Transcriptional repression: lessons from thyroid hormone action and promyelocytic leukaemia

José V Lopez-Liuchi and Christoph A Meier

Division d’Endocrinologie et Diabétique, Département de Médecine Interne, Hôpital Cantonal Universitaire de Genève, Rue Michelli-du-Crest 24, 1211 Geneva 14, Switzerland

(Correspondence should be addressed to C A Meier)

At first sight, hypothyroidism and acute promyelocytic leukaemia (PML) have little in common. However, recent studies on the mechanisms regulating gene transcription by nuclear hormone receptors (NRs) demonstrate that active repression of gene expression is likely to be an important factor in diseases such as hypothyroidism and PML.

Cellular proliferation, differentiation, and organ development are regulated by modulation of gene transcription. NRs are a family of ligand-dependent transcription factors, comprising the receptors for thyroid hormone, steroids, and retinoids. NR homo- or heterodimers bind to specific DNA binding sites in the upstream region of a regulated target gene and they enhance transcription in response to ligand binding by recruiting and activating the basic transcriptional machinery. In the absence of ligand some NRs, such as the thyroid hormone receptor (TR), actively repress transcription by binding co-repressor molecules such as nuclear co-repressor (N-CoR) and silencing mediator for retinoid and thyroid hormone receptor (SMRT). These proteins associate with TRs in the absence of thyroid hormone, but dissociate in its presence, thereby alleviating gene repression.

However, the precise mechanism of this constitutive repression remained unclear until recent studies further elucidated the importance of histone–DNA contacts as a limiting factor to the access of transcriptional factors to nucleosomal DNA. In a nucleosome, 200 base pairs of double-stranded DNA (dsDNA) wind twice around an octamer of histones (two molecules of H2A, H2B, H3, and H4 proteins) and this core particle is externally associated with histone 1. Nucleosomes then adopt a spiral configuration with six nucleosomes per turn. This conformation plays a role in the condensing of DNA packaging, and it may restrict the access of transcription factors to their DNA binding sites.

Examining the role of chromatin structure as a regulator of gene transcription, Lee et al. (1) had already demonstrated in 1993 the enhanced binding of transcription factors to chromatin after the acetylation of core histones. Wong et al. (2) then devised experiments in which either dsDNA or single-stranded DNA (ssDNA) templates containing a TR response element (TRE) were injected into Xenopus oocyte nuclei. A TRE is a defined DNA motif in a tri-iodothyronine (T3)-regulated gene promoter, which mediates the specific binding of TRs to its sequence. The authors hypothesised that microinjected ssDNA would more efficiently adopt a chromatin-like structure than microinjected dsDNA, since the ssDNA will be replicated in a process that is coupled to nucleosome assembly. Thus, if histones play a role in the repression of transcription, the nuclei containing DNA in a nucleosomal structure would repress transcription of the TRαA promoter more efficiently. Both the templates microinjected as ssDNA or as dsDNA were assembled to some degree into nucleosomes, but further analysis revealed better nucleosome packaging for the microinjected ssDNA. The additional microinjection of unliganded TR more efficiently repressed basal transcriptional activity (>15 fold) in nuclei primarily injected with ssDNA relative to those injected with dsDNA. These findings suggest that replication-coupled chromatin assembly plays an important role in transcriptional repression.

The importance of histones and chromatin structures and the discovery of co-repressors led to the hypothesis that these proteins could interact with and modify nucleosomal structures resulting in transcriptional repression. The mechanism of this interaction was further clarified by Nagy et al. (3), Heinzel et al. (4) and Alland et al. (5) when they reported that N-CoR exists in a complex with two other proteins, Sin3 and RPD3. RPD3 is a histone deacetylase (HDAC) that removes acetyl groups from the core histones. To obtain repression in the presence of unliganded TR, all components of the N-CoR/Sin3/RPD3 complex are essential. Furthermore, HDAC inhibitors (such as trichostatin) reduce transcriptional repression. These findings are complementary to previous results showing that co-activator proteins (which enhance transcription) are histone acetylases. Taken together, these data suggest a model for transcriptional repression where the unliganded TR/N-CoR complex binds to the TRE on the surface of histone octamers. The RPD3 protein is then recruited and deacetylates the histones, thereby increasing transcriptional repression by inducing nucleosomal conformations unfavourable to the assembling of basal transcriptional machinery as a functional complex (see Fig. 1). Upon binding of T3, TRs shed N-CoR and recruit co-activator proteins, which contain histone acetyltransferase activity. From this model it can be inferred
that the clinical manifestations of hypothyroidism are not merely due to a lack of gene expression induced by thyroid hormone, but that it is in fact due to the significant basal repression of target genes by unliganded TRs. One could thus hypothesise that the role of thyroid hormone at physiological doses is not to stimulate transcription of genes, but to ensure that basal transcription is not repressed by the unliganded TR/co-repressor complex. If this were the case, the knocking-out of all TRs in an animal should lead to a normal, rather than hypothyroid, phenotype, a hypothesis that is currently being investigated in several laboratories.

