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CROSSTALK BETWEEN ENVIRONMENTAL SIGNALS AND 3D GENOME ORGANIZATION IN THE REGULATION OF GENE EXPRESSION

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CROSSTALK BETWEEN ENVIRONMENTAL SIGNALS AND 3D GENOME ORGANIZATION IN THE REGULATION OF GENE EXPRESSION

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Anna Lewandowska Ronnegren

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To my parents, who always believed in me.

Rodzicom, którzy zawsze we mnie wierzyli

POPULAR SCIENCE SUMMARY OF THE THESIS

The nucleus is the “heart“ of the cell. It contains all the accumulated information about our physical appearances, habits and preferences. Every single detail of our body functions is written in the form of a genetic code, composed of four elements — four types of nucleotides. These nucleotides are aligned in a 2 meter long DNA string. However, this extended molecule has to be tightly packed into the extremely small volume provided by the nucleus and yet be amenable to differentiate and maintain the numerous cell types of our bodies in response to internal and external cues. Each human arises from one single cell, the fertilized oocyte, which during prenatal development generates trillions of cells of different morphology and function, despite that almost (plasma B cells and T cells the exception with rearranged Ig and TCR genes) all of them have exactly the same genetic material. The secret to this lies is in the packaging and spatial organisation of genome and its dynamic regulation. One could compare it to the piano, which symbolizes the genome, while the pianist represents the machinery interpreting the different notes generated by both intrinsic and extrinsic signals to define different cell types. A similar phenomena takes place in the cell — it is using some part of available genetic material to regulate gene expression, and hence its morphology and functions, while storing unnecessary (temporary or permanently) genetic units in transcriptionally repressive nuclear sub-compartments — often at the nuclear periphery. Understanding the principles behind this genome organisation is crucial not only for understanding how our organism works, but also for being able to identify novel therapeutic strategies in case if something goes wrong in the cell — during cancer development, for example.

This thesis focuses on chromatin fibres movements within the nuclear architecture and how such dynamic processes set the stage for encounters regulating gene expression. By exploring “cellular routines” in repositioning certain DNA fragments between active and inactive parts of the nucleus, new principles underlying cellular choices of usage of certain DNA fragments were uncovered. Again, using the pianist metaphor, one could say that the aim of the research is to understand how “the cell” is ”choosing” notes which are going to be played in certain moment of its life, and how the notes might be changing in time or in response to a changing environment. These aims are highly relevent for our understanding of how they can go awry to cause human diseases, such as cancer. By understanding cancer architectural complexity, developmental choices and/or responses to outside stimuli, we

might learn the habits of the “cancer day-scheme” to fight it by cutting off its necessary supplies by identifying targets for therapeutic regiments and their timing.

ABSTRACT

The thesis explores the connection between environmental stimuli and gene expression regulated by the spatial changes in genome organization. In Paper I, by applying state of the art Circular Chromosome Conformation Capture assay (4C) and Chromatin *in situ* Proximity (ChrISP) techniques, we show that transcriptionally active circadian genes meet in space with repressed lamina-associated domains (LADs), and that these interactions are under the control of the circadian clock. External time cues thus synchronised circadian transcriptional oscillations by repositioning clock-controlled genes from the transcriptionally permissive sub-compartment of nuclear interior to the transcriptionally repressive nuclear periphery. These processes relied on the rhythmic formation of complexes between CTCF and PARP1, two master regulators of the genome, to increase the amplitude of circadian gene expression.

In Paper II we took an advantage of the novel, ultrasensitive Nodewalk technique to explore the stochastic nature of *MYC* interactions with its flanking enhancers. By pushing the Nodewalk limits of identification of chromatin interactions in the input material corresponding to less than 8 cells, we could show that *MYC* is likely screening for neighbouring interaction partners rather than *vice versa*. Moreover, we could show that *MYC* does not interact with enhancers, once its transcription had been initiated. These findings suggest that enhancer hubs simultaneously interacting with *MYC* are likely virtual consequences of high cell population analyses and that *MYC* interacts with its enhancers in a mutually exclusive manner.

Paper III concentrates on the role of a CTCF binding site within the oncogenic super-enhancer (OSE) in the regulation of *MYC* gene gating in colon cancer cells. CRISPR induced mutations in the CTCF binding site within the OSE abrogated WNT-dependent nuclear export of *MYC* mRNA, providing genetic evidence to the claim of the OSE-mediated gating of active *MYC* alleles to the nuclear pore.This manuscript documents, moreover, that the communication between OSE and *MYC*, as well as their repositioning to the nuclear pore, involves PARP1 to indicate a more general role for the CTCF :PARP1 complex in gene regulation.

In summary, this thesis has uncovered novel principles underlying the roles of stochastic chromatin interactions and mobility within the 3D nuclear space to regulate gene expression with a focus on circadian transcriptional regulation and the recently discovered gene gating phenomenon in humans. These findings contribute to our understanding of principles in which the nuclear architecture and genome organisation synergize to induce or maintain the properties of the cell. By extrapolation, such findings might form a platform for identifying new therapeutic strategies to battle cancer, for example.

LIST OF SCIENTIFIC PAPERS

I. Zhao H*, Sifakis EG*, Sumida N*, Millán-Ariño L*, Scholz BA, Svensson JP, Chen X, **Ronnegren AL,** Mallet de Lima CD, Varnoosfaderani FS, Shi C, Loseva O, Yammine S, Israelsson M, Rathje LS, Németi B, Fredlund E, Helleday T, Imreh MP, Göndör A.

PARP1- and CTCF-Mediated Interactions between Active and Repressed Chromatin at the Lamina Promote Oscillating Transcription. Mol Cell, 2015.59(6): p. 984-97.

II. Noriyuki Sumida, Emmanouil G Sifakis, Narsis A Kiani, **Anna Lewandowska Ronnegren**, Barbara A Scholz, Johanna Vestlund, David Gomez-Cabrero, Jesper Tegner, Anita Göndör and Rolf Ohlsson.

MYC as a driver of stochastic chromatin networks: Implications for the fitness of cancer cells. Nucleic Acids Research, 2020 Oct 13, gkaa817.

III. Ilyas Chachoua*, Ilias Tzelepis,* Hao Dai*, **Anna Lewandowska Ronnegren***, Jia Pei Lim*, Mirco Martino, Felipe Beccaria Casagrande, Rashid Mehmood, Anita Göndör.

A CTCF binding site within the oncogenic super-enhancer coordinates the WNT-regulated gating of MYC. Manuscript.

CONTENTS

LIST OF ABBREVIATIONS

3C	chromatin conformation capture
3D	three dimensional
4C	circular chromosome conformation capture
4D	four dimensional
5C	chromosome conformation capture carbon-copy
AHCTF1	AT-Hook containing transcription factor 1
Arntl2	aryl hydrocarbon receptor nuclear translocator-like 2
ATP	adenosine triphosphate
BAC	bacterial artificial chromosome
BER	base excision repair
BMAL1	brain and muscle arnt-like 1
CAB	chlorophyll a/b-binding proteins
ChIA-PET	chromatin interaction analysis by paired-end tag sequencing
ChIP	chromatin immunoprecipitation
ChIP-loop	chromatin immunoprecipitation loop
ChIP-Seq	chromatin immunoprecipitation sequencing
ChrISP	chromatin in situ proximity
CLOCK	circadian locomotor output cycles kaput
Co-IP	co-immunopurification
CR	circadian rhythm
CRY	cryptochromes
CT	chromosome territory
CTCF	CCCTC-binding factor
DAPI	4,6-diamidino-2-phenylindole
DBP	D-element binding protein
DMR	differently methylated region
DMSO	dimetylsulfoxid
DNA	deoxyribonucleic acid
DNAse I	deoxyribonuclease I
DTT	ditiotreitol
EDMD	Emery-Dreifuss muscular dystrophy
EGF	epidermal growth factor
EnhD	enhancer D
EU	5-ethynyl uridine
FBS	fetal Bovine Serum
FISH	fluorescence in situ hybridization
GFP	green fluorescent protein
GUN5	genomes uncoupled 5
HAT	histone acetyltransferase
HCEC	human colon epithelial cell
HCoEpiC	colonic epithelial c+B91ell Medium
HDAC	histone deacetylase
HEB	human embryoid body
HESC	human embryonic stem cell
HGPS	Hutchinson-Gilford progeria syndrome
HMT	histone methyltransferase
eRNA	enhancer RNA
ICC	immunocytochemistry
ICR	imprinting control region
IGF2	insulin-like growth factor 2
IgG	immunoglonulin G
INM	inner nuclear membrane
ISPLA	in situ proximity ligation assay

kb	kilobase
LAD	lamina-associated domain
LAP2	lamina associated peptide 2
LBR	lamin B receptor
lncRNA	long non-coding RNA
LOCK	large organized chromatin K9 modification
Mb	megabase
MED1	mediator subunit 1
MEF	mouse embryonic fibroblast cell
MLL1	mixed lineage leukaemia 1
MYC	myelocytomatosis viral oncogene
NPC	nuclear pore complex
NUP	nucleoporin
NuRD	nucleosome remodelling and deacetylase
OCT4	octamer-binding transcription factor 4
OSE	oncogenic super-enhancer
PAR	poly ADP ribose
PARD3	partitioning defective 3 homolog
PARG	poly (ADP-ribose) glycohydrolase
PARP1	poly (ADP-ribose) polymerase 1
PARylation	poly (ADP-ribosyl)ation
PBS	phosphate-buffered saline
PC	plastocyanin
PCR	polymerase chain reaction
PER	period
PGE2	prostaglandin E2
PTM	post-translational modification
RBCS	Rubisco small subunit
REV-ERBα/β	reverse erithroblastosis α and β
RNA	ribonucleic acid
RNAPII	RNA polymerase II
RNP	ribonucleoprotein
RORα	retinoic acid receptor-related orphan receptor-α
RORE	ROR/ REV-ERB -binding element
SOX2	sex determining region Y
SCN	suprachiasmatic nuclei
SE	super-enhancer
SNP	single nucleotide polymorphism
SSC	saline sodium citrate
TARDBP	TAR DNA-binding protein 43
TAD	topologically associating domain
TE	typical enhancer
TCF4	transcription factor 4
TF	transcription factor
TGF-β	transforming growth factor- β
TTFL	transcriptional/translational feedback loop
UV	ultraviolet
VAT1L	vesicle amine transport 1 homolog-like protein

1 INTRODUCTION

1.1 EPIGENETIC PRINCIPLES

A single human genome can give rise to not only numerous different cell types and specialized functions, but also their responses to varying developmental and environmental cues. This epigenetic adaptability in the cellular interpretation of genetic information depends on the establishment of cell type-specific gene expression patterns. With a mere 2% of the genome coding for proteins, the remaining 98% of the genome is replete with regulatory elements that underlie context-specific gene activity(1). Chromatin adaptations implemented by the epigenetic machinery as well as its 3D mobility are key factors in the induction and subsequent stable propagation of gene expression pattern without altering the underlying DNA sequence(2). Moreover, the chromatin fibre is an essential platform on which transcription factors (TF), singling pathways and other chromatin modifications converge and in some instances collaborate in response to environmental stimuli(1). Thus, chromatin states maintain flexible features in response to the appropriate cues and conditions and yet are stable enough to ensure the propagation of robust phenotypes during development. Consequently, the loss of this robustness increases the potential for disease development(1,3–5). However, very little is known about the mechanisms which govern the transitions between normal and pathological epigenetic plasticity. It has been proposed that the compartmentalization of active and inactive domains, co-existence of different epigenetic marks as well as interactions between different chromatin loci or regulatory elements like promoters and enhancers underlie how chromatin states respond to environmental cues, similar to the establishment of developmentally stable gene expression patterns(6,7).

1.1.1 The primary chromatin fiber

The 6 billion bases of coding and non-coding DNA of the diploid genome are wrapped around approximately 30 million nucleosomes to form chromatin, which is the physiological and structural form of human genetic information(1,2). Nucleosomes are formed by wrapping ~145–147 bp of DNA around histone octamer cores separated by a

linker region of ca 20-40 bp to define the 'primary structure' of chromatin fibres. This simplified picture is, however, compounded by the ever-increasing number of histone variants as well as of post-translational histone modifications (PTMs) and DNA modifications(8), to generate an almost astronomical number of theoretically possible variations in the chromatin primary structure. The PTMs play important roles in establishing equilibriums between different structural states, including the regulation of the chromatin compaction as well as the interactions between nucleosomes and non-histone proteins. Among many distinct histone PTMs, acetylation of lysine residues and methylation of arginine residues are best known. Thus, acetylation of lysine neutralizes the positive charge of the amino acid and is often associated with chromatin decompaction and *vice versa*, whereas arginine methylation plays important roles in the regulation of gene expression(6,9). These and other PTMs decorate not only the chromatin of gene bodies, but also regulatory elements, such as enhancers. Regular enhancers are typically defined as short (~100–1000 bp) noncoding DNA sequences, composed of concentrated clusters of transcription factor (TF) recognition motifs. Such complexes tend to bend the DNA to generate so-called DNase I hyper-sensitive sites demarcating such regions(10–13). The type of combinatorial PTMs associated with regulatory regions defines their functions. For example, active enhancers are marked with H3K4me1, H3K27ac, H3K122ac and absent or low levels of H3K27me3 and H2K9me2/3 marks. Conversely, enhancers which are poised to become active enhancers are demarcated with both active and repressive marks, such as H3K4me1 and H3K27me3(6,14,15). This picture is complicated, however, by the demonstrations that some of the enhancer regions can be devoid of the typical enhancer-specific chromatin marks(16,17). It has been estimated that each cell type can have anything from 10,000 to 150,000 enhancers with an accumulated number exceeding 1 million enhancers active in all human cells(11,18,19). Accordingly, enhancers are key regulatory elements controlling tissue-specific transcription programs and thus essential for the robust maintenance of a diverse range of phenotypes(20). More recently, a new class of enhancers, the so-called super-enhancers (SE), has been identified. Such regions are often found near genes that control cell states and have cell type-specific functions in response to a diverse range of signaling pathways(11,18,20). SEs are defined not only by the usual enhancer marks, such as H3K4me1, H3K27ac, master TFs, p300, but also by the prominent presence of the Mediator complex (Med1)(10,11). A schematic representation of the loop formation that such super-enhancers can form with targeted promoters is presented in

Figure 1. In a comparison to typical enhancers (TE), SE typically spreads from 10 kb to over several hundreds of kb (while the median size of TEs ranges from 1 kb to 4 kb), often contain more than one separate region that is bound by multiple TFs (such as OCT4, SOX2 and NANOG in Embryonic Stem Cells), drive targeted gene transcription with a high precision potentially related to the higher expression of enhancer RNAs (eRNAs) than typical enhancers. Accordingly, the number of SEs in each cell type is fewer by one to two orders of magnitude than the corresponding number of TE(10,11,21–23).

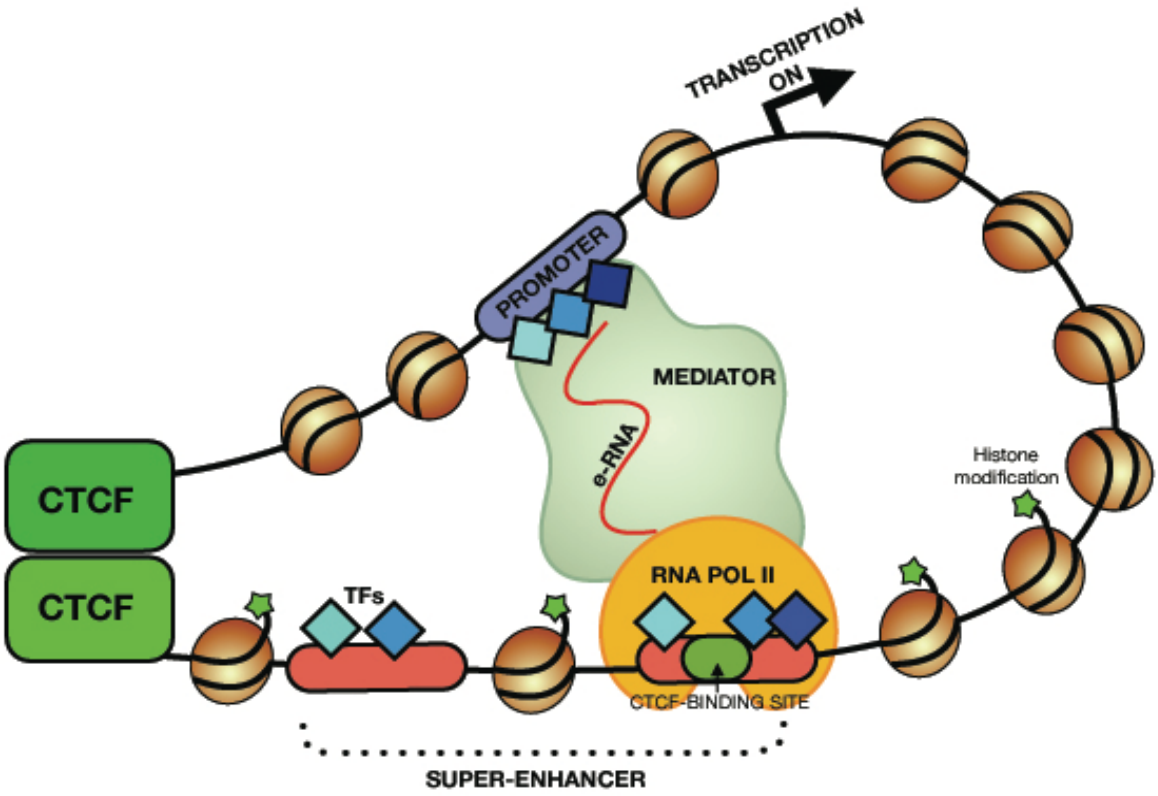

Fig.1 Schematic loop formation between promoter and super-enhancer Super-enhancers are enriched in histones modifications (H3K4me1 and/or H3K27ac) as well as transcription factors, p300 and the Mediator complex. CTCF can bind to chromatin to establish domain boundaries, enhancers and promoters. Bi-directionally transcribed eRNAs play stabilizing roles in loop formation between promoter and super-enhancer by providing a platform for interactions between trans-acting factors.

