

biology, this study is a surprising advance. Never before has such strong evidence existed for neuroendocrine control of bone. Beyond the fact that osteoblasts are the physiologic target, however, the role of leptin is not through direct stimulation of osteoblasts, and the regulation of body weight and bone mass by leptin are apparently mediated through different signaling pathways; we have a lot to learn about this form of regulation. There is also a question regarding how useful this finding is for predicting the risk for low bone mass in populations. A simple and direct application of the data from Ducy et al.'s⁴ animal studies would lead one to believe that bone mass might be directly correlated to serum leptin levels. Odabasi et al.⁶ recently attempted to make such a correlation in postmenopausal women with osteoporosis, but based upon their data they concluded that circulating leptin doesn't have a significant influence on bone mass. However, the data from the db/db mice indicates that serum leptin is only half of the equation. In fact, it has been established that obese people can have leptin resistance owing to impaired transport of leptin to the central nervous system.^{7,8} As such, the correct correlation to look for is between low efficiency of leptin signaling and high bone mass. Because low efficiency of leptin signaling could be due to low serum leptin, poor serum transport of leptin, reduced leptin receptor number, or binding of leptin to its receptor, looking only at serum leptin level would not be informative.

It is not clear how nutritionists will use the discovery of this new regulatory pathway for bone mass. More work needs to be completed to see how leptin and leptin signaling pathways are involved in the control of bone mass under less extreme situations than the total loss of leptin

or leptin receptor. Regardless, it is certain that if the findings of Ducy et al.⁴ are confirmed, the issue of whether this regulator pathway is disrupted by dietary or pharmacologic interventions will need to be considered when one evaluates obesity and weight loss systems. Otherwise, the valuable action of weight loss may ultimately be associated with a detrimental reduction in bone mass.

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Retinoic Acid Induces the Degradation of the Leukemogenic Protein Encoded by the Promyelocytic Leukemia Gene Fused to the Retinoic Acid Receptor α Gene

Acute promyelocytic leukemia (APL) cells carry a mutated gene that is the result of a translocation in which the retinoic acid receptor α (RAR α) gene is fused to the promyelocytic leukemia (PML) gene, coding for a fusion protein, PML/RAR α . Its presence is the single event that causes APL in transgenic mice. All-trans-retinoic acid (atRA) induces the proteolytic degradation of PML/RAR α by ubiquitination and proteolysis. RAR α itself is also

degraded by atRA treatment, a process representing a possible feedback mechanism to turn off RAR α 's stimulation of transcription.

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A relation of vitamin A to cancer has been known since Saffiotti et al.¹ discovered in 1967 that vitamin A inhibited the induction of tracheobronchial cancer in hamsters. This promising beginning raised expectations for treatment of cancer in humans. These expectations were realized mainly for cancer prevention: precancerous keratoses of skin or cervix, leukoplakias, and second primary cancers of the head and neck could be treated successfully with vitamin A derivatives (i.e., retinoids).² Cancer therapy with retinoids, in contrast, is limited to skin cancer and hematologic malignancies.³ Regarding the latter, a striking suc-

cess was the total remission achieved by Lie et al.⁴ in acute myelogenous leukemia in children given high doses of retinyl palmitate. The treatment was based on an earlier finding by Breitman et al.⁵ that showed that all-*trans*-retinoic acid (atRA) induced the differentiation of leukemic cells in culture (HL-60) to normal granulocytes.

In addition, remission of acute promyelocytic leukemia (APL) was achieved with atRA, though relapses occurred invariably after a few months unless treatment was combined with cytostatics. Resistance to atRA developed and appeared to be caused by a decrease of its plasma levels, probably through induction by atRA of its own catabolism.⁶ Successful long-term survival of APL patients was reported recently by Hu et al.,⁷ who combined atRA treatment with administration of arsenic trioxide.

