Dynamic nature of transcriptional regulation of nuclear receptor target genes in the context of chromatin organization

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Abbreviations: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; ChIP, chromatin immunoprecipitation; CoA, co-activator; CoR, co-repressor; GR, glucocorticoid receptor; HAT, histone acetyltransferase; HDAC, histone deacetylase; INF, interferon; LXR, liver X receptor; MGL, memory gene loop; NR, nuclear receptor; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RE, response element; TF, transcription factor; TSS, transcription start site; VDR, vitamin D receptor

Many members of the nuclear receptor (NR) superfamily are expressed in the skin making them a highly interesting subject of dermato-endocrine research. Natural and synthetic NR ligands are used for the treatment of various skin disorders. We discuss here the impact of the dynamic nature of chromatin organization, i.e., the spatio-temporal changes of chromatin region of NR target genes. This dynamics is triggered by environmental changes, of which for NRs the exposure with their ligands is most critical. For an understanding of skin disorders, which involve the actions of NRs, this means that the parameter time should be carefully considered in context of other factors that may influence the chromatin organization, and by this the responsiveness, of key NR target genes.

Nuclear Receptors in the Skin

Nuclear receptors (NRs) form a structurally-related superfamily of transcription factors (TFs) that are involved in the regulation of nearly all biological processes, of which the control of metabolism, of cellular growth and differentiation and of inflammation, may be the most important.¹ The NR superfamily contains 48 human members, half of which are activated by small lipophilic ligands. These ligands are either classical endocrine hormones, such as cortisol, retinoids and 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] or dietary lipids, such as polyunsaturated fatty acids and xysterols.² This makes NRs interesting pharmacological targets for nearly every human tissue and its diseases.

Many members of the NR superfamily, such as the glucocorticoid receptor (GR), retinoic acid receptor (RAR) γ , vitamin D receptor (VDR), peroxisome-proliferator-activated receptor (PPAR) δ and liver X receptors (LXR) β , are expressed in the different cells of the skin, such as keratinocytes, dermal fibroblasts

and infiltrating immune cells (phagocytes, dendritic cells and T lymphocytes).³⁻⁹ This makes these receptors a highly interesting subjects of dermato-endocrine research and their natural ligands as well as their synthetic derivatives targets for the treatment of various common skin disorders, such as psoriasis [where cortisol and synthetic 1,25(OH)₂D₃ derivatives are very effective] and acne (which is often treated with RAR ligands), of photoaging and chronological aging of the skin and even of skin cancer.⁹ Core processes in there are inflammation and disturbed differentiation, which both can be modulated by NRs and their ligands. Interestingly, skin is the place of the synthesis of vitamin D₃ and poses the enzymatic machinery to locally produce 1,25(OH)₂D₃.^{4,10}

Based on their affinity to ligands the members of the NR superfamily can be sorted into three subgroups: (1) classical endocrine receptors that bind their ligands with a K_d of 1 nM or less and that respond rapidly to hormonal stimulus, (2) adopted orphans that bind dietary lipids and xenobiotics with far lower affinity, i.e., a K_d in the range of μ M to mM¹¹ and (3) orphans that do not have any known, natural ligands and behave like regular TFs. Alternatively, the NR superfamily can be divided to sequence homology into seven classes¹² or based on their dimerization and DNA-binding properties into four subgroups.¹³

NRs regulate gene expression via specific DNA sequences called response elements (REs) that locate within regulatory regions of the target genes and to which NRs bind as homo- or heterodimers.¹⁴ Consequently, most REs are formed by two recognition motifs, whose relative distance and orientation define, which NRs can associate with a given RE. Recently published genome-wide chromatin immunoprecipitation (ChIP)-Seq studies suggest that with a given human tissue the genome contains 2,000 to 10,000 binding sites per NR.^{15,16} However, microarrays from the same tissues in average demonstrated only some 200 to 1,000 genes to be direct target of the given NRs, i.e., there seem to be some ten-fold more genomic NR binding sites than primary target genes.^{16,17} Interestingly, most of these sites do not locate within classical promoter regions, but within introns and

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more distal regions,¹⁸ a characteristic that appears to be common for most NRs.¹⁹ These genome-wide data suggest that in average a given primary NR target gene is regulated by multiple binding sites of the NR at various positions relative to its transcription start site (TSS). This confirms the data obtained from single gene studies, where multiple functional REs per gene were described.²⁰⁻²³ Moreover, the spatial organization of the NR binding sites appears to have a critical role in bringing via DNA looping at least one activated NR protein close to the TSS of the respective primary NR target gene.