1.1.2 3D chromatin interactions

The enhancer needs to physically interact with the promoter of its target gene to initiate its transcription. This is an essential step to ensure that the correct set of genes are turned on at the correct time during a developmental window(24). Physical enhancer-promoter contacts generally involve collaboration between the Mediator complex at the enhancer and promoters which usually are marked with the H3K4me3 modification. The resulting chromatin loop involves the cohesin complex that promotes the search for promoters with active marks by a loop extrusion principle(17,25,26). The formation of enhancer-gene loops can be further stabilized by enhancer RNAs (eRNAs), which constitute links between enhancers and their target genes(20,27). The CTCF (CCCTC-binding factor) is another important factor, also for SEs, by being able to physically link distal regions to form chromatin loops with a median size ca. 200kb in convergent orientations. The loss of this feature, which promotes highly specific and precise SE-promoter interactions, affects loop domains and transcriptional patterns(21,27–30). Moreover, the CTCF binding site can provide a structural hierarchy necessary for the function of SEs (by mediating and/or facilitating long-range chromatin interactions) (27,31). However, very little is known about the mechanisms underlying the regulation of such chromatin loops – a situation further compounded by the fact that only 7% of distal regulatory elements control the most proximal promoters with enhancer regions located anywhere from 1kb to tens of Mbs away from their target promoters(6,17,25). Moreover, one enhancer can contact more than one promoter(25). It is also important to note that interactions between promoters and enhancers might be established without leading to overt transcriptional initiation. Indeed, for the large subunit of the RNA polymerase complex to trigger transcription it must acquire a serine 2 phosphorylation mark at its large subunit(17,24).

Apart from promoter-enhancer interactions, chromatin interactions can involve different types of regulatory elements like promoter-promoter, enhancer-enhancer or insulator-insulator loops(24). The chromatin insulator is a sequence element of DNA that blocks enhancer-promoter interactions in a position-dependent manner to potentially contribute to the spatial arrangement of chromosomes in the nucleus(30,32). However, their ability to antagonize enhancer-gene interactions is position-dependent - the promoter thus remains capable of being activated by other enhancers and the enhancer can activate other promoter(32). The *H19* imprinting control region (ICR) functions as a methylation

sensitive, CTCF-dependent chromatin insulator and has been shown to physically interact with a differently methylated region (DMR) with a silencer function at the maternally inherited *Igf2* allele(33,34). The *H19* ICR region is inherited unmethylated from the maternal germline and methylated from the paternal germline to direct parent of origin-specific expression of the proximal *H19* and the more distal *Igf2* alleles. Since only the unmethylated *H19* ICR is able to bind CTCF, only the maternal *Igf2* allele is insulated from downstream enhancers. This feature requires the ability of the CTCF-*H19* ICR complex to form chromatin loops(33–38). Conversely, while the paternally methylated *H19* ICR allele is unable to prevent activation of *Igf2,* it silences the *H19* gene.

1.2 NUCLEAR ORGANIZATION

1.2.1 Spatial separation between active and inactive chromatin states

As discussed above, a fundamental property of the genome is its spatial organization at several, hierarchical levels in cell nucleus. In interphase nuclei, individual chromosomes occupies a limited space called chromosome territories, which are approximately 2-4 μm in diameter(7,39). Despite cell type-specific differences in the localization of individual chromosome territories in individual cells, larger (and gene-poor) chromosomes tend to locate close to the nuclear periphery while smaller and more gene-rich chromosomes inhabit a more central position(40). One of the most striking features of the chromosomal organization is thus the global spatial separation of active and inactive chromatin referred to as A (active) and B (inactive) compartments, which are further divided into sub-compartments with distinctive chromatin states, association with nuclear landmarks and replication timing(40–42). Within nuclear sub-compartments chromatin is organized into topologically-associated domains (TADs) - areas of highly interacting chromatin separated from each other by, for example, CTCF boundaries demarcating the TADs(29,40,43). The constraining of chromatin movements between TADs likely restricts contacts to involve only neighboring enhancers and promoters(44). TADs can be either A-type (with open, gene-rich chromatin) or B-type (gene-poor, closed chromatin)(45). Lamina-associated domains (LADs) are distinct regions of the genome interacting with nuclear lamins (a thin meshwork of filaments that lines the inner nuclear membrane). LADs encompass 0,1 to

10Mb, are AT-rich and contain H3K9me2 and H3K27me3 marks with poor representation of H3K36me3(39,45) to constitute 35-40% of the mammalian genome(29,45,46). Similarly to TAD borders, LAD borders are enriched in CTCF binding sites(45) and approximately 9% of LAD borders contain CTCF within 10kb of the boundary(47). Furthermore, LADs are represented by cell type-specific variants (facultative, fLADs) or cell type-invariant (constitutive, cLADs) LADs(48). These types of LADs differ from each other in gene density and conservation between mouse and human(48). Interestingly LADs overlap to a high degree with Large Organized Chromatin Lysine Modifications (LOCKs), which are regions enriched in the H3K9me2 mark and highly dynamic throughout the differentiation of murine embryonic stem cells (mESC)(45,48). Genes localized within LOCKs are typically silenced and comprise 31% of the genome of differentiated ES cells, but less than 5% in undifferentiated cells(49). Interestingly both LADs and LOCKs might play an important role in the regulation of chromatin movements.

1.2.2 Chromatin movements

Although the distribution of the chromosome territories within the nucleus to a high degree depends on their size and gene-richness, their preferred position is not absolute(50). This also translates to the kind and dynamics of chromatin fiber interactions and distinct loci of particular chromosomes manifest different features in terms of mobility and search for interacting partners(51). Whereas most chromatin interactions take place within the chromosome territory, genes and regulatory regions can also interact in *trans* with inter-chromosomal interactions occurring preferably at the edge of chromosome territories(7). However, particular loci can also loop into the CT of another chromosome or multiple loci can loop out of their CTs(7,38). The mobility of distinct loci depends on their position within nuclear sub-compartments with genes associated with the nuclear periphery or the nucleolus exhibiting significantly lower mobility in comparison to genes within the nucleoplasm(52). Such features may underlie long-range chromatin interactions that have been implicated in the epigenetic regulation of gene expression(51).

Experiments with molecular tethering to the nuclear periphery using an inducible system showed that physical repositioning of examined genes to the nuclear periphery can

reversibly repress the activity of these genes in human cells(53). Although not a universal feature, the tethering of some of genomic loci to the periphery is thus generally promoting silencing of gene activity(54). Conversely, it is generally considered that the mobility of active chromatin to the site of mRNA export, the nuclear pore, might increase expression efficiency(55). The so-called 'gene gating' phenomena thus states that certain loci can be juxtaposed to the nuclear pore and facilitate rapid nuclear export of processed mRNAs. In one such example, it was shown that an oncogenic super-enhancer mediated the repositioning of active *MYC* alleles to nuclear pores, which enabled *MYC* transcripts to escape the more rapid degradation pathway in the nucleus than in the cytoplasm in colon cancer cells(55–57). Of note, Paper III provide genetic evidence in support of this scenario by showing that a single CTCF binding site within the oncogenic super-enhancer coordinates the WNT-regulated gating of *MYC* to the nuclear pores. In contrast to short-range interactions, global mobility of chromatin might depend on the actin/myosin system to seemingly produce active and directed movements(58). The specificity of the repositioning of certain loci to the nuclear periphery is likely dependent on a combination of DNA sequence, trans-acting factors, PTMs and lncRNA(6,59).

1.2.3 Chromatin hubs vs stochastic movements

The nuclear architecture is dynamic in nature and yet stably maintained(7). This may relate to that the nucleus is considered to be built mainly by self-organizing principles(51,60). Such a dynamic environment displays a high level of stochastic collisions between chromatin fibers. Although gene expression itself is a fundamentally stochastic process, transient chromatin fibre interactions don't necessarily imply any regulatory function(7,61). Nonetheless, enhancer interactions both in *cis* and *trans* as well as the establishment of the so-called transcription factories provide well documented examples for the meta-stable character of such functional chromatin networks(17,62,63). However, the frequency of spatial meetings between two interacting genomic regions is not universal for all cells in a cell population(51). Even though chromatin-fiber interactions might display a high level of noise to appear largely non-functional, they might increase the potential for a functional outcome to contribute to cellular plasticity as well as establishing/maintaining stochastic patterns of mono-allelic gene expression(6,7,64,65).

1.2.4 Contribution of CTCF and PARP1 to chromatin interactions and mobility

As noted above, CTCF plays an important role in the formation of chromatin loops. CTCF is an evolutionary conserved 11-zinc-finger protein with ability to bind various DNA motifs and different regulatory proteins(66–68). Its ability to interact with DNA, proteins as well as RNA is a consequence of its domain organization – the central zinc-finger domain allows CTCF to bind DNA with the use of different zinc-fingers combinations, whereas binding to its protein partners is mediated *via* interactions with any part of the entire protein, i.e. its central zinc finger domain or the N- and C-terminal parts. For example, N-terminal region has the ability to bind PARP1 protein, as described later in the text, while the unstructured C-terminal region can bind RNA(66,67). The complexity of the CTCF structure translates into the various significant roles CTCF plays in regulation of cellular processes and development.

CTCF was initially discovered as a transcriptional repressor of chicken c-*myc* gene(69) and its important function as an insulator in the regulation of *Igf2/H19* imprinting has been well studied(35,70–72). Subsequent research showed that CTCF binds to 40,000-80,000 sites genome wide in mouse and human ESCs to play role in alternative splicing, DNA repair, recombination and mediation of enhancer-promoter interactions(17,66,73–75). Many, but not all, of CTCF binding sites are co-occupied by the cohesin complex, which physically interacts with CTCF as a biochemically stable complex. Together they shape chromatin architecture, although CTCF and the cohesin complex differ in the dynamics of chromatin binding and mechanism of contribution to the chromatin organization(76–81). CTCF, which has been characterized as a master weaver of the genome(67), also binds to a wide range of factors including PARP1. Although PARP1 is mostly known for its role in DNA-damage response, it has been implicated in epigenetic modifications of both histones and DNA as well as maintaining the integrity of constitutive and facultative heterochromatin(82–84). Many of these functions are in partnership with CTCF, as witnessed by the PARP1-mediated poly(ADP-ribosyl)ation of its N-terminal region (82,85). Dynamic PARylation of CTCF in response to cellular signals or environmental cues thus plays an important role in CTCF-dependent chromatin insulation and loop formation(86–88) as well as the regulation of chromatin mobility (Paper I).

1.2.5 Technologies exploring 3D chromatin structures

The mapping of the 3D structure of the genome has been possible thanks to technological achievements represented by two main approaches: *In situ* techniques, such as proximity ligation and 3D DNA FISH(89) and chromosome conformation capture (3C) and derived "C" techniques(90). The "C" family of techniques focuses on local and/or genome wide DNA-DNA contacts and involves restriction digestion of formaldehyde-crosslinked chromatin followed by intra-molecular ligation to identify proximities between distal regions (Fig.2). In the original 3C technique, this was performed by PCR, while later versions of the method rely primarily on high throughput DNA sequencing(91,92). The parental 3C technique has been extensively used to demonstrate interactions, such as formation of chromatin loops between two genomic loci(91). However, the drawback of this technology is that it is based on educated guesses that two particular regions might interact. To overcome this biased limitation, Circular 3C (4C) in combination with high throughput sequencing was innovated(38). Strategic placing of the primers within the bait and formation of circular DNA between bait and interacting sequences allowed the identification of unknown intra- and inter-chromosomal interactions of a bait(93,94). Next, the chromosome conformation capture carbon copy (5C) allowed obtaining information on contacts between multiple genomic loci(95). This was possible by introducing a step where 3C products were incubated with a mix of specific oligos which annealed exactly at one of the restriction sites covering an entire genomic region of interest(91). However, the "many-to-many" 5C strategy was subsequently replaced by an "all-to-all" approach represented by the Hi-C technique. The introduction of biotin-labeled nucleotides for filling restriction "sticky-ends" (followed by "blunt-ends" ligation) allowed the selection of ligation junctions by biotin pull-down(89,96). Although the Hi-C technique has been very useful to study genome wide TAD structures, it is less ideal for the examination of individual loci interaction pattern due to low resolution and reduced statistical power(91). Moreover, similarly to almost all of the other 3C-based methods, Hi-C analyses are mostly performed on the cell-population level with low sensitivity to blur the dynamics of chromatin encounters. Thus, Hi-C maps are unable to discriminate between stable 3D contacts present in all cells and an average of stochastic contacts which can differ between particular cells of a cell population(97). The single-cell Hi-C (which introduces an in-nucleus ligation step) helped to overcome the problem of the use of cell population, but the technique still suffers from poor sensitivity and resolution(98). Moreover, the introduction of exponential PCR

amplification steps – a feature shared by almost all "C" techniques, is likely to introduce a bias. This limitation is resolved by including a linear RNA amplification step in the newly developed Nodewalk technique (Paper II), which will be further discussed in chapter 1.2.6.

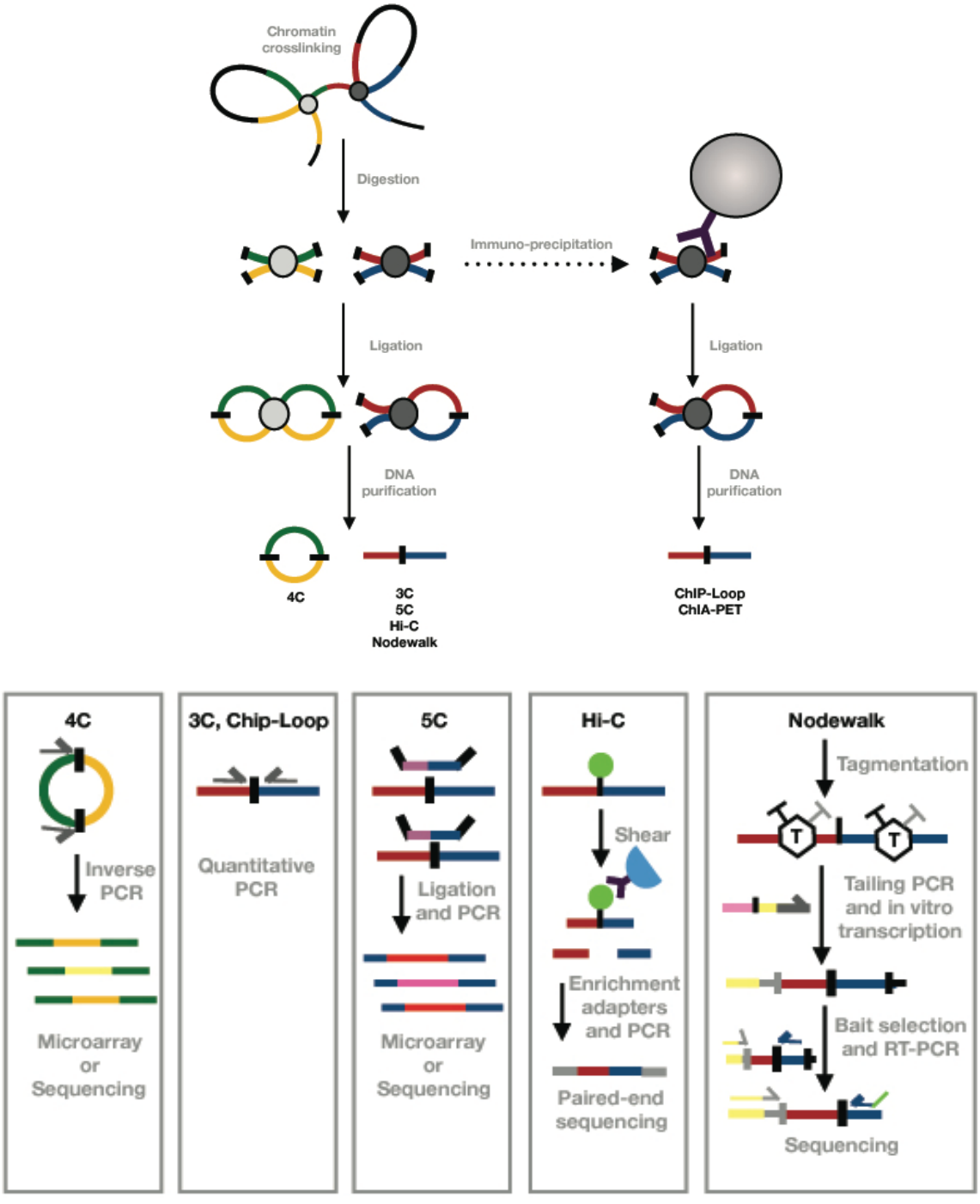

Fig.2 Schematic illustration of the "C" family of techniques

Another common limitation of all the ”C” family techniques is that they do not with few exceptions (Paper I) provide robust information as to where in the nuclear space the interactions take place and their frequency of interactions. This generally acknowledged drawback usually requires validation of identified interactions by microscopy assays, such as 3D fluorescence *in situ* hybridization (3D-FISH). Whereas the power of this technique lies in that it represents a single-cell analysis of gene positioning to provide information of chromatin dynamics in relation to the nuclear architecture, it is limited by the resolution of the fluorophores of choice(89). To quantitatively detect chromatin proximities in single cells with an optimal resolution, chromatin *in situ* proximity (ChrISP) assay is a method of choice(99). This technology detects proximities between two different antibodies (against labeled probes specific to target DNA sequences, or between a DNA sequence and any other epitope represented by a protein, for example) within 160Å of each other and allows the spatial visualization of chromatin interactions or higher order chromatin confirmations in single cells in relation to structural hallmarks(100).

1.2.6 The Nodewalk technique

The Nodewalk method is a member of “C” family techniques which rely on the ligation of digested DNA to generate covalently linked intra-molecular chromatin complexes. However, the DNA tagmentation by transposase allows both further fragmentation of ligated DNA and the incorporation of suitable, tailed primer sequences in Nodewalk, including the promoter for T7 RNA polymerase, followed by only 5-7 amplification cycles. This step avoids amplification biases and allows the generation of a template for the *in vitro* linear, production of large amounts of RNA from very small amount of input material. Following the selection of bait, specific primers positioned close to a restriction enzyme site can be used to prime cDNA synthesis and subsequent high throughput sequencing. Nodewalk thus combines high resolution with high sensitivity improving it >10,000-fold in comparison to other “many-to-all” techniques. This in turn allowed the reproducible identification of chromatin interactions in input material corresponding to less than 8 cells(101) (Paper II). The Nodewalk technique was instrumental in providing key data

underlying the discovery of the gating of the active *MYC* gene to the nuclear pore in human colon cancer cells(56).

