Acute promyelocytic leukemia (APL) cells carry a presence is the single event that causes APL in which the retinoic acid receptor α (RARα) gene encoding for a fusion protein, PML/RARα. Its mutated gene that is the result of a translocation in which the retinoic acid receptor α (RARα) gene is fused to the promyelocytic leukemia (PML) gene, coding for a fusion protein, PML/RARα. Its presence is the single event that causes APL in transgenic mice. All-trans-retinoic acid (atRA) induces the proteolytic degradation of PML/RARα by ubiquitination and proteolysis. RARα itself is also degraded by atRA treatment, a process representing a possible feedback mechanism to turn off RARα’s stimulation of transcription.

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. in acute myelogenous leukemia in children given high doses of retinyl palmitate. The treatment was based on an earlier finding by Breitman et al. 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. Successful long-term survival of APL patients was reported recently by Hu et al., 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., who found that in APL cells a translocation occurred of the gene for the retinoic acid receptor α (RARα) 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 RARα gene on chromosome 17 (Figure 1). This produced a chimeric gene, PML/RARα, coding for a fusion protein, PML/RARα, in which the amino terminus of PML was fused to the carboxyl terminus of RARα. Expression of this protein is the single event for full transcription to leukemia, as shown by Brown et al., who produced transgenic mice harboring PML/RARα in myeloid cells. As a consequence, these mice developed APL.

New light was thrown on the action of atRA by Scita et al., who found that RARα was down-regulated by atRA in a dose- and time-dependent manner in cultured cells, while Yoshida et al. showed that the fusion protein, PML/RARα, 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. Using NB4 cells and Western blots with RARα antibody, the authors demonstrated that atRA (10⁻⁶ M) down-regulated PML/RARα 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/RARα 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 APL, also caused rapid catabolism of PML/RARα, thus strengthening the hypothesis that APL remission is caused by down-regulation of the fusion protein. Protease and caspase inhibitors were found to block degradation of PML/RARα by both atRA and arsenic trioxide, though the latter had no effect on RARα itself. 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 RARα was over-expressed in a series of cell lines (U937, HL-60, CHO), atRA caused its dose-dependent down-regulation. To show that in the fusion protein, PML/RARα, the presence of RARα alone was sufficient for degradation, the authors fused RARα to another protein (enhanced green fluorescent protein [EGFP]) and found that the EGFP/RARα chimera was degraded by atRA just as well as PML/RARα was degraded.

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

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

![Figure 1. Schematic representation of chromosomal translocation.](image-url)
and RXRa were expressed together, both atRA and 9-cis-RA degraded RARα and RXRa equally. Ligand preference is abolished when RARα/RXRα is cotransfected, suggesting that the RARα/RXRα heterodimer is the substrate for degradation.13

To specifically address the involvement of heterodimerization and/or DNA binding in the ligand-mediated degradation of RARα, the group13 then produced a version of RARα that contained mutations at positions 380 and 383, amino acids known to be essential for heterodimer formation with RXRα and which the authors demonstrated could not bind DNA. This mutated protein, RARα(380,383), when coexpressed in COS cells along with RXRa, was not degraded by atRA despite the presence of RXRa. The authors13 then asked whether that was because the mutant protein could not heterodimerize with RXRa or because it could not bind to DNA. To answer this question, they performed an ingenious experiment. They found a way to allow the RARα(380,383) mutant to bind DNA by fusing it to the DNA binding domain of the GAL4 protein. Thus, whereas RARα(380,383) could neither bind DNA nor interact with RXRα, this chimeric protein, GAL4-RARα(380,383), although still unable to bind RXRα, could now bind DNA via the GAL4 DNA-binding domain. COS cells were cotransfected with an expression construct for GAL4-RARα(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 RARα(380,383) to DNA, effectively conferring DNA binding ability to the RARα(380,383) mutant. Western blot analysis demonstrated that DNA-tethered GAL4-RARα(380,383) was degraded by atRA, in contrast with the RARα(380,383) mutant which, as stated above, was not degraded. The authors concluded “hence, the inability of the RARα mutant defective in RXRa 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/RARα fusion protein makes RARα 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/RARα, 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, RARα, 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 RARα, the work of Zhu et al.13 demonstrated that RARα is degraded and its action turned off after activation of transcription, because they found that degrada-


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