A first indication of the relationship between atRA and APL was brought to light by de The et al.,⁸ who found that in APL cells a translocation occurred of the gene for the retinoic acid receptor α (RAR α) from chromosome 17 to chromosome 15 in place of the promyelocytic leukemia (PML) gene; the PML gene from chromosome 15 took the place of the RAR α gene on chromosome 17 (Figure 1). This produced a chimeric gene, *PML/RAR α* , coding for a fusion protein, PML/RAR α , in which the amino terminus of PML was fused to the carboxyl terminus of RAR α . Expression of this protein is the single event for full transformation to leukemia, as shown by Brown et al.,⁹ who produced transgenic mice harboring *PML/RAR α* in myeloid cells. As a consequence, these mice developed APL. Normally, atRA stimulates differentiation of neutrophils into granulocytes by binding to RAR α ; PML acts as a tumor suppressor protein, mediating multiple apoptosis signals (caspases, type I and II interferons) through a nuclear receptor.¹⁰ Both of these activities are blocked by the PML/RAR α fusion protein.

New light was thrown on the action of atRA by Scita et al.,¹¹ who found that RAR α was down-regulated by atRA in a dose- and time-dependent manner in cultured cells, while Yoshida et al.¹² showed that the fusion protein, PML/RAR α , was also catabolized by atRA treatment of cultured human APL cells (NB4).

Further details of the mechanism by which atRA inhibits APL were described by Zhu et al.¹³ Using NB4 cells and Western blots with RAR α antibody, the authors demonstrated that atRA (10^{-6} M) down-regulated PML/RAR α in a biphasic manner: catabolism was rapid in the first hour, followed by a slow phase during the next 12 hours. The rapid phase was caused by the action of caspases, the slow phase by proteases. This was shown to be a posttranslational process because the PML/RAR α mRNA was not affected. Synthetic retinoid agonists were as potent as atRA in down-regulation, whereas RA antagonists prevented the catabolism.

Interestingly, arsenic trioxide, the known therapeutic agent for APL⁷ also caused rapid catabolism of PML/

RAR α , thus strengthening the hypothesis that APL remission is caused by down-regulation of the fusion protein.¹³ Protease and caspase inhibitors were found¹³ to block degradation of PML/RAR α by both atRA and arsenic trioxide, though the latter had no effect on RAR α itself.¹³ This result suggested that the arsenic trioxide degradation followed a different pathway from that initiated by atRA. The action of atRA was not confined only to APL cells (i.e., NB4). Thus, when RAR α was overexpressed in a series of cell lines (U937, HL-60, CHO), atRA caused its dose-dependent down-regulation.¹³ To show that in the fusion protein, PML/RAR α , the presence of RAR α alone was sufficient for degradation, the authors fused RAR α to another protein (enhanced green fluorescent protein [EGFP]) and found that the EGFP/RAR α chimera was degraded by atRA just as well as PML/RAR α was degraded.¹³

Protein degradation generally depends on the proteolytic action of proteasomes; the investigators¹³ determined that proteasome inhibitors prevented atRA-driven catabolism of RAR α . Furthermore, such degradation depends on ubiquitination. To show that both RAR α and PML/RAR α are ubiquitinated upon exposure to atRA, COS cells were cotransfected with expression vectors for RAR α or PML/RAR α together with one for epitope-tagged ubiquitin. Western blot analysis with antibodies to RAR α , PML/RAR α , and the epitope tag revealed the presence of ubiquitinated RAR α and ubiquitinated PML/RAR α after atRA treatment, which proved that atRA stimulates catabolism of RAR α and of PML/RAR α by ubiquitination followed by proteasome proteolysis.