1.3 THE 4TH DIMENSION OF THE REGULATION OF NUCLEAR FUNCTIONS - INTRODUCTION TO THE CIRCADIAN RHYTHM.

Many cellular processes, including chromatin movements or chromatin fiber interactions, exhibit periodic behavior. These time-dependent events are often regulated by the circadian clock machinery that likely has evolved to maximize organismal fitness in response to changing external cues. The circadian clock is a highly conserved system that enables organisms to adjust to daily changes in the environment, such as food availability and night-day cycles(102). It controls a variety of physiological processes like hormone secretion, feeding behavior or sleep-wake cycles. Almost all eukaryotic organisms manifest behavioral, physiological and metabolic oscillations, with a period of ca 24 hours. Circadian rhythms display unique properties – although endogenous free-running periods of approximately 24h, the phase of the rhythm can be entrained or re-set by external time cues. Moreover, the periodicity of circadian rhythm is stable across a wide range of temperatures (termed temperature compensations)(103). The mammalian timing system exhibits a hierarchical architecture with the master pacemaker located in the suprachiasmatic nucleus (SCN). This controls the phase of oscillations at both the tissue and cellular levels(104) by providing an output to peripheral tissues (peripheral clocks) following its synchronization by external time cues, such as light. Peripheral clocks oscillate autonomously and can be entrained also by food intake(104,105). At the cellular level, the molecular clock machinery regulates cellular processes *via* several transcriptional/translational feedback loops (TTFLs).

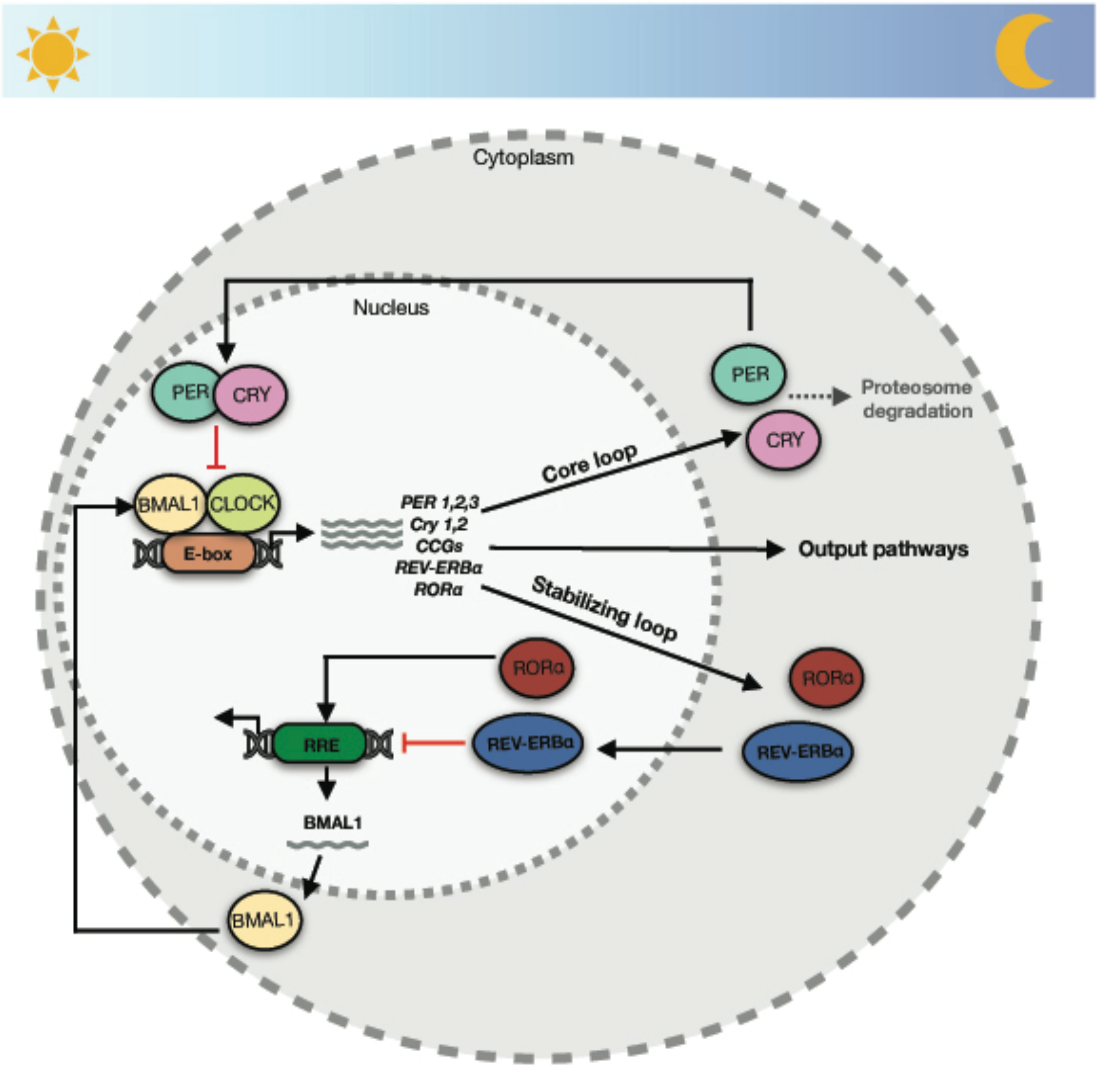

Fig. 3 Schematic representation of mammalian core circadian feedback loops. The positive feedback loop (CLOCK/BMAL1) regulates the transcription of negative feedback loop components – *PER* and *CRY*. PER and CRY proteins enter the nucleus to suppress the transcriptional activity of the CLOCK/BMAL1 complexes. The machinery is stabilized by antagonizing actions of RORα and Rev-erbα.

In mammals, the positive limb of the core TTFL feedback loop consists of two transcriptional activators: the circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1) proteins. They form a heterodimer to promote the expression of the negative elements (PERIOD (PER) and CRYPTOCHROME (CRY) homologs). Following a time delay, dimerized complex between PER and CRY translocate to the nucleus where they inhibit the action of CLOCK/BMAL1

complexes(102,103,106,107). The core TTFL is, moreover, coupled to other sub-loops which contribute to the functions of the circadian clock. One of these sub-loops comprises transcription factors reverse erythroblastosis virus-α (Rev-erbα) and retinoic acid receptor-related orphan receptor-α (RORα) which bind to RORE in the *BMAL1* promoter to either activate (RORα) or repress (Rev-erbα) *BMAL1* transcription(102,103,107,108). The principles of mammalian core circadian feedback loops are schematically shown in Figure 3. The core clock machinery is connected to many cellular pathways and has additional layers of regulation that includes PARP1, for example. This topic is further elaborated on in chapter 1.4.1.

1.3.1 The entrainment of circadian rhythm

As mentioned in chapter 1.3, the synchronization of the clock to 24h oscillations requires entrainment by external timing cues, also called "synchronizers", "zeitgebers" or "entraining agents"(107). Circadian rhythm synchronization can be obtained by variety of factors, which relate to the architectural organization of the clock machinery. Among them, light and food are well studied examples of external cues that regulate the activity of the clock components. Following the recognition of light at the retina, the signal reaches the SCN *via* the retino-hypothalamic tract(109). This "photic entrainment" indirectly regulates light-insensitive peripheral clocks by both humoral and non-humoral pathways(107,110). Food composition and their timing of ingestion, on the other hand, cause periodic changes in the availability of circulating macronutrients, which can reset the circadian clocks specifically in peripheral tissues(103,107). Temporal food restriction can induce circadian rhythmicity of locomotor behavior even in animals with SCN lesions(111). At the molecular level, the feeding process increases blood glucose levels, which can both downregulate the expression of PER1 and PER2, and indirectly regulate CRY stability(112,113). The complexity of the clock machinery is further exemplified by the observation that the CLOCK/BMAL1 binding to DNA can be affected by food restriction-dependent alternations in the cellular redox state(107). Apart from food and light, synchronization of the phase of the circadian rhythm can be obtained by action of other synchronizers, such as arousal stimuli, which include social interactions, exercise, stress or caffeine consumption(107,114). Even though one of the important features of circadian

rhythms is their temperature compensation (the period length is maintained in a wide range of physiological temperatures), peripheral cells and tissues can be synchronized by temperature fluctuations(107). Not only *in vivo* models but also *in vitro* cell cultures or explants can be synchronized in their circadian rhythms, and many factors can function as zeitgebers. It was shown that serum shock, for example, can affect the molecular circadian clock, and various factors in the blood, such as glucose, calcium, EGF, PGE2 or 1α,25-dihydroxyvitamin D3, can affect rhythmic oscillations(107,115). Finally, chemical compounds, such as dexametasone (an artificial glucocorticoid) or forskolin (which acts similarly to the serum shock) can synchronize circadian rhythms by resetting intrinsic biological processes(116,117).

1.3.2 The role of circadian rhythm

Circadian rhythms (CR) play important roles at both the organismal and cellular levels. Their existence provide organisms with selective behavioral advantages, allowing for instance the anticipation of daily food availability and predator avoidance(118). Moreover, CR allow the physiological adaptation of the organisms to food intake, metabolism and detoxification, and the circadian clock of plants prepares them to kick-start photosynthesis in the presence of appearing light energy by ensuring the production of photo-system I and II components already before the sunrise(118–120). Additionally, temporal separation of chemically incompatible cellular processes acts in favor for the organisms. For example, simultaneous photosynthesis and nitrogen fixation in cyanobacteria would cause inefficient nitrogen fixation due to the poisoning of the nitrogenase by oxygen generated during photosynthesis(121). It has also been suggested that the circadian clock underlies the heterogeneity in stem cells populations, to promote developmental potential(122). Studies with mouse models showed, moreover, that epidermal stem cells in hair-follicle bulge coexisted as two subpopulations, which differentially expressed core components of the clock machinery to prevent simultaneous expression of genes responsible for stem cell dormancy, proliferation or differentiation, such as TGFß and WNT pathway members(123–125). The DNA damage response is yet another biological process affected by the circadian rhythm – the ability to repair UV irradiation-induced DNA damages were higher in the late afternoon and early evening than before dawn(126). Such observations are compounded by

the fact that a majority of circadian genes are not only expressed in a tissue-specific manner, but also that different circadian transcripts might accumulate with different phases in different cell populations within a tissue. This heterogeneity is likely the sum of differences both in the spatial distribution of environmental cues and the ability of the cells to respond to these cues(119). Accordingly, the disruption of circadian rhythms by unhealthy lifestyle or shift working, as well as polymorphisms/mutations in the clock genes might contribute to many diseases such as sleep disorders, cardiovascular diseases, diabetes or cancer(127).

1.3.3 Circadian organization of the epigenome

Many studies have made the link between distinct epigenetic regulatory layers and the regulation of circadian transcription. One of the earliest reports showed that the phosphorylation of H3 at Serine 10 is light-dependent in SCN neurons(128). Subsequent studies reinforced this observation by showing that epigenetic modifications, such as DNA methylation and PTMs of histones, play roles in rhythmic transcription. For example, both the activating H3K4me3/H3K9ac and the repressive H3K9me3 marks exhibits circadian rhythmicity at regulatory regions(129–132). Consequently, the recruitment of PolII to promoters of circadian genes as well as its regulation at the elongation step display circadian-depended variations(131,133). To sustain circadian dynamics of the epigenome, the molecular clock collaborates with many epigenetic modifiers. These include mixed lineage leukaemia 1 (MLL1, interacting with CLOCK/BMAL1), nucleosome remodeling deacetylase (NuRD, which interacts with and assists the PER complex) and the Polycomb group enzyme KMT6/EZH2, which co-precipitates with CLOCK/BMAL1(131–134). However, the circadian clock also influences the nuclear architecture and the topology of the genome. One of the first indications of this effect were shown in the studies of *Chlamydomonas reinhardtii* revealing diurnal oscillations of DNA supercoiling in the chloroplasts(135). Similarly, temporal changes in the organization of the albumin D element-binding protein (*Dbp)* circadian gene and its 3D chromatin fibre interactome could be observed in the mouse. Thus, rhythmic changes in the local chromatin condensation conferred oscillating transcription of genes included in the *Dbp* circadian interactome in mouse embryonic fibroblasts (MEF)(136,137).

1.3.4 The roles of CTCF and PARP1 in the circadian cycle

Since the chromatin fibre serves as a platform for the integration and propagation of many signaling and metabolic pathways, many cellular factors, which are not directly categorized as part of the core clock machinery, might be affected by or influenced by the circadian rhythm. This is exemplified by CTCF and PARP1, which influence chromatin interactions and mobility, as described in detail in chapter 1.2.4. Thus, PARP1 can influence food uptake since its loss of function correlates with diet-induced obesity(138,139). Moreover, Asher *et al*. showed that ADP-ribosylation of PARP1 oscillates in a rhythmic manner in synchrony with feeding-fasting cycles in the mouse liver(140,141). These findings provide an important link between circadian rhythms and metabolism, and have inspired the formulation of a molecular model. This posits that at the beginning of the light phase PARP1 binds to and poly(ADP-ribosyl)ates CLOCK. The loss of PARP1 would therefore cause a phase-shift in the interactions between CLOCK/BMAL1 and PER/CRY complexes. In line with this reasoning, mice lacking a functional *Parp1* gene displayed impaired food entrainment of peripheral circadian clocks(140,141). CTCF, one of the partners of PARP1, prominently binds to CLOCK/BMAL1 enhancers implying that these factors join forces to facilitate long-range chromatin interactions between CLOCK/BMAL1 enhancers and their target genes(142). But their ability to join forces to effectuate the circadian rhythm does not end there.

1.3.5 Transcriptional activation and repression directed by chromatin movements

As circadian transcription takes place in the context of the 3D nucleus, mechanisms allowing genome wide cyclic chromatin transitions must relate to the structural hallmarks of the nucleus(129). Paper I demonstrated that such events include a collaboration between PARP1 and CTCF. In short, this novel finding showed that circadian interactions between CTCF and PARP1 were driving the rhythmic recruitment of a subset of genomic loci to the repressive nuclear periphery(143). Subsequently, another study implicated that CTCF and PARP1 influenced the repositioning of the *Arntl2* mouse gene in breast cancer cells(144). Thus, Ha *et al*. showed that the presence of SNP at the promoter of this gene affected its interaction with CTCF and PARP1. They hypothesized that this SNP might therefore affect

the recruitment/release of the *Arntl2* gene to/from the nuclear envelope, thereby altering the timing of *Arntl2* expression(144). Correlation between chromatin mobility and changes in transcriptional state has been observed also in *Arabidopsis thaliana*(145). Feng *et al*. showed that the chlorophyll a/b-binding proteins (CAB) locus migrates from the nuclear interior to the nuclear periphery during its transcriptional activation, and that this relocation was triggered by light. Similar light-induced repositioning during transcriptional activation has been documented for the Rubisco small subunit (RBCS), plastocyanin (PC) and genomes uncoupled 5 (GUN5) loci(145).

1.3.6 Connection between enhancers and circadian chromatin movements

Circadian dynamics altering spatial gene regulation and transcriptional feedback events likely reflect rhythmic enhancer-promoter communications(146). This supposition was borne out in two parallel studies from Mermet et al. and Kim et al., in which both groups highlighted the connections between circadian rhythms and 3D genome folding(147,148). Mermet et al. used the 4C technology to demonstrate that clock-controlled promoter-enhancer interactions act as regulatory layers underlying circadian transcription in mouse liver and kidney. Accordingly, the deletion of intronic enhancer elements in *Cry1* or *Bmal1* influenced circadian rhythmicity(148). Similarly, Kim *et al.* showed that circadian gene expression is in mouse liver controlled by rhythmic interactions between promoter and enhancer elements, and that Rev-erbα contributed to rhythmic gene expression by antagonizing the formation of functional loops between target gene promoters and Rev-erbα-regulated enhancer(147,149). It remains to be seen, however, how such rhythmic enhancer-promoter interactions relate to their position within the nuclear architecture and the potential rhythmic mobility of the involved regions between transcriptionally permissive and repressive nuclear environments. This question is in the focus of Paper III, showing that the gating of *MYC* to nuclear pores required coordination of enhancer-promoter interactions with the recruitment of this complex to nuclear pores by CTCF and PARP1. As the *MYC* gene is expressed in a circadian manner in many different cell types(150), this finding raises the question whether the rhythmic repositioning of circadian genes to the nuclear periphery might initially involve their anchoring to nuclear pores to increase the amplitude of their cytoplasmic mRNA products, followed by their transient

transfer to the repressive compartments surrounding the nuclear pores to attenuate their transcriptional activity.

In summary, accumulated findings from several decades of intense research have broadened our understanding of spatial genome organization and its role in the regulation of many cellular processes, such as gene transcription. The discoveries of the functional divisions of transcriptionally permissive and transcriptionally repressive compartments reinforces the view that the genome is not randomly compacted in the nucleus, but that its compaction is highly controlled to maintain cellular plasticity, ie to provide a preparedness responding to environmental cues. Chromatin fibre interactions and movements emerge as crucial factors underlying the regulation of cellular identities. Crucially, the cell phenotypes must remain robust in their responses to the outside stimuli. These scientific advances have been made possible due to the development of highly sensitive technologies allowing the examination nuclear processes with an increasing precision.

Recently, the Nobel Prize in Physiology and Medicine was awarded to Jeffrey C. Hall, Michael Rosbash and Michael W. Young for their discoveries of molecular mechanisms regulating circadian rhythms. These and many other findings point towards to the future need to emphasize novel 4D research approaches, which focus on how the time plays a crucial role in regulating chromatin transitions within the 3D nuclear architecture in response to Zeitgebers. However, the examination of time-limited processes still represents a considerable technological challenge, and only the development of more sensitive methodology that preserves spatial genome organization will allow scientists to answer remaining key questions, such as: what are the principles behind regulated chromatin movements and interactions, and how can we use this knowledge to improve existing therapies?

2 RESEARCH AIMS

The overall aim of this thesis was to explore the connection between environmental signals and 3D genome organization underlying the regulation of gene expression. To this end, three independent studies were designed with the following questions:

1. How do external cues regulate gene expression in the compartmentalized nuclear architecture? Specifically, does the synchronization of the circadian rhythm regulate the movement of circadian chromatin loci between repressive and permissive nuclear compartments, accompanied by transcriptional changes? What is the underlying molecular mechanism?

2. What is the relationship between stochastic transcriptional bursts and dynamic 3D chromatin states? Specifically, how does *MYC* communicate with its enhancers in relation to its transcriptional activity?

3. What are the molecular mechanisms that coordinate enhancer-promoter communications and the localization of enhancer-promoter interactions within the 3D nuclear architecture? Specifically, does CTCF, a master regulator of chromatin structures and a key partner of PARP1 in regulating chromatin mobility, coordinate multiple steps of the WNT-regulated gene gating of *MYC*?

