015). The Abundant Histone Chaperones Spt6 and FACT Collaborate to Assemble, Inspect, and Maintain Chromatin Structure in Saccharomyces cerevisiae. *Genetics*, *201*, 1031–1045. doi: 10.1534/genetics.115.180794

Melixetian, M., Ballabeni, A., Masiero, L., Gasparini, P., Zamponi, R., Bartek, J., ... Helin, K. (2004). Loss of Geminin induces rereplication in the presence of functional p53. *The Journal of Cell Biology*, *165*(4), 473–482. doi: 10.1083/jcb.200403106

Méndez, J., Zou-Yang, X. H., Kim, S. Y., Hidaka, M., Tansey, W. P., & Stillman, B. (2002). Human origin recognition complex large subunit is degraded by ubiquitin-mediated proteolysis after initiation of DNA replication. *Molecular Cell*, *9*, 481–491. doi: 10.1016/S1097-2765(02)00467-7

Millán-Zambrano, G., Burton, A., Bannister, A. J., & Schneider, R. (2022). Histone post-translational modifications — cause and consequence of genome function. *Nature Reviews Genetics*, *23*(9), 563–580. doi: 10.1038/s41576-022-00468-7

Moyer, S. E., Lewis, P. W., & Botchan, M. R. (2006). Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. *Proc Natl Acad Sci USA*, *103*(27), 10236–10241. doi: 10.1073/pnas.0602400103

Müller, P., Park, S., Shor, E., Huebert, D. J., Warren, C. L., Ansari, A. Z., ... Fox, C. A. (2010). The conserved bromo-adjacent homology domain of yeast Orc1 functions in the selection of DNA replication origins within chromatin. *Genes and Development*, *24*, 1418–1433. doi: 10.1101/gad.1906410

Muramatsu, S., Hirai, K., Tak, Y. S., Kamimura, Y., & Araki, H. (2010). CDK-dependent complex formation between replication proteins Dpb11, Sld2, Pol ϵ, and GINS in budding yeast. *Genes and Development*, *24*, 602–612. doi: 10.1101/gad.1883410

Narlikar, G. J., Sundaramoorthy, R., & Owen-Hughes, T. (2013). Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. *Cell*, *154*(3), 490–503. doi: 10.1016/j.cell.2013.07.011

Nguyen, V. Q., Co, C., & Li, J. J. (2001). Cyclin-dependent kinases prevent DNA rereplication through multiple mechanisms. *Nature*, *411*, 1068–1073.

Nieduszynski, C. A., Knox, Y., & Donaldson, A. D. (2006). Genome-wide identification of replication origins in yeast by comparative genomics. *Genes and Development*, *20*(14), 1874–1879. doi: 10.1101/gad.385306

Noguchi, K., Vassilev, A., Ghosh, S., Yates, J. L., & DePamphilis, M. L. (2006). The BAH domain facilitates the ability of human Orc1 protein to activate replication origins in vivo. *EMBO Journal*, *25*(22), 5372–5382. doi: 10.1038/sj.emboj.7601396

Noll, M. (1974). Subunit structure of chromatin. *Nature*, *251*, 249–251.

Oberbeckmann, E., Krietenstein, N., Niebauer, V., Wang, Y., Schall, K., Moldt, M., ... Eustermann, S. (2021). Genome information processing by the INO80 chromatin remodeler positions nucleosomes. *Nature Communications*, *12*(3231), 1–19. doi: 10.1038/s41467-021-23016-z

Oberbeckmann, E., Niebauer, V., Watanabe, S., Farnung, L., Moldt, M., Schmid, A., ... Korber, P. (2021). Ruler elements in chromatin remodelers set nucleosome array spacing and phasing. *Nature Communications*, *12*(3232), 1–17. doi: 10.1038/s41467-021-23015-0

Oberbeckmann, E., Wolff, M., Krietenstein, N., Heron, M., Ellins, J. L., Schmid, A., ... Korber, P. (2019). Absolute nucleosome occupancy map for the Saccharomyces cerevisiae genome. *Genome Research*, *29*(12), 1996–2009. doi: 10.1101/gr.253419.119.8

Ohta, S., Tatsumi, Y., Fujita, M., Tsurimoto, T., & Obuse, C. (2003). The ORC1 cycle in human cells. *Journal of Biological Chemistry*, *278*(42), 41535–41540. doi: 10.1074/jbc.M307535200