Co-Regulator Proteins

For their effective function in gene activation and repression NR proteins have to interact with a number of co-regulatory proteins, which function either as co-activators (CoAs)²⁴ or as co-repressors (CoRs).²⁵ In the absence of ligand NR can form complexes with CoR proteins, which in turn associate with histone deacetylases (HDACs) leading to a local condensation of chromatin.²⁶ The classical model of NR signaling suggests that ligand binding induces a conformational change within the ligand-binding domain of the NR leading to dissociation of CoRs and formation of interaction surface for CoAs.²⁷ Some CoAs have histone acetyltransferase (HAT) activity or they recruit other CoAs possessing such activity. The action of these enzymes modulates the condensation status of chromatin leading to local chromatin relaxation.²⁸ Subsequently, NRs interact with CoAs that are members of the mediator complex. This connects the NR binding chromatin region to the basal transcriptional machinery assembled on the TSS of the target gene.²⁹ Thus, ligand-activated NRs serve as adaptors between the regulatory genomic region and chromatin modifying enzyme complexes as well as activators of RNA polymerase II.

However, the switch between gene repression and activation is more complex than a simple alternative recruitment of two different regulatory complexes.³⁰ Most co-regulators are co-expressed in the same cell type at relatively similar levels, which raises the possibility of their concomitant recruitment to a specific promoter. Moreover, it is presently not clear, whether co-regulators already mark in some cases active regulatory elements independent of DNA-binding TFs or if they always require active recruitment to target sites. Both aspects emphasize that the spatio-temporal context, i.e., the nuclear organization and the timing of the association of TFs and their co-regulators, plays an important role in controlling gene transcription.

Impact of Histone Modifications, Histone Variants and Chromatin Remodeling

In the eukaryotic cell, genomic DNA is by default tightly packed with nucleosome-forming histone proteins, i.e., in a non-accessible repressive status.^{31,32} Therefore, the pre-requisite for the expression of genes within a given genomic region is the local relaxation of these chromatin proteins, in order to allow TFs to associate with their cognate binding sites within promoter and enhancer regions. Mechanisms to modify chromatin structure include

covalent post-translational histone modifications, replacement of the standard histones with histone variants and nucleosome remodeling.^{33,34}

Histone acetylation. Histone acetylation catalyzed by HATs is strongly associated with transcriptional activation.³⁵ For example, a genome-wide analysis of di-acetylated histone H3K9 and H3K14 in resting and activated human T-cells revealed that chromatin accessibility of a genetic locus is increased by hyper-acetylation of a limited number of regulatory elements, i.e., promoters and enhancers.³⁶ Acetylated histone tails can also bind chromatin remodelers that have bromodomain binding modules.^{33,37} Lysine acetylation is assumed to neutralize the positive charges of the N-terminal histone tails thus weakening the histone-DNA interactions.³⁸ Alternatively, the lysine acetylation may cause a conformational change within the nucleosomes that leads to destabilization of DNA-nucleosome arrangement.³⁹

Histone methylation. Histone methylation may have an even more critical role for transcriptional regulation than histone acetylation, since depending on the amino acid residue within the histone tails to be modified, the effect may be either activating or repressive. Since the addition of a relatively small methyl group is not enough to neutralize the positive charge of lysine or arginine residues,⁴⁰ histone methylation rather creates binding sites for regulatory proteins having chromodomain binding modules, which can either condense or relax the chromatin structure.³⁷ The methylations of histones H3K4, H3K36, H3K79, and H2BK5^{41,42} are associated with transcriptional activation, whereas methylations of histones H3K9, H3K27 and H4K20 are related to inactive chromatin.⁴³ Importantly, the N-terminal histone tails can be methylated to multiple sites. The lysine residues may be mono-, di- or tri-methylated and arginine residues mono- or di-methylated⁴¹ and the level of methylation appears to be a specific landmark for different genomic loci. For example, tri-methylated H3K4 is found at the beginning, di-methylated H3K4 in the middle and mono-methylated H3K4 near the 3' ends of actively transcribed genes.⁴⁴ Furthermore, tri-methylated H3K36 is enriched within the coding regions and tri-methylated H3K79 throughout the transcribed regions. Mono-methylated H3K27 is distributed throughout euchromatin, but selectively removed from the start sites of active genes, suggesting that removal of the above histone mark may be a prerequisite for transcription initiation.