3 MATERIALS AND METHODS

3.1 CELL CULTURES AND TREATMENTS

HCT116 cells, kindly provided by Dr B Vogelstein, were cultured in complete growth medium (McCoy's 5A modified medium (Thermo Fisher Scientific, 26600023)) supplemented with 10 % Fetal Bovine Serum (FBS) (Thermo Fisher Scientific, 16141079) and 1% penicillin-streptomycin (Life technologies). HCEC were grown in the presence of Colonic Epithelial Cell Medium (HCoEpiC, ScienCell, 2950) and Drosophila S2 cells (Thermo Fisher Scientific, R69007) in Schneider's Drosophila medium (Thermo Fisher Scientific, 21720024) at the ambient temperature. hESCs (HS181, female) were cultured on irradiated male feeder fibroblasts and HEBs were generated as described previously(151). Cells were cultured at 37 °C under 5% CO2 and routinely tested mycoplasma contamination using EZ-PCR Mycoplasma Test Kit (Biological Industries, 20-700-20).

Serum shock treatments were performed as described previously(115). Briefly, HCT116 cells were cultured with serum-rich medium (McCoy's 5A modified medium, supplemented with 50% horse serum (Thermo Fisher Scientific, 16050122)) for 2 hours. Cells were cultured with complete growth medium subsequently for indicated periods.

HCT116 cells were treated with Olaparib (0.3 mM final concentration) for 24 hours, Flavopiridol (2 mM final concentration) for 8 hours, or 0.5 μM G9a enzymatic inhibitor BIX 01294 trihydrochloride hydrate (Sigma-Aldrich, B9311) for 72 hours before being harvested, as described in Paper I. The treatment with 10 μM β-catenin/TCF Inhibitor V (BC21) (Merckmillipore, 219334), or corresponding amount of DMSO in control cells were performed for 16h (Paper III).

3.2 MUTATION OF THE OSE-SPECIFIC CTCF BINDING SITE BY CRISPR

Key sequences within the main CTCF binding site at the OSE (chr8:128,219,114-128,219,767) cells in Paper III were mutated using the CRISPR/Cas9 technology custom service of Synthego (CA, USA). Briefly, specific guide RNA (gRNA) of a sequence

UAAACAGCAAUGCCCUCCAA, targeting the CTCF binding site within the OSE, was complexed together with the sp Cas9 to form a ribonucleoprotein (RNP). RNPs and donor DNA were then delivered to the cells *via* electroporation. Guide RNA cut location: chr8:127,215,101. The sequence within the CTCF binding site was modified from CTCACCATTGGAGGGCATTG to TTCATTATTTTATTTCATTG. Donor DNA sequence:
TTCTCACTGACTCTAAAACCTATCCATGCTCCTAAACCTCTTCATTATTTTATTT
CATTGCTGTTTACCCTTTCAGTTTCAGCTGTACTATCAAAAGCAG. Following recovery for 2 days, the edits created were evaluated by PCR amplification off the edited site followed by Sanger sequencing. The edited cell pool was used to seed single cells for clonal expansion. Each well seeded was imaged every 2-3 days and rigorously tracked to ensure the population were truly clonal and only the progeny of a single cell. Resulting clones were verified using Sanger sequencing. Two clones (D3 and E4) were selected and expanded without using any selection agents.

3.3 RNA/DNA FISH ANALYSES

Probes for *H19/IGF2, TLK1, VAT1L, PARD3, TARDBP*, specific LADs and other 4C interactors in Paper I were generated based on bacterial artificial chromosome/clone (BAC). Oncogenic super-enhancer (OSE), enhancer D (EnhD)-and *MYC* probes in Paper II and Paper III were prepared from a pool of PCR products spanning 8 to 10 kb regions of *Hind* III sites encompassing the *MYC* promoter and gene body (chr8:128,746,000-128,756,177), the OSE (chr8:128,216,526-128,225,855) and EnhD (chr8:128413009-128414109), respectively. The PCR products were sonicated to 500-2000 bps range followed by labelling with Green 496-dUTP (Enzo, 42831), Cy3-dCTP (GE Healthcare, PA53031) or Cy5-dCTP (PA55021, GE Healthcare). The BAC probes were labeled with Biotin-16-dUTP (Roche, 11093070910), using Bioprime Array CGH kit (Life technologies, 18095-011). A mixture of equal amounts of each labelled PCR product was used as the FISH probe and hybridized to formaldehyde cross-linked cells as described before(56). Finally, the cells were mounted in Vectashield mounting medium containing 4,6-diamidino-2-phenylindole (DAPI) (Vector Labs, H-1200).

RNA FISH was carried on cells cultured on chamber slides (Thermo Fisher Scientific, 154534) and crosslinked with 3% formaldehyde for 15 minutes at room temperature (RT). To inhibit ribonuclease activity, Ribonucleoside Vanadyl Complex (NEB, S1402S) was added to the buffers at all steps. Cells were permeabilized with 0.5% Triton X-100 in 2xSSC for 10 minutes at RT. The FISH probe was mixed with a 10-fold excess of human Cot-1 DNA (Thermo Fisher Scientific, 15279011) and hybridization was carried on the slides, overnight at 37°C in a buffer containing 50% formamide, 10% dextran sulphate and 2xSSC. Cells were washed twice first with 2xSSC/50% formamide at 40°C for 15 minute and then with 2xSSC for 15 minutes also twice at 40°C, followed by mounting with Vectashield mounting medium containing 4,6-diamidino-2-phenylindole (DAPI) (Vector Labs, H-1200).

Crosslinking and permeabilization of the samples for DNA FISH analyses were performed as described for RNA FISH. After denaturation in 2 x SSC/ 50% formamide for 40 minutes at 80°C, cells were kept in ice cold 2 x SSC for 5 minutes. The hybridization and washing steps were prepared as described for RNA FISH.

3.4 IN SITU PROXIMITY LIGATION ASSAY (ISPLA)

To detect proximities between different proteins: CTCF-PARP1, CTCF-CTCF, PARP1-PARP1 in Paper I and CTCF-AHCTF1 in Paper III cells that were crosslinked with 1% formaldehyde, blocked in 10% goat serum in PBS, 1h RT and incubated with antibodies of choice in 10% goat serum in PBS overnight at 4°C. Modified antibodies (termed R+ and M-) were added to the slides and further steps including hybridization of backbone and splint oligo DNAs, ligation with T4 ligase and rolling-circle amplification were perfumed as described before(152).

3.5 CHROMATIN IN SITU PROXIMITY (CHRISP)

ChrISP assays were performed and quantitated as previously described(153). Briefly, cells were crosslinked and permeabilized as for RNA/DNA FISH. After the hybridization of the FISH probes, the incubation with primary antibodies of choice was performed overnight at 4°C. Next, similarly to the ISPLA technology, the cells were incubated with modified antibodies (termed R+ and M-), hybridized with backbone and green splinter and ligated, as described(153).

3.6 GRID WIDE-FIELD MICROSCOPY

In Paper I, cell imaging and generation of optical section in 3D were performed with the use of Leica DMI 3000B fluorescent microscope with OptiGrid device (Grid confocal) and analyzed in Volocity software (Quorum Technologies Inc). In Paper II and Paper III Leica DMi8 microscope with the Thunder Imaging System (Leica Microsystems) was used and pictures were analyzed with the use of Leica Application Suite X (LasX) software. Stacks were taken at 0.3 μm intervals in the Z-axis. 150-300 alleles were counted for distance measurements and/or ChrISP and ISPLA signal intensity in each case in Paper I, in Paper II app. 1800 alleles in three independent experiments and in Paper III 2740 alleles in two independent experiments were examined for DNA FISH proximity analysis. RNA FISH signals were scored for by subtracting the intensity of the background in the immediate surroundings.

3.7 CHROMATIN NETWORKS AND INTEGRATION ANALYSES

3.7.1 Circular chromatin conformation capture sequencing (4C-Seq)

4C-seq analyses of chromatin interactomes in both HESCs and HEBs were performed as previously described(93), using the human *H19* imprinting control region (ICR) region as bait. Briefly, formaldehyde crosslinking of examined cell lines was performed in the presence of presence of 4 mM Ribonucleoside Vanadyl Complex (S1402S, New England Biolabs) with or without Olaparib (0.3 mM final concentration). The digestion of chromatin

representing 10^6 formaldehyde-fixed hESCs or EB cells was carried for 2 weeks at 37°C with *Bgl*II, in the presence of 1 U/ml RNasin Plus (Promega). Removal of poly(ADP-ribose) (PAR) was achieved by incubating the crosslinked chromatin with 25 ng/ml (final concentration) recombinant PARG 5 (catalog no. 4680-096-01, Trevigen) in the presence of 2 mM DTT in *Bgl*II restriction buffer at 25°C prior to *Bgl*II digestion for 24h. For the RNase-treated samples, crosslinked chromatin was incubated with RNase A (0.8 mg/ml final concentration) during enzyme restriction. Further steps, including intra-molecular ligation, reversing crosslinking of 4C material and DNA purification, were performed as previously described(93)

3.7.2 Nodewalk

The identification of chromatin networks impinging on *MYC* and flanking enhancers was performed as previously described by Sumida *et al.*(154). Adaptation of the Nodewalk protocol for small input material included following steps: after formaldehyde fixation HCT116 cells were counted and diluted in nuclear isolation buffer with a final concentration of 600 cells/µl (corresponding to ca 3ng of genomic DNA/µl). The resulting cell suspension aliquots (0.5µl) were mixed and incubated for 10min, followed by direct 10times x1.2 Buffer 2 dilution (New England Biolabs, Ipswich, MA. B7002S). Digestion with *Hind* III and subsequent ligation events were performed as previously described(56,154), but in a smaller reaction volume (20µl for Hind III digestion and 200µl for 3C ligation). After reverse cross linking, the 3C-DNA material was purified using the ChIP DNA Clean and Concentrator kit (Zymo Research, D5205), pre-heated at 65°C with the elution buffer to increase the recovery of large DNA fragments. The control of digestion efficiency of cross linked chromatin(93) was performed by designing F1/R1 and F2/R2 PCR primers (Table 1) flanking the *Hind* III sites at the 5` and 3`ends of the *MYC* promoter and gene body. Total DNA was quantified using the primers F3/R3 (Table 1) to produce a PCR fragment which lacks an internal *Hind* III site. To determine the digestion efficiency, the following formula was used: $(1- (PCR_{F1+R1/F2+R2} / PCR_{F3+R3}) \times 100$ (%). Libraries were generated by tagmentation as described earlier(56,154). The size distribution of the tagmented 3C DNA ranged from 200 to 300 bp. For the small input samples, Nextera XT DNA sample prep kit (Illumina, San Diego, CA. FC-121–1031, FC-131–1024) were used. The input amount was validated by qPCR using the F3 and R3 primers. Sequencing of each

library was performed on Illumina Miseq (Illumina) using the Miseq reagent cartridge v2 (Illumina) that generated 140–150 bp paired-end reads.

Table 1 PCR primers and conditions

Type of primers	**Primer ID**	**Forward**	**Reverse**	**Cycle**
ChIP assay	PARD3	CAAAAGATTTAAA AGGCAGCAAAA	TGCTATAAAAATT TGGGGAAACAAA	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
	Vat1L	TCAGTCATATTGG AGATGGAA	GGCCAAAATGCAC CCAAGTT	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
	CDC42EP3 (Neg. site)	CATGTGGCAGGCA GACAGGT	GCAACCCGGCTCT CTTCCTT	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
	H19 ICR	TCACCCTGAGGCC AAGATCC	CACGGGGGTCATC AGGGATA	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
	MPP4	TCAGAAGGGCCTC GCTCAGT	GCAGCTGGGGAGT GGTGAGT	95 °C, 5 min; (95 °C, 30 s; 62 °C, 30s; 72 °C, 30 s) x 36
	AK021484	GACCACAGAATAG TTTCCACAGAGAC C	TGCAAACTCTTGG TTCTTTTAAAGTG	95 °C, 5 min; (95 °C, 30 s; 62 °C, 30s; 72 °C, 30 s) x 36
	HOXA7	CCACTGCTCAGCC TTAGAGGAA	GTTTGTCTGGTTTT TGCGTGTG	95 °C, 5 min; (95 °C, 30 s; 62 °C, 30s; 72 °C, 30 s) x 36
For allelic discrimination	AD1 F1, R1	CCTCTCATCTCCCC AACCC	CACCCGGATGGTG CAGAATT	95 °C, 5 min; (95 °C, 30 s; 57 °C, 30s; 72 °C, 30 s) x 25
	AD2 F2, R2	CAACCCTCAATAG TGCACCCTG	AGTGCAGGCTCAC ACATCACAG	95 °C, 5 min; (95 °C, 30 s; 68 °C, 60s) x 25
	Taqman probe	Maternal: Cy3-TGGCTCCCATGAATGTCCTATCCCT-BHQ		
		Paternal: FITC-TGGCTCCCATGATTGTCCTATCCCT-BHQ		
Assessment of restriction enzyme digestion	F1, R1	GACCCGGGGCCAC GGGGCT	TTTCAGCCTCCAG ATGTGTG	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
	F2, R2	CAAGATCGAGCCA TTGGAC	AGGCAGGAAGAG GGTCTGTC	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
	F3, R3	CCAACTCTGTCTT GCCTTCTT	ACCCAAGCCACGC GTCGCAG	95 °C, 5 min; (95 °C, 30 s; 65 °C, 30s; 72 °C, 30 s) x 36
Detection of fibroblast DNA	XY1F, R	CTGATGGTTGGCC TCAAGCCTGTG	TAAAGAGATTCAT TAACTTGACTG	95 °C, 5 min; (98 °C, 10 s; 60 °C, 30s; 72 °C, 60 s) x 36

Quantification of fibroblast DNA	X1, X2	ATGATAGAAACGG AAATATG	AGTAGAATGCAAA GGGCTC	95 °C, 5 min; (95 °C, 10 s; 57 °C, 30s; 72 °C, 30 s.) x 36
	Y11, Y22	AATCATCAAATGG AGATTTG	GTTCAGCTCTGTG AGTGAAA	95 °C, 5 min; (95 °C, 30 s; 57 °C, 30 s 72 °C, 30 s) x 36
ChIP-loop	ICR-Vat1L	GCCTGCCTCTGGA CTCTGAGACTGG	CCACTGCTCAGCC TTAGAGGAA	95 °C, 5 min; (98 °C, 10 s; 60 °C, 20 min; 68 °C, 5 min). x 5 ; (98 °C, 10 s; 61 °C, 60 s; 68 °C, 5 min.) x 35

4C	ICR	Core	GATTAGGCTCCCA GCCATGCATG	GGGTCATCTGGGA ATAGGACACTC	94 °C, 3 min (98 °C, 20 s; 70 °C, 20 min; 68 °C, 20 min.) x4; (98 °C, 20 s; 70 °C 90 s; 68 °C, 20 min.) x 25
		Nest 1	GATTAGGCTCCCA GCCATGCATG	GGGTCATCTGGGA ATAGGACACTC	94 °C, 3 min (98 °C, 20 s; 58 °C, 90 s; 68 °C, 20 min.) x 27
		Nest 2	GATAAGAGCGAA ACTCTGTC	CACTCATGGGAGC CGCAC**	94 °C, 3 min (98 °C, 20 s; 56 °C, 60 s; 68 °C, 20 min.) x 26
		Nest 3	CAGAAAATTATGA CAATGAAAG	CACTCATGGGAGC CGCAC**	94 °C, 3 min (98 °C, 20 s; 59.5 °C, 30 s; 68 °C, 20 min.) x23
	Vat1L	Core	GCCTGCCTCTGGA CTCTGAGACTGG	CAGTCCTGGCCAA AATGCACCC	94 °C, 3 min (98 °C, 20 s; 70 °C, 20 min; 68 C, 20 min.) x 4; (98 °C, 20 s; 70 °C, 90 s; 68 °C, 20 min.) x 19
		Nest 1	GGGTAATAAAGGA ATAACTTGGGTGC	GCACCCAAGTTAT TCCTTTATTACCC	94 °C, 3 min (98 °C, 20 s; 60 °C, 90 s; 68 °C, 20 min.) x 20
		Nest 2	GGCTTGGACATAT TTGCTATTTTG	CTATTCAGTGTGC TGCTGCAAG	94 °C, 3 min (98 °C, 20 s; 56 °C, 60 s; 68 °C, 20 min.) x 20
		Nest 3	GGAGCAAAGTCAA AGGAGAGATC	CCATCTCCAATAT GACTGAAGATC	94 °C, 3 min (98 °C, 20 s; 55 °C, 60 s; 68 °C, 20 min.) x 19

3.8 IMMUNOPRECIPITATION ANALYSIS

3.8.1 ChIP-qPCR

The collection of cells and their fixation with freshly prepared 1% formaldehyde solution was performed as described previously(93). The immuno-purification of the DNA-protein complexes used the following antibodies: in Paper I - PAR polymer (4336-BPC-100, Trevigen), CTCF (sc-15914, SantaCruz Biotechnology, BD), Rad 21 (Abcam) or PARP1 (ALX-210-221-R100, Alexis) and Dynabeads~~®~~,R Protein G (Invitrogen); in Paper III - CTCF (Abcam, ab155990), AHCTF1 (Novusbio, NBP1-87952), ß-catenin (Novus Bio #NBP1-87952) or TCF4 (Santa Cruz, sc-8631) and Dynabeads protein G (Thermo Sciences, 10004D), as described(155). The purification was performed with ChIP DNA Clean and Concentrator (Zymo Research, D5205), and quantification by standard qPCR analysis using primer sequences and PCR conditions, as previously described(56).

3.8.2 Co-immunoprecipitation (co-IP) assay

Co-IP assays were performed using the Nuclear Complex Co-IP kit, following the manufacturer's recommendation (54001, Active Motif). Briefly, 400 µg (Paper I) or 250 µg (Paper III) of nuclear lysates were used in the analyses. The pre-clearing of the material was done by the addition of Dynabeads Protein G (Thermo Fisher Scientific, 10004D). In Paper I, anti-CTCF (2899S, Cell Signalling) and in Paper III anti-CTCF (Abcam, ab37477, mouse), anti-NUP133 (Abcam, ab114096, rabbit), anti-AHCTF1 (Novus Bio, NB600-238, rabbit), normal rabbit IgG (Cell Signaling, 2729S) and normal mouse IgG (Santa Cruz, sc-2025) were used. Where indicated, extracts were incubated with Olaparib at final concentration (0.3 µM) during the washes. The immunoprecipitated material was analyzed by Western blot (Paper I) or Simple Western assay using the WESTM system (ProteinSimple, Bio-Techne) (Paper III).