Parker, M. W., Bell, M., Mir, M., Kao, J. A., Darzacq, X., Botchan, M. R., & Berger, J. M. (2019). A new class of disordered elements controls DNA replication through initiator self-assembly. *eLife*, *8*(e48562), 1–35. doi: 10.7554/eLife.48562

Ptashne, M., & Gann, A. A. (1990). Activators and targets. *Nature*, *346*(6282), 329–331. doi: 10.1038/346329a0

Rao, H., & Stillman, B. (1995). The origin recognition complex interacts with a bipartite DNA binding site within yeast replicators. *Proc Natl Acad Sci USA*, *92*, 2224–2228. doi: 10.1073/pnas.92.6.2224

Remus, D., Beall, E. L., & Botchan, M. R. (2004). Dna topology, not dna sequence, is a critical determinant for drosophila orc–dna binding. *The EMBO Journal*, *23*(4), 897-907. doi: 10.1038/sj.emboj.7600077

Remus, D., Beuron, F., Tolun, G., Griffith, J. D., Morris, E. P., & Diffley, J. F. (2009). Concerted Loading of Mcm2-7 Double Hexamers around DNA during DNA Replication Origin Licensing. *Cell*, *139*, 719–730. doi: 10.1016/j.cell.2009.10.015

Risca, V. I., Denny, S. K., Straight, A. F., & Greenleaf, W. J. (2017). Variable chromatin structure revealed by in situ spatially correlated DNA cleavage mapping. *Nature*, *541*, 237–241. doi: 10.1038/nature20781

Robinson, N. P., Dionne, I., Lundgren, M., Marsh, V. L., Bernander, R., & Bell, S. D. (2004). Identification of Two Origins of Replication in the Single Chromosome of the Archaeon *Sulfolobus solfataricus*. *Cell*, *116*, 25–38. doi: 10.1016/S0092-8674(03)01034-1

Rowley, A., Cocker, J., Harwood, J., & Diffley, J. (1995). Initiation complex assembly at budding yeast replication origins begins with the recognition of a bipartite sequence by limiting amounts of the initiator, ORC. *The EMBO Journal*, *14*(11), 2631–2641. doi: 10.1002/j.1460-2075.1995.tb07261.x

Schalch, T., Duda, S., Sargent, D. F., & Richmond, T. J. (2005). X-ray structure of a tetranucleosome and its implications for the chromatin fibre. *Nature*, *436*, 138–141. doi: 10.1038/nature03686

Schmidt, J. M., & Bleichert, F. (2020). Structural mechanism for replication origin binding and remodeling by a metazoan origin recognition complex and its co-loader Cdc6. *Nature Communications*, *11*(4263), 1–17. doi: 10.1038/s41467-020-18067-7

Shen, Z., Sathyan, K. M., Geng, Y., Zheng, R., Chakraborty, A., Freeman, B., ... Prasanth, S. G. (2010). A WD-repeat protein stabilizes ORC binding to chromatin. *Molecular Cell*, *40*, 99–111. doi: 10.1016/j.molcel.2010.09.021

Sheu, Y. J., & Stillman, B. (2010). The Dbf4-Cdc7 kinase promotes S phase by alleviating an inhibitory activity in Mcm4. *Nature*, *463*, 113–117. doi: 10.1038/nature08647

Siddiqui, K., & Stillman, B. (2007). ATP-dependent assembly of the human origin recognition complex. *Journal of Biological Chemistry*, *282*(44), 32370–32383. doi: 10.1074/jbc.M705905200

Sima, J., Chakraborty, A., Dileep, V., Michalski, M., Klein, K. N., Holcomb, N. P., ... Gilbert, D. M. (2019). Identifying cis elements for spatiotemporal control of mammalian dna replication. *Cell*, *176*(4), 816-830. doi: 10.1016/j.cell.2018.11.036

Simpson, R. T. (1990). Nucleosome positioning can affect the function of a cis-acting DNA elementin vivo. *Nature*, *343*(6256), 387–389. doi: 10.1038/343387a0

Siow, C. C., Nieduszynska, S. R., Müller, C. A., & Nieduszynski, C. A. (2012). OriDB, the DNA replication origin database updated and extended. *Nucleic Acids Research*, *40*(Database issue), D682–D686. doi: 10.1093/nar/gkr1091

Stinchcomb, D. T., Struhl, K., & Davis, R. W. (1979). Isolation and characterisation of a yeast chromosomal replicator. *Nature*, *282*, 39–43. doi: 10.1038/282039a0