Histone variants. All of the four canonical histones have variants, of which H2A.Z and H3.3 are associated with transcription.⁴⁵ H3.3 is related to transcriptionally active genomic loci throughout the cell cycle.⁴⁶ H2A.Z is suggested to have two functions. It maintains the transcription when associated with promoters and, on the other hand, it represses the transcription when associated with the coding regions.^{42,47} The different effects depend on the other histones that form the histone octamer together with H2A.Z. The combination of H2A.Z and H3.3 provides an unusually labile chromatin conformation, whereas H2A.Z and H3 form more stable nucleosomes.⁴⁸ H2A.Z is also highly enriched at insulator sites that separate functional chromatin regions from each other.⁴² The benefit of using histone

variants might lie in a rapid requirement of changing the chromatin environment into a more active or repressed state.

Chromatin remodeling. In addition to the post-translational histone modifications, transcriptional activation often requires remodeling of nucleosomes within the regulatory regions and the TSS of a gene to be activated. This is achieved via function of specific ATP-dependent chromatin remodeling complexes.⁴⁹⁻⁵¹ However, continuous presence of chromatin remodelers is not required for maintenance of nucleosome repositioning.⁵² All eukaryotes have at least five families of chromatin remodelers (SWI/SNF, ISWI, NURD/Mi-2/CHD, INO80 and SWR1), all of which are related to transcriptional regulation among many other functions.³³ The most widely studied family in mammalian cells is SWI/SNF, which contains either BRG1 or BRM as an ATPase subunit along with several BRG-associated factors. Chromatin remodeling by SWI/SNF appears not to be necessary for the activation of primary responding genes.⁵³ Consistently, most of these genes have high basal levels of tri-methylated H3K4 and acetylated H3K9.⁵⁴ Secondary responding genes need to be acetylated before chromatin remodeling, since histone acetylation has been observed to be a pre-requisite for the function of the SWI/SNF complex.⁵² Indeed, SWI/SNF ATPases contain bromodomains that bind to acetylated histone tails.³³ The presence of active chromatin markers at the regulatory regions of SWI/SNF-independent primary response genes is dependent on the GC-content of the DNA sequence.⁵⁵ These CpG islands are associated with a low nucleosome density and are present at 72% of the mammalian promoters.⁵⁶ In addition, GC-rich primary responding genes are characterized by the presence of CoR complexes, such as NCoR/HDAC3 and CoREST/HDAC1, which may counteract the activity of chromatin while in basal state.⁵⁴

Dynamics of Transcriptional Regulation

In higher eukaryotes successful regulation of gene expression in response to environmental cues requires the co-operation of three kinds of regulatory DNA elements: core promoters contain the TSS, proximal regulatory regions and enhancers.⁵⁷ Recently, several studies have suggested that mRNA transcription is a highly dynamic process, in which TFs cyclically associate with regulatory chromatin regions.^{21-23,58} In some cases, the cycling of TFs appears to mirror to the mRNA expression level of regulated genes.

Figure 1. Model of transcriptional cycling. Three different levels of mRNA stability are distinguished (inset on top): stable mRNA (solid line, half-life more than 120 min), medium stable mRNA (dashed line, half-life clearly above 60 min) and labile mRNA (pointed line, half-life clearly below 60 min). This results in either steady accumulation of mRNA levels, stair case-type of accumulation or cyclical accumulation (central graph), although in all three cases cyclical TF binding and chromatin looping can be observed (bottom graph).