3.9 WESTERN BLOT ANALYSIS

In Paper I, protein analyses were performed by SDS-PAGE using “any kD” Criterion TGX Gel (567-1125, Biorad). Transfer of separated proteins to polyvinylidene difluoride membranes (162-0174, Biorad) was carried out overnight at 4°C at 200 mA. The membranes were blocked overnight incubation in TBST (10mM Tris-HCl, pH 8.0, 150mM NaCl, 0.05% Tween 20) containing 7% skim milk followed by overnight incubation at 4°C with antibodies against alpha-tubulin (T6199, Sigma Aldrich), beta-actin (4967L, Cell Signaling Technology), CTCF (2899S, Cell Signaling), PAR (4336- BPC-100, Trevigen), or PARP1 (ALX-210-221-R100, Alexis) diluted in TBST and 7% skim milk. The membranes were washed with TBST 3 times, 10 minutes each. Identification of the signal was achieved by incubation with secondary antibodies conjugated to horseradish peroxidase against mouse IgG (AP308P, Millipore) or rabbit IgG (7074S, Cell Signaling Technology), and its visualization by using ECL Prime Western Blotting Detection reagent (RPN2232, GE Healthcare) captured by LAS-1000 (Fuji Film). The signals were quantified by Quantity One software (BioRad).

In Paper III, protein analyses were performed with Simple Western assay using the WES™ system (ProteinSimple, Bio-Techne) using the anti-CTCF (Cell Signaling, 2899S, rabbit), anti-PARP1 (Abcam, ab32071, rabbit), anti-NUP133 (Abcam, ab155990, rabbit), anti-AHCTF1 (Novus Bio, NB600- 238, rabbit) and anti-β-catenin (Cell Signaling, 8480S, rabbit) antibodies. The acquired chemiluminescence signal was measured and analyzed using the Compass software for Simple Western (ProteinSimple, Bio-Techne). The identification of chemiluminescence peaks corresponding to analyzed protein was obtained by configuration of default setting and production of standard curve based on serial dilutions of the input.

3.10 RNA ANLYSES

3.10.1 siRNA transfection

Where indicated, CTCF siRNA(h) (sc-35124), PARP1 siRNA(h) (sc-29437) or GFP siRNA (sc- 45924) from Santa Cruz Biotechnology were transfected into the analyzed cells by using Lipofectamine RNAiMAX Transfection Reagent (13778075, Life Technologies), following the manufacturer's instructions. Briefly, when cells reached 20% confluency, 20 pmol of each siRNA- Lipofectamine complex was added to the cell culture. After 6h of lipofection, medium was replaced with McCoy's 5A modified medium (16600-082) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin and cells were harvested for further analysis 48 hours after transfection. The efficiencies of the siRNA transfections were validated by immunostaining analysis.

3.10.2 Pulse labeling of RNA and nuclear RNA export assay

Newly synthesized RNA samples were obtained by incubating the cells with 0.5 mM (at final concentration) 5-ethynyl uridine (EU, Thermo Fisher Scientific, E10345) for 15 or 30 minutes. Following this pulse, the cells were washed with 5xPBS and incubated with pre-warmed normal growing medium for indicated time periods. The fractionation of EU-labelled nuclear and cytoplasmic RNA followed by cDNA synthesis and RTQPCR-analyses of the presence of intronic and exonic regions of *MYC* enabled analyses of the kinetics of the nuclear export of newly synthesized RNA (determined by the cytoplasmic/nuclear ratios). The separation of the nuclear and cytoplasmic fraction as well as the isolation of newly labeled RNA used the Ambion® PARISTM system (Thermo Fisher, AM1921), according to the manufacturer's instructions. The capturing of EU-labelled RNAs was performed by Click-iT Nascent RNA capture kit (Thermo Fisher, C10365) followed by conversion into cDNA using SuperScript VILO cDNA Synthesis Kit (Life Technology, 11754050).

3.10.3 RT-qPCR analysis of transcription

The quality of purified RNA samples was measured before cDNA synthesis by SuperScript VILO cDNA Synthesis Kit (Life Technology, 11754050) using Bioanalyzer 2100 (Agilent). All the qPCR examinations were done by using 10-fold diluted cDNA and iTaq Universal SYBR Green Supermix (Bio-Rad, 1725125) on RotorGene 6000 (Corbett Research). Serial dilutions of sonicated genomic DNA were used to ensure the linear range of the PCR amplification.

4 RESULTS

4.1 PAPER I: PARP1- AND CTCF-MEDIATED INTERACTIONS BETWEEN ACTIVE AND REPRESSED CHROMATIN AT THE LAMINA PROMOTE OSCILLATING TRANSCRIPTION

4.1.1 Inter-chromosomal interactome and connection between circadian loci and repressed domains

The analyses of dynamic chromatin crosstalk was performed using the 4C technique, which allows the capturing of more than two simultaneously interacting chromatin fibers(38,93,94). The *H19* ICR, used as a bait in human embryonic stem cells (hESCs) and human embryoid bodies (hEBs), was chosen as it confers epigenetic changes in *trans* in the mouse(156) (G&D 2009 paper). We identified 518 different regions which were reproducibly interacting with the bait in a developmentally regulated manner. The interactome included both intra- and inter-chromosomal interactions, the latter of which dominated the chromatin network(157). The sequences within the 4C libraries covered both genes and intergenic regions and were enriched in transcriptional units controlling cell adhesion/synaptic processes. The created network was approximate scale-free and characterized by a high modularity. Although the interactome represented the sum of interactions within a cell population, where individual interactions are likely variable and dynamic at the single cell level, multiple interactions emerged reproducibly in two or more 4C samples as central nodes of the network.

To validate the network, 3D DNA FISH analyses were performed. Analysis of the physical distance between the bait and its interactors showed that the nodes with high count reads in 4C library (representing central nodes with higher connectivity) were significantly closer to the bait than the sequences having low read counts. To further validate the topology of the network we generated new chromatin interactome data using new 4C bait, the locus coding vesicle amine transport 1 homolog-like protein (*VAT1L*), which connected to every module of the *H19* ICR interactome. Indeed, apart from the reproduction of the modules discovered using the *H19* ICR as bait, the *VAT1L* bait discovered new regions independent from the *H19* ICR interactome. Finally, 3D DNA FISH analyses showed that regions more distal in the network were less frequent in physical proximity than directly interacting nodes, with

the *H19* ICR bait in contact with only a subset of loci at any given time. A unique feature of the *H19* ICR 4C network was its coverage of various chromatin states. Interactions with both transcriptionally active and inactive chromatin components neither segregated away from each other neither showed preferences in both human embryonic stem cells (hESC) and derived embryoid bodies (hEBs). This observation uncovered an exceptional feature of the *H19* ICR interactome by questioning the dogma that active and inactive domains are always separated(39,157)

4.1.2 Role of CTCF and PARP1 interactions in connecting circadian loci to LADs

The high reproducibility of the 4C network highlighted the possibility that encounters between the nodes might be regulated. Taking into the account the role of PARylated CTCF, accompanied by its ability to complex with PARP1 to regulate the insulator of the maternal *H19* ICR(158), we hypothesized that PARylation and/or PARP1 together with CTCF might organize chromatin network in *trans*. Indeed, removal of PAR chains from formaldehyde cross-linked chromatin by PAR glycohydrolase (PARG)(159) disassembled the network in both hESC and hEBs in a manner distinct from that produced by RNase treatment. These data indicated that PAR is present in the *H19* ICR chromatin fibre interactome. However, this observation didn't discriminate whether PARylation was the cause or consequence of upcoming chromatin fibers interaction. To explain the mechanism of PAR deposition as well as the role of CTCF/PARP1 interactions in establishing the inter-chromosomal network, several experiments were performed. First, we could show that CTCF can activate PARP1 but not PARP3 in the absence of DNA damage, reinforcing the notion that PARylation of chromatin depends on CTCF/PARP1 interactions. Moreover down-regulation of CTCF by small interfering RNA (siRNA) reduced cellular PAR levels, indicating that CTCF is a major regulator of PARP1 activity in the living cell. In parallel, ChIP-loop analysis documented that PARP1 is a part of *H19* ICR-*VAT1L* complex, whereas 3D DNA FISH revealed that Olaparib treatment not only inhibits the enzymatic activity of PARP1 but also disrupted CTCF/PARP1 interactions in both co-immunoprecipitation and *in situ* proximity ligation assay experiments(160). Moreover, Olaparib treatment significantly reduced the proximities between *IGF2/H19-VAT1L* loci as well as between *IGF2/H19-PARD3*, the latter of which encoding a cell polarity regulator under circadian

control. Since Olaparib treatment affected also the relative positions of *VAT1L* and *IGF2/H19* in relation to their chromosome territories we concluded that CTCF/PARP1 interactions and/or PARP1 activity regulates chromatin movements. Accordingly, chromatin immunoprecipitation (ChIP) analysis demonstrated that PARP1 binds to many of the chromatin hubs in the 4C network, including *VAT1L* and *H19* ICR. Moreover, CTCF binding to some of the *H19* ICR-interactors was disrupted by a 24-hours Olaparib treatment indicating indirect binding to these regions. Even only 10-min incubation with Olaparib during the formaldehyde cross-linking of 4C material disassembled most of the chromatin interactions in hESCs. All these observations taken together demonstrate that the CTCF/PARP1 complex plays important roles in the establishment of the *H19* ICR chromatin network and that PAR chains likely both stabilize these interactions and diversify the network by providing a platform for interactions.

Discovery of extensive interactions between transcriptionally active and inactive chromatin components within 4C network suggested that the communication between examined loci might function to fine-tune transcription. Due to the fact that PARP1 was found to be involved in the entrainment of circadian rhythms to feeding in mouse(161) we examined our network for circadian genes. Indeed, permutation-based enrichment analyses showed an over-representation of circadian genes involved in lipid metabolisms, particularly in flanking regions 10 kb or less from the site of interaction. Remarkably, circadian genes frequently interacted with repressed domains and constitutive LADs, and the percentage of these interactions was higher than between circadian genes. 3D DNA FISH analyses confirmed this pattern by demonstrating frequent, PARP1-dependent proximities between *VAT1L* and four different constitutive LADs at the nuclear periphery.

4.1.3 CTCF/PARP1 mediated circadian transcriptional attenuation by oscillating repositioning of circadian loci to the nuclear periphery

We therefore considered the link between the role of the nuclear periphery in the dynamic regulation of gene transcription(46,48), and repressive chromatin modifiers interacting with the core clock machinery (co-regulating the negative limb of the circadian feedback loop)(162,163). Specifically, we hypothesized that circadian regulation of transcription

might require the recruitment of circadian loci to the nuclear lamina to attenuate their transcription in oscillating manner. To explore this possibility, we used the method of serum shock synchronization of circadian gene expression in cultured HCT116 cells(115). In contrast to hESCs, for example, the HCT116 cells displayed prominent circadian expression profiles. Using *in situ* proximity ligation (ISPLA) we first documented that the proximities between CTCF and PARP1 were under circadian control peaking at 8 and 32h and could be found primarily at the nuclear periphery. Similarly, 3D DNA FISH analyses showed that the serum-shock induced the rhythmic juxtaposition of *IGF2/H19, VAT1L, PARD3* and *TARDBP* to the nuclear periphery. Interestingly, the examined loci showed different timing of recruitment to the periphery - 0h and 24h peaks for *IGF2/H19* and *VAT1L*, while *PARD3* and *TARDBP* peaked around 8h later. Because *IGF/H19* and *VAT1L* exhibited low transcriptional activity in contrast to *PARD3* and *TARDBP* in HCT116 cells, we examined if inhibition of transcriptional elongation by Flavopiridol(164) would affect *PARD3* and *TARDBP* tethering to nuclear lamina. Indeed, Flavopiridol treatment 8h before harvesting the serum-shocked cells not only inhibited transcription, but also accelerated the movement of these two loci to the nuclear periphery. These results show that transcriptional activity or transcription-associated processes delay the mobility of circadian loci towards the nuclear periphery. Olaparib treatment and CTCF or PARP1 knock-down, on the other hand, not only abolished rhythmic juxtaposition of circadian loci to the lamina, but also disrupted rhythmic transcription of *PARD3*. All of these findings document that CTCF and PARP1 join forces to control both the rhythmic repositioning of circadian loci to the nuclear lamina and the entrainment of circadian transcription.

Interestingly, transcriptional attenuation of *PARD3* did not occur immediately following its recruitment to the nuclear periphery. Using a higher precision in the time course, we could show that its transcriptional activity was highest when juxtaposed to the lamina to significantly drop a few hours later while still juxtaposed to the nuclear periphery. To further investigate this observation, we examined the presence of repressive chromatin marks on the PARD3 locus in HCT116 cells, which are typically enriched at nuclear periphery. Since ChIP analyses revealed the presence of the H3K9me2 mark but not the H3K27me3 mark at *PARD3*, we used the ChrISP technique(153) to document that the H3K9me2 mark peaks at *PARD3* at the time of its transcriptional attenuation while still remaining at the nuclear periphery. Conversely, lower levels of the H3K9me2 signal

coincided with an increase of *PARD3* transcriptional activity when returned to the interior of the nucleus. Importantly, the inhibition of enzymatic activity of G9a/Glp by BIX01294(165) not only depleted the H3K9me2 mark and reduced the amplitude of oscillation in *PARD3* transcription, but also abolished its movement to the periphery. In summary, the data demonstrates that the juxtaposition of *PARD3* to the nuclear periphery is accompanied by a time-dependent acquisition of the repressive H3K9me2 mark and rhythmic transcriptional attenuation.

4.2 PAPER II: MYC AS A DRIVER OF STOCHASTIC CHROMATIN NETWORKS: IMPLICATIONS FOR THE FITNESS OF CANCER CELLS

4.2.1 *MYC*-driven chromatin networks as a sum of stochastic interactions

The active *MYC* locus, which is under circadian control(150), has an ability to form networks enriched in flanking enhancers(56,101). To identify the most important nodes in the *MYC* network, we used the Nodewalk technique to create virtual chromatin networks in primary cultures of human colon epithelial cells (HCECs) as well as in a colon cancer cell line (HCT116). Due to the fact that *MYC* is diploid in HCECs but triploid in HCT116 cell line we randomly sampled two-thirds of the interactions from the HCT116 network, hence compensating for any possible bias. The analysis showed that the most connected nodes are more prominent in HCT116 than in HCEC, and increasing k-core (a parameter which identifies the most connected nodes(166)) correlated with strong enrichment of primed and active enhancers. To uncover which nodes were crucial for establishing the topology of the network, and hence the removal of which nodes would globally affect the network structure, we used the dynamic index approach for distinguishing the most influential regions(167). Contrary to expectations that enhancers would drive the network, *MYC* itself came up as the most dynamic information spreader within the network indicating that *MYC* itself is searching for the enhancers rather than other way around.

To pursue this finding to the next level, we wanted to rule in or out that the *MYC* chromatin network represented the sum of stochastic interactions present only in large cell population. To this end, we developed and applied a modified Nodewalk protocol(56,154), to enable the analyses of very small input samples and yet preserve high reproducibility. We generated three types of samples - 1. A set of samples with large input material, representing technical replicates; 2. Ten samples with small input material derived from a large pool of ligated DNA corresponding to one million cells, which was aliquoted to generate ten samples, each containing 0,88ng of DNA (app. 176 cells), also representing ten technical replicates; 3. Nine biological replicates starting from independently prepared small cell populations, corresponding to 177 cells. As the *MYC* chromatin network is dominated by interactions in *cis*, our focus on the presence of flanking interactors in technical replicates from the same initial RNA library (prepared from 0,88ng of 3C DNA), generated a technical reproducibility >90%. Additionally, analyses of nine 177-cells samples showed that >70% of *MYC* interactions were present in only one library, while in ten 0,88ng 3C DNA technical replicates >85% of interactors were reproduced in two or more libraries. Importantly both set of samples exhibit a comparable proportion of interaction categories, and the overlap between the pooled 177-cells biological replicates or the pooled 0,88 technical replicates and the large input technical replicates generated from 3C DNA aliquots (corresponding to 10 000 cells) exceeded 91%. These data indicated that the small-input Nodewalk protocol could be reliably used to recapitulate the interactors already present in high-input network. Thus, interactomes uncovered in the large input cell populations represented only the sum of stochastic interactions present in individual cells.

4.2.2 Mutually exclusive interactions of *MYC* with flanking enhancers

Examination of stochastic interactions faces significant challenges - the smaller the sample is the more variable results can be obtained to compound reproducibility. However, having proven the high reproducibility of the modified Nodewalk technique we decided to further reduce the input sample to 34,8pg which corresponds to 21 alleles in seven cells. By comparing 23 aliquots of such samples the 9x177 batch of cells, 6 out of 8 different interactors within TAD1 or TAD2 flanking *MYC* overlapped. Moreover, the number of different enhancers interacting with *MYC* (in single aliquot) ranged from 0 to 1. Given that

the average recovery of the bait was 36,2%, 0,7 enhancer region interacted with 7.6 different *MYC* alleles. These numbers and the high similarity between the binned data of high and small-input material demonstrate that *MYC* interacts with its enhancers in a dynamic and mutually exclusive manner. This conclusion was reinforced by 3D DNA FISH analyses demonstrating that the proximities between *MYC* and its two major interactors – the oncogenic super-enhancer (OSE) and enhancer D (EnhD)(56,154) – rarely, if at all, co-localize in the nuclear space, within the limits of microscopic resolution. All of these observations taken together disprove the concept of enhancer hubs simultaneously impinging on *MYC*, showing that synchronous interactions of many enhancers with the *MYC* locus are only virtual consequence of larger cell population analysis and represent the sum of stochastic events occurring in smaller populations.

