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Pulling rank with RNA: RANKL promotes the association of PGC-1β/RNA complexes with NCoR/HDAC3 to activate gene expression in osteoclasts

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In this issue, Abe et al[1] report a novel mechanism by which RANKL stimulates osteoclast differentiation and bone resorption through non-coding RNAs that bind PGC-1β and convert the NCoR/HDAC3 co-repressor complex into a co-activator of AP-1- and NFκB-regulated genes.

The ability of cells to regulate the expression of specific genes at the right time and place is essential for developmental and physiological processes throughout life. Approximately 1,650 DNA-binding transcription factors are encoded in the human genome[2] and control the specificity of gene expression in different cell types. Transcription factors provide a platform for macromolecular protein complexes (which do not bind DNA directly) to assemble and alter chromatin structure and RNA polymerase activity. Up to 2,500 chromatin-associated proteins are present in the human genome to fine-tune transcription factor activity and gene expression in response to a vast array of environmental cues.[3] A question that has intrigued molecular biologists for decades is how a transcription factor or combination of transcription factors can differentially assemble activator or repressor complexes in response to various stimuli. Nuclear hormone receptors provide a relatively simple paradigm where a hormone ligand induces conformational changes in the transcription factor to displace nuclear receptor co-repressor (NCoR)-histone deacetylase (HDAC) complexes and promote the association of co-activators and histone acetyltransferases (HATs).[4] Other molecular switches involving co-repressor displacement from ligand-independent transcription factors have been described, such as for the Notch-regulated transcription factor Rbpj[5] and signal-dependent phosphorylation of c-Jun, part of the AP-1 transcription factor family.[6] In this issue, Abe et al.[1] describe a new mechanistic switch in myeloid cells involving non-coding RNAs (e.g., *Dancr*, *Rnu12*) and PGC-1β that do not displace NCoR/HDAC3 co-repressors from gene regulatory regions, but rather subvert them into a co-activator/HAT complex in response to RANKL stimulation (Figure 1A).

Osteoclasts are specialized multinucleated cells that resorb both the organic and inorganic components of bones to regulate calcium homeostasis, bone health, and other physiological processes. Increased osteoclast activity is associated with bone loss, frailty, and osteoporotic fractures, while reductions in osteoclast function may cause bones to become dense, brittle, and fracture prone. The combination of M-CSF (monocyte-colony stimulating factor) and RANKL (receptor activator of nuclear factor kappa-B ligand) induces the expression of genes regulated by NFκB, AP-1, and other transcription factors[7] in myeloid progenitor cells and promotes their differentiation into osteoclasts. In this report, Abe et al. demonstrate that NCoR is required for RANKL-stimulated formation of multi-nucleated osteoclasts *in vitro*. They also show that conditional deletion of NCoR, or NCoR and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor; aka NCoR2) in myeloid progenitors and other LysM-Cre expressing cells suppresses bone resorption and causes a high-bone-mass phenotype in adult mice (Figure 1B). This is consistent with the report that HDAC3 deletion in LysM-Cre positive cells blocks RANKL-induced activation of NFκB, the formation of multi-nucleated osteoclasts, and cortical bone healing.[8] Thus, NCoR/HDAC3 complexes are necessary for proper osteoclast differentiation.

NCoR and SMRT are well-studied co-repressors that bind transcription factors and recruit HDAC3, which removes acetyl groups from lysines in histones and other proteins.[4] Displacement of this macromolecular complex from chromatin is often observed in genes that are derepressed in response to extracellular stimuli. However, Abe and colleagues found that NCoR and HDAC3 were paradoxically present at H3K27ac-enriched chromatin regions after RANKL exposure and were required for the expression of RANKL-induced genes.[1] Binding sites for AP-1, NFκB-p65, and PU.1 were common in the NCOR/HDAC3-regulated genes. The authors hypothesized that a PGC-1 (peroxisome proliferator-activated receptor-gamma coactivator-1) protein may be involved in these events because HDAC3 was previously linked to PGC-1α-dependent gene activation in brown adipose tissue.[9] PGC-1 family members are master regulators of mitochondrial biogenesis and cellular metabolism. Abe et al. find that osteoclasts express PGC-1β, not PGC-1α, and confirm previously reported data showing that PGC-1β expression increases during RANKL-induced osteoclastogenesis.[10] PGC-1β was found in macromolecular complexes (~2MDa in size) containing NCoR and HDAC3 and co-located with NCoR, HDAC3, and H3K27ac at RANKL-upregulated genes with binding sites for AP-1 and NFκB-p65 transcription factors. These PGC-1β complexes contained histone acetyl transferase activity, but a specific HAT was not