Which domains in the multidomain molecule of RAR α are essential for the catabolic response to atRA? By mutational analysis of RAR α , Zhu et al. showed that the essential regions for degradation were the DNA-binding domain and the transcription-activation (AF-2) domain.¹³ When the investigators expressed RAR α and RXR α separately in COS cells, they discovered that both atRA and 9-*cis*-RA degraded RAR α , but 9-*cis*-RA degraded RXR α much more rapidly than RAR α . By contrast, when RAR α

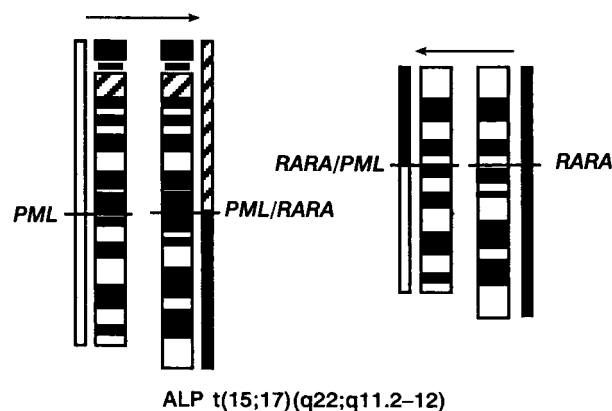


Figure 1. Schematic representation of chromosomal translocation.

and RXR α were expressed together, both atRA and 9-*cis*-RA degraded RAR α and RXR α equally. Ligand preference is abolished when RAR α /RXR α is cotransfected, suggesting that the RAR α /RXR α heterodimer is the substrate for degradation.¹³

To specifically address the involvement of heterodimerization and/or DNA binding in the ligand-mediated degradation of RAR α , the group¹³ then produced a version of RAR α that contained mutations at positions 380 and 383, amino acids known to be essential for heterodimer formation with RXR α and which the authors demonstrated could not bind DNA. This mutated protein, RAR α (380,383), when coexpressed in COS cells along with RXR α , was not degraded by atRA despite the presence of RXR α . The authors¹³ then asked whether that was because the mutant protein could not heterodimerize with RXR α or because it could not bind to DNA. To answer this question, they performed an ingenious experiment. They found a way to allow the RAR α (380,383) mutant to bind DNA by fusing it to the DNA binding domain of the GAL4 protein. Thus, whereas RAR α (380,383) could neither bind DNA nor interact with RXR α , this chimeric protein, GAL4-RAR α (380,383), although still unable to bind RXR α , could now bind DNA via the GAL4 DNA-binding domain. COS cells were cotransfected with an expression construct for GAL4-RAR α (380,383) and with a CAT reporter gene whose transcriptional activity was under the control of a GAL4 DNA-binding element, thus allowing for monitoring of functional DNA binding. The reporter was activated by atRA treatment, indicating that the GAL4 domain had tethered RAR α (380,383) to DNA, effectively conferring DNA binding ability to the RAR α (380,383) mutant. Western blot analysis demonstrated that DNA-tethered GAL4-RAR α (380,383) was degraded by atRA, in contrast with the RAR α (380,383) mutant which, as stated above, was not degraded. The authors concluded "hence, the inability of the RAR α mutant defective in RXR α binding to be degraded likely results from its inability to bind DNA."¹³

In summary, the gene translocation that results in the formation of the PML/RAR α fusion protein makes RAR α unavailable for neutrophil differentiation and makes PML unavailable for apoptosis and causes uninhibited growth of leukemic cells. Zhu et al.¹³ established that atRA stimulates catabolism of PML/RAR α , removing the leukemogenic fusion protein by ubiquitination and proteasome proteolysis. But they have shown much more. Whereas the function of RA is to bind to the receptor, RAR α , and activate transcription, a mechanism must also exist to turn off transcription when completed. Thus, following the initial discovery of Scita et al.,¹¹ that RA can stimulate degradation of RAR α , the work of Zhu et al.¹³ demonstrated that RAR α is degraded and its action turned off after activation of transcription, because they found that degrada-

tion required binding to DNA. DNA binding may mediate conformational or other changes needed for degradation. It thus appears that degradation affects only transcriptionally active receptors, resulting in a feedback control of receptor action.

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