4.3 PAPER III: A CTCF BINDING SITE WITHIN THE ONCOGENIC SUPER-ENHANCER COORDINATES THE WNT-REGULATED GATING OF MYC

4.3.1 CTCF regulates the WNT-dependent pre-nucleoporin-OSE interactions

Scholz *et al.* (56) documented the existence of an oncogenic super-enhancer-mediated mechanism that recruits the *MYC* gene to nuclear pores under the control of the WNT signaling pathway in human colon cancer cells. To explore the inner workings of this process, we focused on CTCF, which regulates both enhancer-promoter communication(168) and chromatin mobility within the nuclear architecture (Paper I). Our initial analyses in HCT116 cells showed that CTCF not only interacts with Nucleoporin 133 (NUP133) and AT Hook Containing Transcription Factor 1 (AHCTF1 or ELYS), which are pre-nucleopore complex members involved in *MYC* gating, but also with ß-catenin, a key player in the WNT-dependent gene gating of *MYC*. Interestingly, ISPLA analysis showed that periphery-specific proximity between CTCF and AHCTF1 can be significantly reduced upon treatment with the BC21 drug(169) which interferes with downstream ß-catenin and TCF4 interactions in the WNT canonical pathway. We therefore focused on a single CTCF binding site within a region of the oncogenic super-enhancer (OSE) physically interacting with *MYC* (Paper II). Using CRISPR technology(170) to change 8 bases within this CTCF binding site, we obtained two clones (D3 and E4)

carrying mutant OSE alleles which lost almost all of their ability to bind CTCF while leaving CTCF binding to the *MYC* promoter unaffected.

4.3.2 CTCF coordinates several steps of the WNT-regulated gating of *MYC*

Next, we wanted to examine if the mutation of the OSE-specific CTCF binding site had an impact on the nuclear export of MYC mRNA into the cytoplasm. To this end, we performed nuclear export assays described before(56), exploring the cytoplasmic/nuclear ratios between newly synthesized *MYC* transcripts, The results showed that even though the overall transcriptional rate of *MYC* was not significantly affected in both mutant cell clones compared to control cells, the mutant OSE alleles were unable to support the facilitated nuclear export of *MYC* mRNA, showing a three-fold reduction in comparison to the wild type allele. Moreover, since the addition of BC21, which inhibits the canonical function of the WNT signaling pathway (163), did not further change the export rate in the D3 and E4 clones, we conclude that WNT regulates *MYC* gating *via* the OSE-specific CTCF binding site. Moreover, computer simulation showed that the observed changes in total *MYC* mRNA expression between wild type and mutant cells was solely dependent on the loss of the rapid nuclear export of *MYC* mRNA

Our lab has previously shown that the *MYC* and OSE regions display highest potential for interactions when juxtaposed to the nuclear periphery(56). While we could confirm this observation in wild type cells, the mutant cells showed both loss of OSE and MYC proximity to each other and a reduced presence of these regions at the nuclear periphery. These findings were independently validated by using the ChrISP technique, which has a higher resolution in all three dimensions. Finally, Olaparib treatment of the cells revealed loss of facilitated nuclear export of *MYC* mRNA, indicating that the CTCF-PARP1 complex may play a functional role also in the pathological gating of *MYC* in cancer cells.

5 DISCUSSION

5.1 NUCLEAR PERIPHERY AND ITS ROLE IN THE REGULATION OF GENE EXPRESSION

Despite its huge size, the human genome enclosed by the nuclear double membrane is tightly compacted but non-randomly organized within the nucleus. In mammals, the nuclear periphery is enriched in a unique set of inner nuclear membrane (INM) proteins (e.g., lamin B receptor – LBR and lamina associated peptide 2 - Lap2; emerin) which interact with the nuclear lamina consisting of a filamentous meshwork of nuclear lamins: A-type lamins (lamina A and C) and B-type lamins (lamin B1 and B2)(171–173).

Accumulating evidence supports a role for the nuclear membrane to function not just as a passive barrier, but also as a dynamic interface which modulates gene activity and chromatin organization. Thus, both experimental (3D-DNA FISH) and bioinformatic analyses showed that gene-rich chromosomes and early replicating genes are more centrally deposed, while gene-poor chromosomes and late replicating genes are preferentially located at the nuclear periphery being part of LADs(171,174,175). It therefore appears that LADs are formed not just as a result of gene inactivity but represent an intrinsically repressive nuclear domain. In line with this reasoning, the insertion of a reporter gene within a LAD or gathering an entire chromosome to the nuclear periphery reduces its expression compared to that when they are positioned in the nucleoplasm(53,176). Moreover randomly integrated sequences delivered from LADs have an ability to tether to the lamina and undergo transcriptional repression(172,177).

Nevertheless, despite these examples of primary repressive functions, the nuclear periphery has also been shown to function in gene activation(178). While association with the lamina is almost always repressive(179), relocation of certain loci to the Nuclear Pore Complex (NPC) might either activate or repress genes(172). NPCs are major transport tracks between the nucleus and the cytoplasm(180) consisting of 32 copies of each of ca 30 nucleoporins proteins (NUPs) in mammals to form a huge protein complex. The complexity of the nuclear pore function is reinforced by demonstrations that it contributes also to the creation

of heterochromatin exclusion zones, regulation of RNA splicing as well as RNA export and its associated phenomenon of gene gating(56,172).

The importance of the regulatory functions of the nuclear periphery is highlighted in Paper I and Paper III. We could thus show that the rhythmic migration of circadian loci to the nuclear periphery and their interactions with LADs promotes circadian transcriptional attenuation, indicating that the repositioning of active circadian genes to the repressive sub-compartment facilitates chromatin transitions from the positive to the negative limb of the circadian rhythm. These findings also highlight a novel function of the repressive gene deserts at the lamina in the regulation of the circadian expressivity of the genome. As circadian genes remained active for several hours at the lamina before their repression, we hypothesized that they might first land on transcriptionally permissive environments, such as nuclear pores. Paper III explores the mechanism of such gene gating events at the *MYC* gene, highlighting the role of CTCF and PARP1 in this process. Given the circadian expression of *MYC* in several model systems, it is thus plausible that the gating process is also under the control of the circadian clock. It remains to be seen whether the circadian genes that undergo rhythmic recruitment to the lamina also undergo facilitated export to the cytoplasm and to what extent this mechanism contributes to oscillating gene expression genome wide.

Given the role of CTCF7PARP1 complexes in the recruitment of circadian loci to the lamina, circadian-dependent changes in the nuclear architecture contribute not only to transcriptional plasticity, but also connect the 3D chromatin structure at the lamina with the metabolic states of the cell (PAPER I, III,(181)). These considerations include also the CTCF- and PARP1-regulated gating of *MYC* to the nuclear pores (PAPER III). As metabolic processes tend to be under circadian control, these findings indicate that there might be a two-way relationship between cellular metabolic states and circadian 3D genome folding at the nuclear periphery.

5.2 COORDINATION OF ENHANCER-PROMOTER INTERACTIONS AND SUB-NUCLEAR LOCALISATION AT THE *MYC* LOCUS

It remains a fundamental question how enhancer-promoter interactions are integrated within the 3D nuclear architecture and how such processes are coordinated with chromatin mobility between active and repressive sub-compartments. Paper II shows that *MYC* screens for enhancers in its flanking TADs in a stochastic process resulting in that *MYC* interacts with one enhancer at a time. The often observed existence of the so-called enhancer hubs are thus likely only a virtual feature of large cell populations that does not exist in single cells. Application of the ultra-sensitive Nodewalk protocol also revealed that <10% of *MYC* alleles interacted with an enhancer at any given time to highlight the dynamic character of enhancer-gene interactions. Interestingly, the loss of the functional cohesin complex has earlier been shown to generate super-enhancer clusters forming hundreds of links within and across chromosomes(182). This data therefore suggests that in the presence of the cohesin complex, genes are preferred partners for enhancers, but that in its absence enhancer hubs might be formed even in single cells. By extrapolation, the enhancer hubs night therefore represent situations with a normally occurring, transiently reduced presence of the cohesin complex at these regions. This discussion is compounded by a previous observation of our lab, that different enhancers display different functions depending on the intra-nuclear position of *MYC*(56). One category of enhancers, such as Enhancer D, thus showed proximity to *MYC* preferentially in intra-nucleoplasmic locations to potentially contribute to the transcriptional activation of *MYC*. Conversely, the oncogenic super-enhancer showed preferential proximity to *MYC* at the nuclear periphery/pore to post-transcriptionally facilitate the nuclear export of its derived mRNA(56).

These observations raise a Pandora's box of questions. For example, how is the dynamic process of the repositioning of the *MYC* locus to the nuclear periphery regulated and coordinated with its interactions in *cis* and *trans*? The observations that partly or completely processed *MYC* transcripts are highly enriched while still proximal to their templates at the nuclear periphery indicate that *MYC* transcript processing requires movement of their templates(56). Since transcriptional activation of *MYC* is largely occurring in the nuclear interior, we speculate that the elongation process might be inhibited to speed up the migration of *MYC* alleles to the nuclear pores. This is incidentally

analogous to our earlier observation, that transcriptional inhibition by Flavopiridol facilitates the migration of circadian loci to the nuclear periphery (Paper I). Similarly, X chromosome inactivation in female mammalian cells is accompanied by relocation of the inactive X to the nuclear lamina during early development(172,183). It is also noteworthy that damaged DNA, represented by compromised telomeres, for example, can explore large volumes of the nucleus within minutes to hours(184). However, it remains to be established if the migration of *MYC* to the nuclear pores is the result of inhibition of transcriptional elongation and/or controlled DNA damage. In line with the latter reasoning, the role of PARP1 in the migration of circadian loci to the nuclear periphery (Paper I) might provide an opening. Finally, it has been shown that nuclear ß-actin is involved in the regulation of interactions between heterochromatin and the nuclear envelope(185). Our unpublished ISPLA data show that CTCF and G-actin have a high potential to form complexes with each other. This is of particular interest since the rhythmic pattern of nuclear ß-actin levels is inverse to that of the cytoplasmic F-actin(186). Taken together, these observations indicate that the migration of *MYC* to the nuclear pores might be indirectly regulated by cytoplasmic F-actin polymers to promote or antagonize nuclear CTCF-ß-actin complexes.

5.3 THE ROLES OF CTCF AND PARP1 IN THE REGULATION OF CHROMATIN NETWORKS AND THEIR SUB-NUCLEAR LOCALIZATION

In Paper I and III we make the point that CTCF and PARP1 play important roles in both the regulation of chromatin interactions and the mobility of the interactors. In Paper I we uncovered novel principles, in which the repositioning of active circadian loci to inactive LADs at the nuclear periphery is regulated by CTCF and its binding partner PARP1, thereby promoting transcriptional attenuation. In Paper III we show that a single CTCF-binding site within the OSE coordinates WNT-regulated *MYC* gating. Although it is well established that CTCF functions as a master regulator of the genome, setting up chromatin boundaries and gene expression domains, its involvement in regulating chromatin mobility is currently an unchartered territory. One clue to this feature might be provided by the involvement of PARP1 in these processes, as highlighted in Papers I and III. However, the inner workings of this process are completely unknown. Although the CTCF-ß-actin complex might play a role in this process, it is not clear how this complex would affect the

directionality of the migration to the nuclear pore. It is important to note that *MYC* appears to be a passive passenger with the oncogenic super-enhancer driving the migration of the entire region to the nuclear periphery. Thus, whereas the cohesin complex is likely involved in the formation of the OSE-*MYC* complex at the nuclear periphery, it does not likely provide the main driving force of the OSE juxtaposition to the nuclear pores. Interestingly, however, the cohesin complex harbors ATPase activity and that its chromatin loop extrusion function requires ATP(187,188). The activation of PARP1 by CTCF results in the production of poly(ADP-ribose) chains that have previously been shown to provide local supplies of ATP, coordinated by the PARG enzyme, during the DNA repair process, for example(189,190). Such locally high levels of ATP might fuel the function of the cohesin complex to coordinate subnuclear localization with OSE-*MYC* interactions. All of these data suggest that CTCF/PARP1 interactions and the consequent generation of PAR might play an important role in the coordination of chromatin mobility and enhancer-promoter communication.

5.4 THE DYSREGULATION OF CHROMATIN STRUCTURES IN DISEASES

The 3D chromatin structure plays an important role in orchestrating nuclear processes, particularly those involved in gene expression outputs stabilizing phenotypes. Although still underestimated, abnormalities in the spatial organization of the genome might underlie many diseases by impeding correct sets or promoting unscheduled sets of interactions between genes and regulatory elements(171,172,191). As a master genome organizer, unscheduled methylation events of CTCF binding sites to antagonize CTCF binding are a common source of abnormal chromatin organization, by globally disrupting TAD organization and chromatin insulation(191). CTCF is a key factor in life, as homozygous knockouts are lethal already during pre-implantation development(192). Moreover, patients displaying CTCF haploinsufficiency exhibit developmental defects such as intellectual disability or growth retardation(191,193). Mutations in one or more of the eleven zinc-fingers of CTCF are frequently associated with different cancer types, and CTCF sensitivity to CpGs hypermethylation could be linked to human gliomas (with global hypermethylation of CTCF-binding sites caused by mutations at the isocitrate dehydrogenase locus)(194,195).

Importantly, we could show that CTCF is also involved in the circadian rhythm dependent chromatin movement regulation. Paper I thus highlights a new principle of gene regulation that should be considered when treating cancer patients, for example. Due to the rhythmic transcriptional activity of circadian genes, timed chemotherapy treatments might be beneficial for the patient by optimizing the function of the treatment when circadian genes are at the periphery, for example, while simultaneously reducing its side effects. This approach could increase the efficiency of cancer cell elimination to the provided drug, thus increasing the chance for the successful treatment.

The unscheduled disruption of nuclear compartments might also lead to various pathologies. Over 400 different mutations of nuclear lamina components were linked to different laminopathies, where the majority of them affected lamin A/C (191). Lamina-associated mutations are the cause of disorders such as Hutchinson-Gilford progeria syndrome (HGPS) characterized by premature aging or Emery-Dreifuss muscular dystrophy (EDMD) myopathy(191). Although the molecular mechanisms underlying the pathological phenotype in such diseases remain the subject of intensive research, the observation of connections between nuclear lamina alternations and the disruption of chromatin organization(191) provide a promising platform for future studies.

Understanding the principles of the regulated chromatin movements toward nuclear periphery is of a high importance, as it provides a new approach in developing novel therapeutic targets. Such reasoning is particularly relevant in reducing the pathological function of *MYC* by reducing its gating to the nuclear pore. Importantly, the gating of the *MYC* gene has so far been found to exist only in cancer cells. By antagonizing the gating process, we might be able to remove the driver of cancer evolution without adversely affecting the normal and important function of *MYC* in regenerating tissues and hematopoietic cells.

6 CONCLUSIONS

Paper I describes the novel finding that rhythmic chromatin repositioning between active and repressive nuclear compartments facilitates entrainment of circadian transcription. We could thus show that CTCF and PARP1 join forces to regulate the formation of the *H19* ICR chromatin network. This interactome is significantly enriched in inter-chromosomal interactions to connect active circadian loci with repressed LADs at nuclear periphery. Additionally, rhythmic recruitment of clock control genes to the nuclear lamina is facilitated by the entrainment by the serum shock, and it is followed by transcriptional attenuation mediated by the acquisition of repressive H3K9me2 mark in a time-dependent manner.

Paper II demonstrates the dynamic and stochastic nature of the *MYC* chromatin network impinging on flanking enhancers, and its organization with *MYC* likely screening for neighboring interaction partners. This observation goes counter to the dogma that enhancer hubs simultaneously promote transcriptional activation. To find further evidence in favor of this conclusion, we modified the Nodewalk protocol to increase its sensitivity while retaining its ability to quantitatively determine frequencies of chromatin interactions. Our results show that *MYC* indeed seeks out enhancer regions one at a time to suggest the evolution of redundant mechanisms of *MYC* activation in cancer cells to increase their adaptability to a changing environment.

Paper III identifies a single CTCF binding site within the oncogenic colorectal super-enhancer to mediate increased levels of *MYC* expression in cancer cells. We also provide genetic evidence for that this OSE recruits *MYC* to the nuclear pores to thereby facilitate the nuclear export of *MYC* mRNAs. Moreover, we show that the mutation of the single, OSE-specific CTCF-binding site abrogates the WNT-regulated *MYC* gating to identify a new target of this pivotal pathway. Additionally, we identified PARP1 as an essential partner of CTCF in the functional gating of *MYC*.

7 POINTS OF PERSPECTIVE

Future research will attempt to further uncover the mechanisms and order of events regulating circadian chromatin movements between transcriptionally permissive and repressive nuclear sub-compartments and how these relate to the CTCF-PARP1 complex. Particularly interesting are mechanisms which cause the release of circadian loci from the nuclear periphery and the subsequent reversal of the repressive chromatin mark(s) when intra-nucleoplasmic, as well as the link between CTCF-PARP1 and the core clock machinery. Additionally, further examination of principles underlying the novel principle of *MYC* gene gating in cancer cells might show how the cancer cells have exploited the clock machinery to hijack the gating process in order to increase their fitness. It will also be important to go beyond model systems and show these principles in action in patient materials. By extrapolation, it will be very interesting to examine to what extent circadian rhythm and gene gating are affected in laminopathies. In the end, the most important outcome of such endeavors will be to find new therapeutic strategies based on the dynamics of chromatin transitions in the 3D nuclear architecture.

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9 REFERENCES

1. Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. Vol. 357, Science. American Association for the Advancement of Science; 2017.

2. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet [Internet]. 2016;17(8):487–500. Available from: http://dx.doi.org/10.1038/nrg.2016.59

3. Raj A, Oudenaarden A van. Stochastic gene expression and its consequences. Cell 2. 2008;135(2):216–26.

4. Han R, Huang G, Wang Y, Xu Y, Hu Y, Jiang W, et al. Increased gene expression noise in human cancers is correlated with low p53 and immune activities as well as late stage cancer. Oncotarget. 2016;7(44):72011–20.

5. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. Nature [Internet]. 2019;571(7766):489–99. Available from: http://dx.doi.org/10.1038/s41586-019-1411-0

6. Göndör A. Chromatin Regulation and Dynamics. Vol. 11, London, Elsevier, Academic Press. 2017.

7. Göndör A, Ohlsson R. Chromosome crosstalk in three dimensions. Nature. 2009;461(7261):212–7.

8. Luger K, Dechassa ML, Tremethick DJ. New insights into nucleosome and chromatin structure: An ordered state or a disordered affair? Nat Rev Mol Cell Biol. 2012;13(7):436–47.

9. Bowman GD, Poirier MG. Post-translational modifications of histones that influence nucleosome dynamics. Chem Rev. 2015;115(6):2274–95.

10. Tang F, Yang Z, Tan Y, Li Y. Super-enhancer function and its application in cancer targeted therapy. npj Precis Oncol [Internet]. 2020;4(1):1–7. Available from: http://dx.doi.org/10.1038/s41698-020-0108-z

11. Pott S, Lieb JD. What are super-enhancers? Vol. 47, Nature Genetics. Nature Publishing Group; 2015. p. 8–12.

12. Mannervik M, Nibu Y, Zhang H, Levine M. Transcriptional Coregulators in Development. Science (80-). 1999;284(April):606–10.