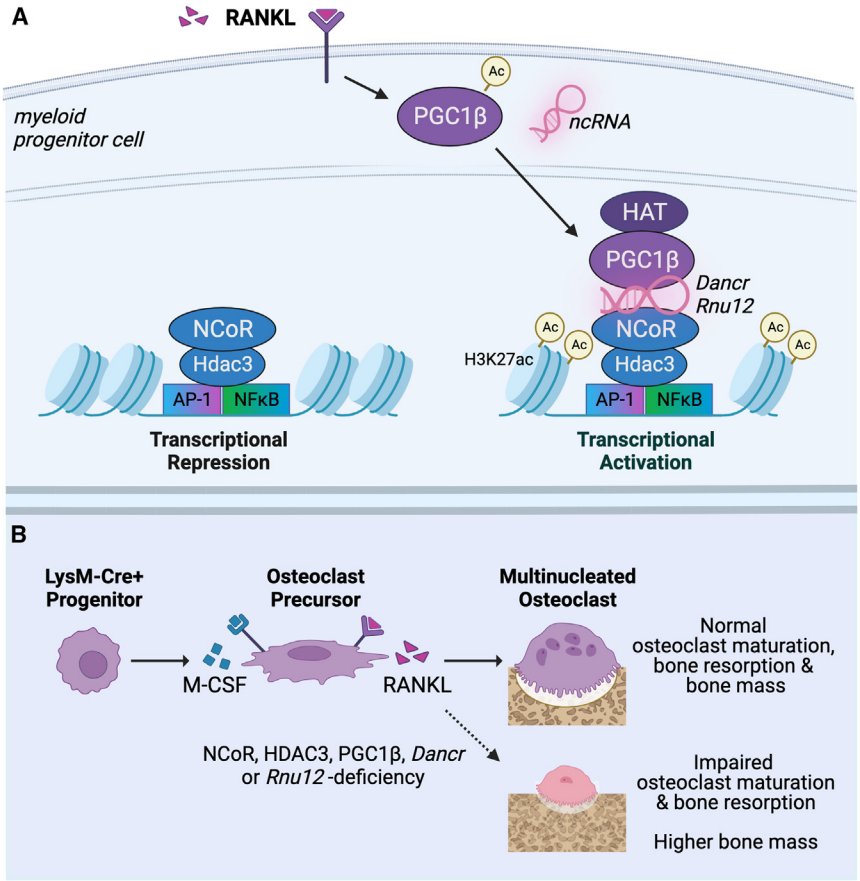

Figure 1. RANKL promotes the association of PGC-1β and non-coding RNAs with NCoR/HDAC3 to activate gene expression and induce osteoclast maturation

(A) NCoR/HDAC3 co-repressor complexes associate with NFκB and AP-1 transcription factors in myeloid cells within the bone marrow. RANKL induces PGC-1β expression and its association with non-coding RNAs, including *Dancr* and *Rnu12*, via its RRM domain. This complex then associates with NCoR/HDAC3. HDAC3 deacetylates PGC-1β, allowing for HAT recruitment and transcription of NFκB- and AP-1-regulated genes.

(B) M-CSF and RANKL induce the differentiation of myeloid progenitor cells into multinucleated osteoclasts that resorb bone and regulate bone homeostasis. Osteoclast differentiation is impeded by the inactivation or deletion of any component of the NCoR/HDAC3/PGC-1β/*Dancr*/*Rnu12* complex. The figure was created with BioRender.

identified. Depletion of PGC-1β attenuated the expression of RANKL-induced and NCoR/HDAC3-dependent genes in bone marrow cells. Interestingly, PGC-1β switched the substrate specificity of NCoR/HDAC3 complexes from H3K27 to PGC-1β itself in a signal-dependent fashion. Consistent with phenotypes of NCoR and HDAC3 depleted mice, PGC-1β knockout mice have increased bone mass and impaired RANKL-induced osteoclastogenesis.[10]