In order to complement these elegant in vitro studies, it was crucial to find an in vivo example of transcriptional repression, allowing assessment of its relevance for tissues and organisms. Acute PML turned out to be an instructive functional model. In this rare disease, altered forms of the retinoic acid receptor (RAR) block myeloid maturation through the active repression of gene transcription (6, 7). Various chromosomal translocations between the RAR locus and the PML or PLZF (for promyelocytic leukaemia zinc finger) gene loci result in fusion proteins (see Fig. 2), leading to acute PML through a mechanism of excessive transcriptional repression. Although the PML and PLZF forms of leukaemia are clinically identical, patients harbouring the PML/RAR translocation respond to all-trans retinoic acid (ATRA) and enter remission, whereas those with the PLZF/RAR fusion are resistant to ATRA. Two recent papers by Lin et al. (6) and Grignani et al. (7) examined the molecular mechanisms of transcriptional repression in PML/RAR and PLZF/RAR patients. Unliganded RAR was known for some time to repress transcription by binding SMRT or N-CoR. Both mutant chimaerical receptors (PML/RARa and PLZF/RARa) are able to bind co-repressors, thus blocking myeloid differentiation. Both fusion proteins also bind, and probably recruit, HDAC1 in vitro. Upon adjunction of ATRA, PML–RARa dissociates from HDAC1, whereas ATRA does not alter the binding of PLZF/RARa to HDAC1. This was now shown to be due to a second co-repressor binding site in the amino terminus of PLZF–RARa. Hence, a functional correlation exists between the capacity of ATRA to

Figure 1 In the absence of T₃, TR is bound by NcoR and the Sin3/RPD3 complex that deacetylates histones inducing a compact DNA conformation unfavourable to transcription. When bound by T₃, TR releases NcoR and binds a co-activator (histone acetylase) and enhances gene transcription.
induce remission and its capability to dissociate co-repressors from the respective abnormal fusion proteins. To clarify the contribution of histone deacetylation further, a specific inhibitor of deacetylases (trichostatin A), was used on cells. Trichostatin A greatly enhanced the differentiation effect of ATRA on cells harbouring the PML–RARα fusion. Moreover, trichostatin A was also able to restore nearly the full effectiveness of ATRA in cells overexpressing the PLZF–RARα fusion. These two papers nicely illustrate the (patho)physiological importance of nuclear co-factors in the oncogenesis of PML and, as suggested by the authors, pharmacological manipulation of these factors might provide novel therapeutical approaches to certain diseases.

The complex system of co-activators and co-repressors, which are highly conserved from yeast to human, is unlikely to represent an oddity of nature and such a refined system comprising multiple interacting proteins is quite likely to play fundamental roles in the regulation of cellular growth and differentiation. This accounts for the great interest in the field of transcriptional co-factors, which will certainly result in further studies unravelling the physiological roles of the co-repressor/co-activator systems, e.g. by employing conditional and tissue specific gene knock-out animal technologies.

Figure 2 The chimaeras resulting from translocations between chromosomes 17 and 15 (t(15:17)), and 17 and 11 (t(11:17)). Each has a 3’ co-repressor binding site from chromosome 17 (vertical stripes). The PLZF–RARα chimaera has a second co-repressor binding site, from chromosome 11, resistant to ATRA-mediated receptor/co-repressor dissociation.

References

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