13. Holmqvist P, Mannervik M. Genomic occupancy of the transcriptional co- activators p300 and CBP. Transcription. 2013;1264(4:1):18–23.

14. Nguyen MLT, Jones SA, Prier JE, Russ BE. Transcriptional enhancers in the regulation of T cell differentiation. Front Immunol. 2015;6(SEP).

15. Heinz S, Romanoski CE, Benner C, Glass CK. The selection and function of cell type-specific enhancers. Nat Rev Mol Cell Biol. 2015;16(3):144–54.

16. Atlasi Y, Stunnenberg HG. The interplay of epigenetic marks during stem cell differentiation and development. Nat Rev Genet [Internet]. 2017;18(11):643–58.

Available from: http://dx.doi.org/10.1038/nrg.2017.57

17. Göndör A, Ohlsson R. Enhancer functions in three dimensions: Beyond the flat world perspective [version 1; referees: 3 approved]. F1000Research. 2018;7(May):1–11.

18. Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-André V, Sigova AA, et al. Super-enhancers in the control of cell identity and disease. Cell. 2013 Nov 7;155(4):934.

19. The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489(7414):57–74.

20. Ko JY, Oh S, Yoo KH. Functional enhancers as master regulators of Tissue-Specific gene regulation and cancer development. Mol Cells. 2017;40(3):169–77.

21. Wang X, Cairns MJ, Yan J. Super-enhancers in transcriptional regulation and genome organization. Vol. 47, Nucleic Acids Research. Oxford University Press; 2019. p. 11481–96.

22. Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, et al. Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell [Internet]. 2013;153(2):307–19. Available from: http://dx.doi.org/10.1016/j.cell.2013.03.035

23. Jia Q, Chen S, Tan Y, Li Y, Tang F. Oncogenic super-enhancer formation in tumorigenesis and its molecular mechanisms. Exp Mol Med [Internet]. 2020;52(5):713–23. Available from: http://dx.doi.org/10.1038/s12276-020-0428-7

24. Mora A, Sandve GK, Gabrielsen OS, Eskeland R. In the loop: promoter-enhancer interactions and bioinformatics. Brief Bioinform. 2016;17(6):980–95.

25. Snetkova V, Skok JA. Enhancer talk. Epigenomics. 2018;10(4):483–98.

26. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, Van Berkum NL, et al. Mediator and cohesin connect gene expression and chromatin architecture. Nature. 2010;467(7314):430–5.

27. Huang J, Li K, Cai W, Liu X, Zhang Y, Orkin SH, et al. Dissecting super-enhancer hierarchy based on chromatin interactions. Nat Commun. 2018 Dec 1;9(1).

28. Rudebeck SR, Bor D, Ormond A, O'Reilly JX, Lee ACH. A Potential Spatial Working Memory Training Task to Improve Both Episodic Memory and Fluid Intelligence. PLoS One. 2012;7(11):1–9.

29. Matharu N, Ahituv N. Minor Loops in Major Folds: Enhancer–Promoter Looping, Chromatin Restructuring, and Their Association with Transcriptional Regulation and Disease. PLoS Genet. 2015;11(12):1–14.

30. Tokuda N, Sasai M, Chikenji G. Roles of DNA looping in enhancer-blocking activity. Biophys J [Internet]. 2011;100(1):126–34. Available from: http://dx.doi.org/10.1016/j.bpj.2010.11.016

31. Willi M, Yoo KH, Reinisch F, Kuhns TM, Lee HK, Wang C, et al. Facultative CTCF sites moderate mammary superenhancer activity and regulate juxtaposed gene in non-mammary cells. Nat Commun [Internet]. 2017;8(May):1–13. Available from:

http://dx.doi.org/10.1038/ncomms16069

32. Maksimenko OG, Chetverina DA, Georgiev PG. Insulators of higher eukaryotes: Properties, mechanisms of action, and role in transcriptional regulation. Russ J Genet. 2006;42(8):845–57.

33. Yoon YS, Jeong S, Rong Q, Park K-Y, Chung JH, Pfeifer K. Analysis of the H19ICR Insulator. Mol Cell Biol. 2007;27(9):3499–510.

34. Kurukuti S, Tiwari VK, Tavoosidana G, Pugacheva E, Murrell A, Zhao Z, et al. CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. Proc Natl Acad Sci U S A. 2006;103(28):10684–9.

35. Chen Y, Dhupelia A, Schoenherr CJ. The Igf2/H19 imprinting control region exhibits sequence-specific and cell-type-dependent DNA methylation-mediated repression. Nucleic Acids Res. 2009;37(3):793–803.

36. Banerjee S, Smallwood A, Lamond S, Campbell S, Nargund G. Igf2/H19 imprinting control region (ICR): an insulator or a position-dependent silencer? ScientificWorldJournal. 2001;1:218–24.

37. Kim S, Yu NK, Kaang BK. CTCF as a multifunctional protein in genome regulation and gene expression. Exp Mol Med. 2015;47(February):e166.

38. Zhao Z, Tavoosidana G, Sjölinder M, Göndör A, Mariano P, Wang S, et al. Circular chromosome conformation capture (4C) uncovers extensive networks of epigenetically regulated intra- and interchromosomal interactions. Nat Genet. 2006;38(11):1341–7.

39. Cavalli G, Misteli T. Functional implications of genome topology. Nat Struct Mol Biol. 2013;20(3):290–9.

40. Bouwman BAM, De Laat W. Architectural hallmarks of the pluripotent genome. FEBS Lett [Internet]. 2015;589(20):2905–13. Available from: http://dx.doi.org/10.1016/j.febslet.2015.04.055

41. Fortin JP, Hansen KD. Reconstructing A/B compartments as revealed by Hi-C using long-range correlations in epigenetic data. Genome Biol. 2015;16(1):1–23.

42. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell [Internet]. 2014;159(7):1665–80. Available from: http://dx.doi.org/10.1016/j.cell.2014.11.021

43. Lazaris C, Aifantis I, Tsirigos A. On Epigenetic Plasticity and Genome Topology. Trends in Cancer [Internet]. 2020;6(3):177–80. Available from: https://doi.org/10.1016/j.trecan.2020.01.006

44. Gómez-Díaz E, Corces VG. Architectural proteins: Regulators of 3D genome organization in cell fate. Trends Cell Biol. 2014;24(11):703–11.

45. Madakashira BP, Sadler KC. DNA methylation, nuclear organization, and cancer. Front Genet. 2017;8(JUN):1–7.

46. Lochs SJA, Kefalopoulou S, Kind J. Lamina Associated Domains and Gene Regulation in Development and Cancer. Cells. 2019;8(3):271.

47. Ong C-T, Corces VG. CTCF: an architectural protein bridging genome topology and function. Nat Rev Genet [Internet]. 2014 Apr [cited 2014 Jul 10];15(4):234–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24614316

48. van Steense B, Belmont S. A. Lamina-associated domains: links with chromosome architecture, heterochromatin and gene repression. Cell. 2017;169(5):780–91.

49. Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. Nat Genet. 2009;41(2):246–50.

50. Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, Fauth C, et al. Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. PLoS Biol. 2005;3(5):0826–42.

51. Lanctôt C, Cheutin T, Cremer M, Cavalli G, Cremer T. Dynamic genome architecture in the nuclear space: Regulation of gene expression in three dimensions. Nat Rev Genet. 2007;8(2):104–15.

52. Marshall WF, Straight A, Marko JF, Swedlow J, Dernburg A, Belmont A, et al. Interphase chromosomes undergo constrained diffusional motion in living cells. Curr Biol. 1997;7(12):930–9.

53. Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, et al. Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet. 2008;4(3).

54. Soutoglou E, Misteli T. Mobility and immobility of chromatin in transcription and genome stability. Curr Opin Genet Dev. 2007;17(5):435–42.

55. Blobel G. Gene gating: A hypothesis. Proc Natl Acad Sci U S A. 1985;82(24):8527–9.

56. Scholz BA, Sumida N, de Lima CDM, Chachoua I, Martino M, Tzelepis I, et al. WNT signaling and AHCTF1 promote oncogenic MYC expression through super-enhancer-mediated gene gating. Nat Genet [Internet]. 2019;51(12):1723–31. Available from: http://dx.doi.org/10.1038/s41588-019-0535-3

57. Burns LT, Wente SR. From Hypothesis to Mechanism: Uncovering Nuclear Pore Complex Links to Gene Expression. Mol Cell Biol. 2014;34(12):2114–20.

58. Chuang CH, Carpenter AE, Fuchsova B, Johnson T, de Lanerolle P, Belmont AS. Long-Range Directional Movement of an Interphase Chromosome Site. Curr Biol. 2006;16(8):825–31.

59. Gordon MR, Pope BD, Sima J, Gilbert DM. Many paths lead chromatin to the nuclear periphery. BioEssays. 2015;37(8):862–6.

60. Misteli T. Concepts in nuclear architecture. BioEssays 275 [Internet]. 2005;27:477–87. Available from: http://www.cs.bham.ac.uk/~jxb/NN/l16.pdf

61. Raj A, van Oudenaarden A. Nature, Nurture, or Chance: Stochastic Gene Expression

and Its Consequences. Cell. 2008;135(2):216–26.

62. Müller HP, Sogo JM, Schaffner W. An enhancer stimulates transcription in Trans when attached to the promoter via a protein bridge. Cell. 1989;58(4):767–77.

63. Rieder D, Trajanoski Z, McNally JG. Transcription factories. Front Genet. 2012;3(OCT):1–12.

64. Lenchenko A, Nemenman I. Cellular noise and information transmission. Curr Opin Biotechnol. 2014;August(28):156–64.

65. Ohlsson R. Widespread Monoallelic Expression. Science (80-). 2007;318(November):1077–8.

66. Arzate-Mejía RG, Recillas-Targa F, Corces VG. Developing in 3D: the role of CTCF in cell differentiation. Development. 2018;145(6).

67. Ohlsson R, Renkawitz R, Lobanenkov V. CTCF is a uniquely versatile transcription regulator linked to epigenetics and disease. Trends Genet. 2001;17(9):520–7.

68. Ohlsson R, Lobanenkov V, Klenova E. Does CTCF mediate between nuclear organization and gene expression? BioEssays. 2010;32:37–50.

69. Lobanenkov V V., Nicolas RH, Adler V V., Paterson H, Klenova EM, Polotskaja A V., et al. A novel sequence-specific DNA binding protein which interacts with three regularly spaced direct repeats of the CCCTC-motif in the 5'-flanking sequence of the chicken c-myc gene. Oncogene. 1990;5(12):1743–53.

70. Pant V, Kurukuti S, Pugacheva E, Shamsuddin S, Mariano P, Renkawitz R, et al. Mutation of a Single CTCF Target Site within the H19 Imprinting Control Region Leads to Loss of Igf2 Imprinting and Complex Patterns of De Novo Methylation upon Maternal Inheritance. Mol Cell Biol. 2004;24(8):3497–504.

71. Guibert S, Zhao Z, Sjölinder M, Göndör A, Fernandez A, Pant V, et al. CTCF-binding sites within the H19 ICR differentially regulate local chromatin structures and cis-acting functions. Epigenetics. 2012;7(4):361–9.

72. Kanduri C, Pant V, Loukinov D, Pugacheva E, Qi CF, Wolffe A, et al. Functional association of CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. Curr Biol. 2000;10(14):853–6.

73. Marina RJ, Sturgill D, Bailly MA, Thenoz M, Varma G, Prigge MF, et al. TET-catalyzed oxidation of intragenic alternative splicing. EMBO J. 2016;35(3):335–55.

74. Han D, Chen Q, Shi J, Zhang F, Yu X. CTCF participates in DNA damage response via poly(ADP-ribosyl)ation. Sci Rep. 2017;7(January):1–10.

75. Hu J, Zhang Y, Zhao L, Frock RL, Du Z, Meyers RM, et al. Chromosomal Loop Domains Direct the Recombination of Antigen Receptor Genes. Cell. 2015;163(4):947–59.

76. Zuin J, Dixon JR, Van Der Reijden MIJA, Ye Z, Kolovos P, Brouwer RWW, et al. Cohesin and CTCF differentially affect chromatin architecture and gene expression in human cells. Proc Natl Acad Sci U S A. 2014;111(3):996–1001.

77. Hansen AS, Pustova I, Cattoglio C, Tjian R, Darzacq X. CTCF and cohesin regulate chromatin loop stability with distinct dynamics. Elife. 2017;6:1–33.

78. Pugacheva EM, Kubo N, Loukinov D, Tajmul M, Kang S, Kovalchuk AL, et al. CTCF mediates chromatin looping via N-terminal domain-dependent cohesin retention. Proc Natl Acad Sci U S A. 2020;117(4):2020–31.

79. Ohlsson R, Bartkuhn M, Renkawitz R. CTCF shapes chromatin by multiple mechanisms: The impact of 20 years of CTCF research on understanding the workings of chromatin. Chromosoma. 2010;119(4):351–60.

80. Göndör A, Ohlsson R. Chromatin insulators and cohesins. EMBO Rep. 2008;9(4):327–9.

81. Dorsett D, Ström L. The Ancient and Evolving Roles of Cohesin in Gene Expression and DNA Repair. Curr Biol 22. 2012;April 20:R240–50.

82. Ciccarone F, Zampieri M, Caiafa P. PARP1 orchestrates epigenetic events setting up chromatin domains. Semin Cell Dev Biol [Internet]. 2017;63:123–34. Available from: http://dx.doi.org/10.1016/j.semcdb.2016.11.010

83. Benabdallah NS, Williamson I, Illingworth RS, Boyle S, Grimes GR, Therizols P, et al. PARP mediated chromatin unfolding is coupled to long-range enhancer activation. bioRxiv. 2017;

84. Lupey-Green LN, Caruso LB, Madzo J, Martin KA, Tan Y, Hulse M, et al. PARP1 Stabilizes CTCF Binding and Chromatin Structure To Maintain Epstein-Barr Virus Latency Type. J Virol. 2018;92(18):1–15.

85. Pavlaki I, Docquier F, Chernukhin I, Kita G, Gretton S, Clarkson CT, et al. Poly(ADP-ribosyl)ation associated changes in CTCF-chromatin binding and gene expression in breast cells. bioRxiv. 2017;44(0):1–26.

86. Farrar D, Rai S, Chernukhin I, Jagodic M, Ito Y, Yammine S, et al. Mutational Analysis of the Poly(ADP-Ribosyl)ation Sites of the Transcription Factor CTCF Provides an Insight into the Mechanism of Its Regulation by Poly(ADP-Ribosyl)ation. Mol Cell Biol. 2010;30(5):1199–216.

87. Yu W, Ginjala V, Pant V, Chernukhin I, Whitehead J, Docquier F, et al. Poly(ADP-ribosyl)ation regulates CTCF-dependent chromatin insulation. Nat Genet. 2004;36(10):1105–10.

88. Klenova E, Ohlsson R. Poly(ADP-ribosyl)ation and epigenetics: Is CTCF PARt of the plot? Cell Cycle. 2005;4(1):96–101.

89. de Wit E, de Laat W. A decade of 3C technologies: Insights into nuclear organization. Genes Dev. 2012;26(1):11–24.

90. Dekker J, Rippe K, Dekker M, Kleckner N. Capturing chromosome conformation. Methods Mol Biol. 2002;295(15 February):1306–11.

91. Sati S, Cavalli G. Chromosome conformation capture technologies and their impact in understanding genome function. Chromosoma [Internet]. 2017;126(1):33–44. Available from: http://dx.doi.org/10.1007/s00412-016-0593-6

92. Dekker J, Marti-Renom MA, Mirny LA. Exploring the three-dimensional organization of genomes: Interpreting chromatin interaction data. Nat Rev Genet. 2013;14(6):390–403.

93. Göndör A, Rougier C, Ohlsson R. High-resolution circular chromosome conformation capture assay. Nat Protoc. 2008;3(2):303–13.

94. Ohlsson R, Göndör A. The 4C technique: the “Rosetta stone” for genome biology in 3D? Curr Opin Cell Biol. 2007;19(3):321–5.

95. Dostie J, Richmond TA, Arnaout RA, Selzer RR, Lee WL, Honan TA, et al. Chromosome Conformation Capture Carbon Copy (5C): A massively parallel solution for mapping interactions between genomic elements. Genome Res. 2006;16(10):1299–309.

96. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, et al. Comprehensive mapping of long range interactions reveals folding principles of the human genome. Science (80-). 2009;326(5950):289–93.

97. Risca VI, Greenleaf WJ. Unraveling the 3D genome: Genomics tools for multiscale exploration. Trends Genet [Internet]. 2015;31(7):357–72. Available from: http://dx.doi.org/10.1016/j.tig.2015.03.010

98. Nagano T, Lubling Y, Stevens TJ, Schoenfelder S, Yaffe E, Dean W, et al. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature. 2013;502(7469):59–64.

99. Chen X, Shi C, Yammine S, Göndör A, Rönnlund D, Fernandez-Woodbridge A, et al. Chromatin in situ proximity (ChrISP): Single-cell analysis of chromatin proximities at a high resolution. Biotechniques. 2014;56(3):117–24.

100. Chen X, Yammine S, Shi C, Tark-Dame M, Göndör A, Ohlsson R. The visualization of large organized chromatin domains enriched in the H3K9me2 mark within a single chromosome in a single cell. Epigenetics. 2014;9(11):1439–45.

101. Sumida N, Sifakis EG, Scholz BA, Fernandez-Woodbridge A, Kiani NA, Gomez-Cabrero D, et al. The ultra-sensitive Nodewalk technique identifies stochastic from virtual, population-based enhancer hubs regulating MYC in 3D: Implications for the fitness of cancer cells. bioRxiv. 2018;

102. Bellet MM, Sassone-Corsi P. Mammalian circadian clock and metabolism - The epigenetic link. J Cell Sci. 2010;123:3837–48.

103. Andreani TS, Itoh TQ, Yildirim E, Hwangbo D-S, Allada R. Genetics of Circadian Rhythms. Sleep Med Clin. 2015;10(4):413–21.

104. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. Vol. 72, Annual Review of Physiology. 2009. 517–549 p.

105. Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, et al. PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A.

2004;101(15):5339–46.

106. Ye R, Selby CP, Chiou YY, Ozkan-Dagliyan I, Gaddameedhi S, Sancar A. Dual modes of CLOCK:BMAL1 inhibition mediated by Cryptochrome and period proteins in the mammalian circadian clock. Genes Dev. 2014;28(18):1989–98.