To regulate the target gene, the enhancer must physically contact the core promoter by looping out the intervening chromatin.^{59,60} Recent studies have suggested that during transcription the distant enhancers do not simply connect with the promoter via static chromatin looping, but this chromatin looping appears to be a highly dynamic process, in which the loops form and release with the same periodicity as cycling of TFs at the enhancer regions.^{21,22} Thus, chromatin loops form between distant enhancers and TSS regions in average with a periodicity of 40–60 min, suggesting that the looping cannot be a random process, but it must be somehow controlled.

Presently, the exact mechanism behind chromatin looping is not known and needs to be clarified. It has been proposed that TFs associated with enhancers interact directly with protein bound to the TSS or that the TF complex migrates along the chromatin fiber until it reaches the TSS region. Another suggestion is that DNA sequences next to enhancer and promoter regions recruit proteins that are not needed in transcriptional regulation per se, but are responsible for chromatin loop formation.^{61,62} Interestingly, when transcription is initiated, the promoter and the 3'-end of the gene interact to form a looped gene conformation.⁶³ This loop is maintained as a memory gene loop (MGL) for

up to 1 h during intervening periods of transcriptional repression and is required for faster RNA polymerase II recruitment upon re-induction. Consequently, the transcription rate is possibly controlled both by MGL formation and TF cycling. Even inter-chromosomal interactions have been observed. Spilianakis and co-workers reported that the interferon (IFN) γ gene and a multi-gene complex encoding interleukins IL-4, IL-5 and IL-13, which locate on different chromosomes, are in close proximity within naive helper T-cells, but after stimulation either into T_H1 or T_H2 subtypes they move away from each other, so that the genomic region that needs to be activated (IFN γ for T_H1 or IL-4, IL-5 and IL-13 for T_H2) can start gene expression, whereas the region to remain silent is moved to a more repressed region of the nucleus.⁶⁴

There is increasing evidence suggesting that, in addition to above-mentioned regulation, the higher order chromatin hierarchy has a significant impact in gene regulation. Here insulators, i.e., genomic regions that separate genomic DNA into territories, have an important role.^{65,66} According to present understanding, enhancer regions within one chromatin loop cannot interact and regulate genes located within other chromatin loops due to the insulators' enhancer blocking activity. In addition, insulators may represent barrier activity meaning that they can block repressive chromatin effects on the adjacent regions.⁶⁷ The above characteristics of insulators suggest that disappearance of a given insulator may cause defects in gene regulation leading to a serious malfunction of the cell. The TF CTCF and its associated protein cohesin have important roles for insulator properties.⁶⁷ CTCF and cohesin are also involved in the formation of chromatin loops⁶⁵ suggesting that these proteins may have a role in the dynamic chromatin looping that connects the distant enhancers and gene promoters during transcription.

Biological Impact of Transcriptional Cycling

Although cycling of TFs, their co-regulators and of chromatin markers has been observed with a number of NR target genes,^{21-23,58,68} not all of them show cycling at mRNA expression

level. A requirement for mRNA cycling is that, in addition to TF cycling, the transcript has relatively short half-life. If the transcribed mRNA has longer half-life than the periodicity of binding of the TFs, a stepwise or continuous accumulation of mRNA can be observed (Fig. 1). In addition, in order to observe cycling, mRNA transcription within the cell population must be synchronized. This can be achieved either by pre-treatment with the RNA polymerase II inhibitor α -amanitin^{21,58} or, in the case of some NR target genes, simply by treating the cells with their ligand.^{22,69}

The reason for cycling may be that it allows more accurate control of the transcription and limits the duration of activation or repression. This is highly important for genes that need to respond quickly to the changes of the environment, such as many NR target genes to their hormonal stimuli. Thus, transcription of these genes appears to have evaluation points, where the need of further mRNA transcription is considered. In cases when the amount of gene product is not sufficient, the next transcriptional cycle is initiated, but when correct levels have been reached, transcription terminates.

Conclusions

In this short review we discussed the impact of the dynamic nature of chromatin organization, i.e., the spatio-temporal changes of chromatin region of NR target genes. This dynamics is triggered by environmental changes, of which in case of NRs the exposure to its natural and synthetic ligands is the most important. For an understanding of skin disorders that involve the actions of NRs this means that the parameter time should be carefully considered in context of other factors that may influence the chromatin organization (and by this the responsiveness) of key NR target genes.

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