Sun, J., Evrin, C., Samel, S., Fernández-Cid, A., Riera, A., Kawakami, H., ... Li, H. (2013). Cryo-EM structure of a helicase loading intermediate containing ORC-Cdc6-Cdt1-MCM2-7 bound to DNA. *Nature Structural and Molecular Biology*, *20*(8), 944–951. doi: 10.1038/nsmb.2629.Cryo-EM

Tanaka, S., Nakato, R., Katou, Y., Shirahige, K., & Araki, H. (2011). Origin association of Sld3, Sld7, and Cdc45 proteins is a key step for determination of origin-firing timing. *Current Biology*, *21*, 2055–2063. doi: 10.1016/j.cub.2011.11.038

Tanaka, T., Knapp, D., & Kim, N. (1997). Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. *Cell*, *90*, 649–660. doi: 10.1016/S0092-8674(00)80526-7

Taylor, J. H. (1968). Rates of chain growth and units of replication in DNA of mammalian chromosomes. *Journal of Molecular Biology*, *31*(3), 579–594. doi: 10.1016/0022-2836(68)90429-4

Theis, J. F., & Newlon, C. S. (1994). Domain B of ARS307 contains two functional elements and contributes to chromosomal replication origin function. *Molecular and Cellular Biology*, *14*(11), 7652–7659. doi: 10.1128/mcb.14.11.7652-7659.1994

Tocilj, A., On, K. F., Yuan, Z., Sun, J., Elkayam, E., Li, H., ... Joshua-Tor, L. (2017). Structure of the active form of human origin recognition complex and its ATPase motor module. *eLife*, *6*(e20818), 1–23. doi: 10.7554/elife.20818

Tsankov, A., Yanagisawa, Y., Rhind, N., Regev, A., & Rando, O. J. (2011). Evolutionary divergence of intrinsic and trans -regulated nucleosome positioning sequences reveals plastic rules for chromatin organization. *Genome Research*, *21*(11), 1851–1862. doi: 10.1101/gr.122267.111

Tsukiyama, T., Becker, P. B., & Wu, C. (1994). ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature*, *367*, 525–532. doi: 10.1038/367525a0

Valouev, A., Johnson, S. M., Boyd, S. D., Smith, C. L., Fire, A. Z., & Sidow, A. (2011). Determinants of nucleosome organization in primary human cells. *Nature*, *474*, 516–522. doi: 10.1038/nature10002

Van Houten, J. V., & Newlon, C. S. (1990). Mutational analysis of the consensus sequence of a replication origin from yeast chromosome III. *Molecular and Cellular Biology*, *10*(8), 3917–3925. doi: 10.1128/mcb.10.8.3917-3925.1990

Vashee, S., Cvetic, C., Lu, W., Simancek, P., Kelly, T. J., & Walter, J. C. (2003). Sequence-independent DNA binding and replication initiation by the human origin recognition complex. *Genes & Development*, *17*(15), 1894-1908. doi: 10.1101/gad.1084203

Vashee, S., Simancek, P., Challberg, M. D., & Kelly, T. J. (2001). Assembly of the Human Origin Recognition Complex. *Journal of Biological Chemistry*, *276*(28), 26666–26673. doi: 10.1074/jbc.M102493200

Vaziri, C., Saxena, S., Jeon, Y., Lee, C., Murata, K., Machida, Y., ... Dutta, A. (2003). A p53-dependent checkpoint pathway prevents rereplication. *Molecular Cell*, *11*, 997–1008. doi: 10.1016/S1097-2765(03)00099-6

Vogelauer, M., Rubbi, L., Lucas, I., Brewer, B. J., & Grunstein, M. (2002). Histone Acetylation Regulates the Time of Replication Origin Firing. *Molecular Cell*, *10*, 1223–1233.

Wal, M., & Pugh, B. F. (2012). Genome-Wide Mapping of Nucleosome Positions in Yeast Using High-Resolution MNase ChIP-Seq. *Methods Enzymol.*, *513*, 233–250. doi: 10.1016/B978-0-12-391938-0.00010-0.Genome-Wide

Wiechens, N., Singh, V., Gkikopoulos, T., Schofield, P., Rocha, S., & Owen-Hughes, T. (2016). The Chromatin Remodelling Enzymes SNF2H and SNF2L Position Nucleosomes adjacent to CTCF and Other Transcription Factors. *PLoS Genetics*, *12*(3), e1005940. doi: 10.1371/journal.pgen.1005940

Wilmes, G. M., & Bell, S. P. (2002). The B2 element of the Saccharomyces cerevisiae ARS1 origin of replication requires specific sequences to facilitate pre-RC formation. *Proc Natl Acad Sci USA*, *99*(1), 101–106. doi: 10.1073/pnas.012578499