107. Xie Y, Tang Q, Chen G, Xie M, Yu S, Zhao J, et al. New insights into the circadian rhythm and its related diseases. Front Physiol. 2019;10(JUN):1–19.

108. Dierickx P, Laake LW Van, Geijsen N. Circadian clocks : from stem cells to tissue homeostasis and regeneration. EMBO Rep. 2017;1–11.

109. Golombek DA, Rosenstein RE. Physiology of circadian entrainment. Physiol Rev. 2010;90(3):1063–102.

110. Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. Annu Rev Neurosci. 2012;35:445–62.

111. Stephan FK, Swann JM, Sisk CL. Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. Behav Neural Biol. 1979;25(4):545–54.

112. Lamia KA, Sachdeva UM, Di Tacchio L, Williams EC, Alvarez JG, Egan DF, et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science (80-). 2009;326(5951):437–40.

113. Hirota T, Okano T, Kokame K, Shirotani-Ikejima H, Miyata T, Fukada Y. Glucose down-regulates Per1 and Per2 mRNA levels and induces circadian gene expression in cultured rat-1 fibroblasts. J Biol Chem. 2002;277(46):44244–51.

114. Mistlberger RE, Skene DJ. Social influences on mammalian circadian rhythms: Animal and human studies. Biol Rev Camb Philos Soc. 2004;79(3):533–56.

115. Balsalobre a, Damiola F, Schibler U. A serum shock induces circadian gene expression in mammalian tissue culture cells. Cell [Internet]. 1998 Jun 12;93(6):929–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9635423

116. Balsalobre A, Marcacci L, Schibler U. Multiple signaling pathways elicit circadian gene expression in cultured Rat-1 fibroblasts. Curr Biol. 2000;10(20):1291–4.

117. Yagita K, Okamura H. Forskolin induces circadian gene expression of rPer1, rPer2 and dbp in mammalian rat-1 fibroblasts. FEBS Lett. 2000;465(1):79–82.

118. Levi F, Schibler U. Circadian Rhythms: Mechanisms and Therapeutic Implications. Annu Rev Pharmacol Toxicol. 2007;47:593–628.

119. Gachon F, Nagoshi E, Brown SA, Ripperger J, Schibler U. The mammalian circadian timing sytem: From gene expression to physiology. Chromosoma. 2004;113(3):103–12.

120. Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, et al. Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. Science (80-). 2000;290(5499):2110–3.

121. Berman-Frank I, Lundgren P, Chen YB, Küpper H, Kolber Z, Bergman B, et al.

Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium Trichodesmium. Science (80-). 2001;294(5546):1534–7.

122. Weger M, Diotel N, Dorsemans A-C, Dickmeis T, Weger BD. Stem cells and the circadian clock. Dev Biol. 2017;431:111–23.

123. Janich P, Toufighi K, Solanas G, Luis NM, Minkwitz S, Serrano L, et al. Human epidermal stem cell function is regulated by circadian oscillations. Cell Stem Cell [Internet]. 2013;13(6):745–53. Available from: http://dx.doi.org/10.1016/j.stem.2013.09.004

124. Janich P, Pascual G, Merlos-Suarez A, Batlle E, Ripperger JA, Albrecht U, et al. The circadian molecular clock creates epidermal stem cell heterogeneity. Nature. 2011;480(8 December):209–16.

125. Brown SA. Circadian clock-mediated control of stem cell division and differentiation: beyond night and day. Co Biol Ltd. 2014;141:3105–11.

126. Kang TH, Reardon JT, Kemp M, Sancar A. Orcadian oscillation of nucleotide excision repair in mammalian brain. Proc Natl Acad Sci U S A. 2009;106(8):2864–7.

127. Brainard J, Gobel M, Scott B, Koeppen M, Eckle T. Health implications of disrupted circadian rhythms and the potential for daylight as therapy. Anesthesiology. 2015;122(5):1170–5.

128. Crosio C, Cermakian N, Allis CD, Sassone-Corsi P. Light induces chromatin modification in cells of the mammalian circadian clock. Nat Neurosci. 2000;3(12):1241–7.

129. Aguilar-Arnal L, Sassone-Corsi P. Chromatin landscape and circadian dynamics: Spatial and temporal organization of clock transcription. Proc Natl Acad Sci U S A. 2015;112(22):6863–70.

130. Koike N, Yoo SH, Huang HC, Kumar V, Lee C, Kim TK, et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science (80-). 2012;338(6105):349–54.

131. Pacheco-Bernal I, Becerril-Pérez F, Aguilar-Arnal L. Circadian rhythms in the three-dimensional genome: Implications of chromatin interactions for cyclic transcription. Clin Epigenetics. 2019;11(1):1–13.

132. Zhu Q, Belden WJ. Molecular Regulation of Circadian Chromatin. J Mol Biol [Internet]. 2020;432(12):3466–82. Available from: https://doi.org/10.1016/j.jmb.2020.01.009

133. Partch CL, Green CB, Takahashi JS. Molecular Architecture of the Mammalian Circadian Clock. Trends Cell Biol. 2014;24(2):90–9.

134. Feinberg AP, Koldobskiy MA, Göndör A. Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. Nat Rev Genet. 2016;17(5):284–99.

135. Salvador ML, Klein U, Bogorad L. Endogenous Fluctuations of DNA Topology in the Chloroplast of Chlamydomonas reinhardtii. Mol Cell Biol. 1998;18(12):7235–42.

136. Ripperger JA, Schibler U. Rhythmic CLOCK-BMAL1 binding to multiple E-box motifs drives circadian Dbp transcription and chromatin transitions. Nat Genet. 2006;38(3):369–74.

137. Aguilar-Arnal L, Hakim O, Patel VR, Baldi P, Hager GL, Sassone-Corsi P. Cycles in spatial and temporal chromosomal organization driven by the circadian clock. Nat Struct Mol Biol. 2013;20(10):1206–15.

138. Devalaraja-Narashimha K, Padanilam BJ. PARP1 deficiency exacerbates diet-induced obesity in mice. J Endocrinol. 2010;205(3):243–52.

139. Vida A, Márton J, Mikó E, Bai P. Metabolic roles of poly(ADP-ribose) polymerases. Semin Cell Dev Biol [Internet]. 2017;63:135–43. Available from: http://dx.doi.org/10.1016/j.semcdb.2016.12.009

140. Kumar V, Takahashi JS. PARP around the Clock. Cell. 2010;142(17):841–3.

141. Asher G, Reinke H, Altmeyer M, Gutierrez-Arcelus M, Hottiger MO, Schibler U. Poly(ADP-Ribose) Polymerase 1 Participates in the Phase Entrainment of Circadian Clocks to Feeding. Cell [Internet]. 2010;142(6):943–53. Available from: http://dx.doi.org/10.1016/j.cell.2010.08.016

142. Trott AJ, Menet JS. Regulation of circadian clock transcriptional output by CLOCK:BMAL1. Vol. 14, PLoS Genetics. 2018. 1–34 p.

143. Zhao H, Sifakis EG, Sumida N, Millán-Ariño L, Scholz BA, Svensson JP, et al. PARP1- and CTCF-Mediated Interactions between Active and Repressed Chromatin at the Lamina Promote Oscillating Transcription. Mol Cell. 2015;59(6):984–97.

144. Ha NH, Long J, Cai Q, Shu XO, Hunter KW. The Circadian Rhythm Gene Arntl2 Is a Metastasis Susceptibility Gene for Estrogen Receptor-Negative Breast Cancer. PLoS Genet. 2016;12(9):1–20.

145. Feng CM, Qiu Y, Van Buskirk EK, Yang EJ, Chen M. Light-regulated gene repositioning in Arabidopsis. Nat Commun. 2014;5:3027.

146. Weidemann BJ, Ramsey KM, Bass J. A day in the life of chromatin: How enhancer–promoter loops shape daily behavior. Genes Dev. 2018;32(5–6):321–3.

147. Kim YH, Marhon SA, Zhang Y, Steger DJ, Won KJ, Lazar MA. Rev-erba dynamically modulates chromatin looping to control circadian gene transcription. Science (80-). 2018;359(6381):1274–7.

148. Mermet J, Yeung J, Hurni C, Mauvoisin D, Gustafson K, Jouffe C, et al. Clock-dependent chromatin topology modulates circadian transcription and behavior. Genes Dev. 2018;32(5–6):347–58.

149. Diettrich Mallet de Lima C, Göndör A. Circadian organization of the genome. Science (80-). 2018;359(March 16):1212–3.

150. Kelleher FC, Rao A, Maguire A. Circadian molecular clocks and cancer. Cancer Lett [Internet]. 2014;342(1):9–18. Available from: http://dx.doi.org/10.1016/j.canlet.2013.09.040

151. Imreh MP, Wolbank S, Unger C, Gertow K, Aints A, Szeles A, et al. Culture and expansion of the human embryonic stem cell line HS181, evaluated in a double-color system. Stem Cells Dev. 2004;13(4):337–43.

152. Söderberg O, Gullberg M, Jarvius M, Ridderstråle K, Leuchowius KJ, Jarvius J, et al. Direct observation of individual endogenous protein complexes in situ by proximity ligation. Nat Methods. 2006;3(12):995–1000.

153. Chen X, Shi C, Yammine S, Göndör A, Rönnlund D, Fernandez-Woodbridge A, et al. Chromatin in situ proximity (ChrISP): Single-cell analysis of chromatin proximities at a high resolution. Biotechniques. 2014;56(3):117–24.

154. Sumida N, Sifakis E, Scholz B, Fernandez-Woodbridge A, Kiani N, Gomez-Cabrero D, et al. The ultra-sensitive Nodewalk technique identifies stochastic from virtual, population-based enhancer hubs regulating MYC in 3D: Implications for the fitness of cancer cells. bioRxiv. 2018;286583.

155. Pant V, Mariano P, Kanduri C, Mattsson A, Lobanenkov V, Heuchel R, et al. The nucleotides responsible for the direct physical contact between the chromatin insulator protein CTCF and the H19 imprinting control region manifest parent of origin-specific long-distance insulation and methylation-free domains. Genes Dev. 2003;17(5):586–90.

156. Sandhu KS, Shi C, Sjölinder M, Zhao Z, Göndör A, Liu L, et al. Nonallelic transvection of multiple imprinted loci is organized by the H19 imprinting control region during germline development. Genes Dev. 2009;23(22):2598–603.

157. Bickmore W a, van Steensel B. Genome architecture: domain organization of interphase chromosomes. Cell [Internet]. 2013 Mar 14 [cited 2014 Jul 15];152(6):1270–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23498936

158. Zlatanova J, Caiafa P. CTCF and its protein partners: Divide and rule? J Cell Sci. 2009;122(9):1275–84.

159. Brochu G, Duchaine C, Thibeault L, Lagueux J, Shah GM, Poirier GG. Mode of action of poly(ADP-ribose) glycohydrolase. BBA - Gene Struct Expr. 1994;1219(2):342–50.

160. Bochum S, Berger S, Martens UM. Olaparib. Recent Results Cancer Res. 2018;211:217–33.

161. Asher G, Reinke H, Altmeyer M, Gutierrez-Arcelus M, Hottiger MO, Schibler U. Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. Cell [Internet]. 2010 Sep 17 [cited 2014 Sep 4];142(6):943–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20832105

162. Takahashi HJS. Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet. 2017;18(3):164–79.

163. Kim JY, Kwak PB, Weitz CJ. Specificity in Circadian Clock Feedback from Targeted Reconstitution of the NuRD Corepressor. Mol Cell [Internet]. 2014;56(6):738–48. Available from: http://dx.doi.org/10.1016/j.molcel.2014.10.017

164. Senderowicz AM. Flavopiridol: the first cyclin-dependent kinase inhibitor in human clinical trials. Invest New Drugs. 1999;17(3):313–20141.

165. Rahman Z, Bazaz MR, Devabattula G, Khan MA, Godugu C. Targeting H3K9 methyltransferase G9a and its related molecule GLP as a potential therapeutic strategy for cancer. J Biochem Mol Toxicol. 2020;(October):1–11.

166. Seidman SB. Network structure and minimum degree. Soc Networks. 1983;5(3):269–87.

167. Luo Y, He H, Duan M, Huang H, Hu Z, Wang H. Dynamic Functional Connectivity Strength Within Different Frequency- Band in Schizophrenia. Front Psychiatry. 2020;10(February):1–13.

168. Braccioli L, De Wit E. CTCF: A Swiss-army knife for genome organization and transcription regulation. Essays Biochem. 2019;63(1):157–65.

169. Tian W, Han X, Yan M, Xu Y, Duggineni S, Lin N, et al. Structure-based discovery of a novel inhibitor targeting the β-catenin/Tcf4 interaction. Biochemistry. 2012;51(2):724–31.

170. Hsu PD, Lander ES, Zhang F. Development and Applications of CRISPR-Cas9 for Genome Engineering. Cell. 2014;157(6):1262–78.

171. Reddy KL, Feinberg AP. Higher order chromatin organization in cancer. Semin Cancer Biol [Internet]. 2013;23(2):109–15. Available from: http://dx.doi.org/10.1016/j.semcancer.2012.12.001

172. Buchwalter A, Kaneshiro JM, Hetzer MW. Coaching from the sidelines: the nuclear periphery in genome regulation. Nat Rev Genet [Internet]. 2019;20(1):39–50. Available from: http://dx.doi.org/10.1038/s41576-018-0063-5

173. Thanisch K, Song C, Engelkamp D, Koch J, Wang A, Hallberg E, et al. Nuclear envelope localization of LEMD2 is developmentally dynamic and lamin A / C dependent yet insu ffi cient for heterochromatin tethering. Differentiation. 2017;94(December 2016):58–70.

174. Göndör A, Ohlsson R. Replication timing and epigenetic reprogramming of gene expression: a two-way relationship? Nat Rev Genet [Internet]. 2009 Apr;10(4):269–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19274048

175. Cremer T, Kreth G, Koester H, Fink RHA, Heintzmann R, Cremer M, et al. Chromosome territories, interchromatin domain compartment, and nuclear matrix: An integrated view of the functional nuclear architecture. Crit Rev Eukaryot Gene Expr. 2000;10(2):179–212.

176. Reddy KL, Zullo JM, Bertolino E, Singh H. Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature. 2008;452(7184):243–7.

177. Harr JC, Luperchio TR, Wong X, Cohen E, Wheelan SJ, Reddy KL. Directed targeting of chromatin to the nuclear lamina is mediated by chromatin state and A-type lamins. J Cell Biol. 2015;208(1):33–52.

178. Taddei A. Active genes at the nuclear pore complex. Curr Opin Cell Biol.

2007;19(3):305–10.

179. van Steensel B, Belmont AS. Lamina-associated domains: links with chromosome architecture, heterochromatin and gene repression. Cell. 2017;169(5):780–91.

180. Hoelz DHL and A. The Structure of the Nuclear Pore Complex (An Update) Daniel. Physiol Behav. 2017;176(3):139–48.

181. Becker KG, Barnes KC, Bright TJ, Wang A. The Genetic Association Database. Nat Genet. 2004;36(5):431–2.

182. Rao SSP, Huang SC, Glenn St Hilaire B, Engreitz JM, Perez EM, Kieffer-Kwon KR, et al. Cohesin Loss Eliminates All Loop Domains. Cell [Internet]. 2017;171(2):305-320.e24. Available from: https://doi.org/10.1016/j.cell.2017.09.026

183. Rego A, Sinclair PB, Tao W, Kireev I, Belmont AS. The facultative heterochromatin of the inactive X chromosome has a distinctive condensed ultrastructure. J Cell Sci. 2008;121(7):1119–27.

184. Lottersberger F, Karssemeijer RA, Dimitrova N, De Lange T. 53BP1 and the LINC Complex Promote Microtubule-Dependent DSB Mobility and DNA Repair. Cell [Internet]. 2015;163(4):880–93. Available from: http://dx.doi.org/10.1016/j.cell.2015.09.057

185. Xie X, Almuzzaini B, Drou N, Kremb S, Yousif A, Östlund Farrants A-K, et al. b - Actin – dependent global chromatin organization and gene expression programs control cellular identity. FASEB J. 2018;Mar;32((3)):1296–314.

186. Hoyle NP, Seinkmane E, Putker M, Feeney KA, Toke P, Chesham JE, et al. Circadian actin dynamics drive rhythmic fibroblast mobilisation during wound healing. Sci Transl Med. 2018;9(415).

187. Rudra S, Skibbens R V. Cohesin codes - Interpreting chromatin architecture and the many facets of cohesin function. Vol. 126, Journal of Cell Science. 2013. p. 31–41.

188. Ball AR, Chen YY, Yokomori K. Mechanisms of cohesin-mediated gene regulation and lessons learned from cohesinopathies. Vol. 1839, Biochimica et Biophysica Acta - Gene Regulatory Mechanisms. 2014. p. 191–202.

189. Tanuma S ichi, Shibui Y, Oyama T, Uchiumi F, Abe H. Targeting poly(ADP-ribose) glycohydrolase to draw apoptosis codes in cancer. Biochem Pharmacol [Internet]. 2019;167(April):163–72. Available from: https://doi.org/10.1016/j.bcp.2019.06.004

190. Shiao Li Oei, Ziegler M. ATP for the DNA ligation step in base excision repair is generated from poly(ADP-ribose). J Biol Chem. 2000;275(30):23234–9.

191. Anania C, Lupiáñez DG. Order and disorder: Abnormal 3D chromatin organization in human disease. Brief Funct Genomics. 2020;19(2):128–38.

192. Moore JM, Rabaia NA, Smith LE, Fagerlie S, Gurley K, Loukinov D, et al. Loss of maternal CTCF is associated with peri-implantation lethality of CtCf null embryos. PLoS One. 2012;7(4).

193. Kemp CJ, Moore JM, Moser R, Bernard B, Teater M, Smith LE, et al. CTCF

haploinsufficiency destabilizes DNA methylation and predisposes to cancer. Cell Rep [Internet]. 2014;7(4):1020–9. Available from: http://dx.doi.org/10.1016/j.celrep.2014.04.004

194. Flavahan WA, Drier Y, Liau BB, Gillespie SM, Venteicher AS, Stemmer-Rachamimov AO, et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. Nature. 2016;529(7584):110–4.

195. Filippova GN, Ulmer JE, Moore JM, Ward MD, Hu YJ, Neiman PE, et al. Tumor-associated zinc finger mutations in the CTCF transcription factor selectively alter its DNA-binding specificity. Cancer Res. 2002;62(1):48–52.