As interesting as these results are, it is not the end of this story. PGC1 proteins contain a conserved RNA-recognition motif (RRM) in their C termini. The authors found that removal of the RRM domain from PGC-1β or RNase treatment reduced RANKL-induced interactions of PGC-1β with NCoR/HDAC3, suggesting that RNAs are key to the transformation of NCoR/HDAC3 complexes from repressors to activators of gene transcription. Several non-coding RNAs were identified in PGC-1β-binding molecules. *Dancr* and *Rnu12* piqued the interest of the authors because they had previously been linked to low bone mass and elevated osteoclast activity in humans. Abe and colleagues nicely show that *Dancr* and *Rnu12* facilitate the association of PGC-1β with NCoR/HDAC3 and that deletion of both ncRNAs substantially reduces the formation of this complex on AP1/NFκB-dependent enhancers in response to RANKL. Deletion of *Dancr* and *Rnu12* also impairs RANKL-induced bone resorption activity of osteoclasts. These results suggest that *Dancr* and *Rnu12* are crucial mediators of RANKL induced differentiation of myeloid cells to osteoclasts.

The novel mechanisms revealed in this study should launch many new avenues of investigation. A broad question is how frequently PGC1/RNA complexes switch NCoR/HDAC3 from transcriptional co-repressors to co-activators in response to signals that regulate cellular growth and differentiation. RANKL is most likely stimulating RANK-dependent signaling in monocyte precursors to induce PGC-1β expression, and it will be of interest to determine how intermediate events in the signaling pathway occur and vary in different cell types that respond to RANKL. For bone biologists, this opens new avenues of research as to how RANKL and non-coding RNAs contribute to osteoclast formation and whether these pathways can be manipulated to treat diseases associated with high bone resorption.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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How the circadian nuclear orphan receptor REV-ERBα represses transcription: Temporal and spatial phase separation combined

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In this issue of *Molecular Cell*, Zhu et al.[1] demonstrate that REV-ERBα and its co-repressor NCOR1 are assembled into daytime-dependent liquid droplets that constitute hubs in which the transcription of multiple REV-ERBα target genes is simultaneously repressed.

The mammalian circadian timing system orchestrates daily oscillations of virtually all bodily processes. It is organized in a hierarchical fashion, in that a master pacemaker located in the brain's suprachiasmatic nucleus (SCN) controls biological rhythms in the entire body, partly by synchronizing slave oscillators operative in nearly all peripheral cell types.[2] Circadian clocks function in a self-sustained and cell-autonomous fashion, and their molecular makeup is virtually identical in all cell types, including SCN neurons. The molecular clock circuitry is composed of two interconnected negative feedback loops in clock gene expression. In the primary feedback loop, the transcriptional activators CLOCK (or paralog NPAS2) and BMAL1 activate the transcription of genes encoding Period (PER1,2,3) and Cryptochrome (CRY1,2) proteins, their own repressors. The robustness of the primary feedback loop is increased by a secondary negative feedback loop, which involves the antagonistic action of ROR (RORα,β,γ) and REV-ERB (REV-ERBα,β) nuclear orphan receptors on cis-acting regulatory elements in the promoters of *Clock*, *Npas2*, and *Bmal1*. The rhythmic expression of REV-ERBα and REV-ERBβ is driven by the primary feedback loop, which tightly couples the two negative feedback loops. Beyond their roles as core clock transcription factors, REV-ERBα and REV-ERBβ control the transcriptional ups and downs of hundreds of genes in synergy with other transcription factors. In the liver many of them participate in lipid and bile acid homeostasis.[3,4] In this issue of *Molecular Cell*, Zhu et al.[1] present compelling evidence for the coalescence of REV-ERBα and its corepressor NCOR1 into liquid droplets in nuclei of hepatocytes and cultured cells. Importantly, a REV-ERBα version devoid of the peptide region responsible for the punctae formation inhibits the transcription of target genes less efficiently than its wild-type counterpart. Hence, phase separation appears to be required for the efficient repression of REV-ERBα target genes.

Eukaryotic transcription factors (TFs) are typically composed of linearly arranged functional domains. At the very least, TFs harbor a DNA-binding domain, a nuclear translocation signal, and an "effector domain." For transcriptional activators and repressors the effector domains consist of peptide regions recruiting co-activator and co-repressor complexes, respectively. While all known DNA-binding domains assume specific and well-defined three-dimensional structures, most transactivation domains of positively acting TFs consist of low complexity peptide sequences termed intrinsically disordered regions (IDRs). IDRs of many proteins have been shown to foster the generation of molecular condensates, such as liquid