Xu, J., Yanagisawa, Y., Tsankov, A. M., Hart, C., Aoki, K., Kommajosyula, N., ... Rhind, N. (2012). Genome-wide identification and characterization of replication origins by deep sequencing. *Genome Biol*, *13*, R27. doi: 10.1186/gb-2012-13-4-r27

Xu, W., Aparicio, J. G., Aparicio, O. M., & Tavaré, S. (2006). Genome-wide mapping of ORC and Mcm2p binding sites on tiling arrays and identification of essential ARS consensus sequences in S. cerevisiae. *BMC Genomics*, *7*(276). doi: 10.1186/1471-2164-7-276

Xu, Y., Ayrapetov, M. K., Xu, C., Gursoy-Yuzugullu, O., Hu, Y., & Price, B. D. (2012). Histone H2A.Z Controls a Critical Chromatin Remodeling Step Required for DNA Double-Strand Break Repair. *Molecular Cell*, *48*(5), 723–733. doi: 10.1016/j.molcel.2012.09.026

Yamada, K., Frouws, T. D., Angst, B., Fitzgerald, D. J., Deluca, C., Schimmele, K., ... Richmond, T. J. (2011). Structure and mechanism of the chromatin remodelling factor ISW1a. *Nature*, *472*(7344), 448–453. doi: 10.1038/nature09947

Yan, C., Chen, H., & Bai, L. (2018). Systematic Study of Nucleosome-Displacing Factors in Budding Yeast. *Molecular Cell*, *71*, 294–305. doi: 10.1016/j.molcel.2018.06.017

Yeeles, J. T., Deegan, T. D., Janska, A., Early, A., & Diffley, J. F. (2015). Regulated eukaryotic DNA replication origin firing with purified proteins. *Nature*, *519*, 431–435. doi: 10.1038/nature14285

Yeeles, J. T., Janska, A., Early, A., & Diffley, J. F. (2017). How the Eukaryotic Replisome Achieves Rapid and Efficient DNA Replication. *Molecular Cell*, *65*, 105–116. doi: 10.1016/j.molcel.2016.11.017

Zegerman, P., & Diffley, J. F. (2007). Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. *Nature*, *445*, 281–285. doi: 10.1038/nature05432

Zhang, Z., Wippo, C. J., Wal, M., Ward, E., Korber, P., & Pugh, F. (2011). A Packing Mechanism for Nucleosome. *Science*, *332*, 977–980.

Acknowledgements

I would like to first thank my Doctoral Advisor Prof. Dr. Philipp Korber for his support, valuable scientific advice and fascinating discussions about chromatin during all these years.

I'm also very grateful to my supervisor, Dr. Christoph Kurat, for allowing me to work in such a fascinating project in his lab; for his constant support and advice throughout these years, but also for the engaging discussions about science and life. I couldn't have asked for a better supervisor during my doctoral studies and his easy-going personality fostered a collaborative and fun atmosphere in the lab.

I would also like to extend my gratitude to Prof. Dr. Peter B. Becker for his support and scientific advise, as well as for maintaining a great work environment in the Molecular Biology department at the BMC. I will always cherish my time as a member of this department with nice and helpful colleagues.

Many thanks to Dr. Tobias Straub for being part of my TAC and all the helpful advise on bioinformatics. I very much appreciate all our collaborators that helped us to push forward the projects described in this thesis and still ongoing studies, it was a great pleasure to work together with all of you. Many thanks to the Deutsche Forschungsgemeinschaft (DFG) for funding my doctoral studies, and the IRTG1064, especially to Elizabeth and Carolin.

I'm deeply thankful to Elisa, from whom I inherited the *in vitro* reconstitution knowledge she developed in the Korber lab, and to Iris for her radiant personality and being always there for me. Thank you very much my *Korberlinge* friends for all the laughs and fun times discussing all kinds of topics over coffee and cake! Also many thanks to Petra for her advice on protein purification and for introducing me to yoga, which was very useful during the PhD. I am also very thankful to the Kurat lab members Priyanka, Jessica, and Silvia for their help in the lab and enjoyable discussions.

A special thank goes to Moisés, who supported me unconditionally throughout all these years. He is truly my personal cheerleader! I am deeply thankful to my mother and *nonna* who always motivated and supported me in my scientific endeavors. Also, many thanks to all my friends outside the lab for helping me with my work-life balance.

Thank you again to all the members of the Molecular Biology department from the BMC, it was a true pleasure to work with all of you!