Cell Death and Differentiation (1998) 5, 1–3

Editorial

When complex worlds collide: retinoic acid and apoptosis

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Retinoids are lipid-soluble compounds derived from, and including, vitamin A (retinol) and synthetic derivatives thereof. Retinol is delivered to cells bound to serum retinol binding protein (RBP), and once taken up it binds to cellular retinol binding protein (CRBP). The retinol-CRBP complex serves as a substrate for enzymes involved in retinoid metabolism, one product of which is retinoic acid (RA), the metabolite that mediates most of the non-visual functions of vitamin A (Napoli, 1996). RA binds cellular retinoic acid binding protein (CRABP), a molecule that appears to function as a regulator of RA metabolism and perhaps its access to the nucleus (Napoli, 1996). Once in the nucleus, RA binds nuclear-resident transcription factors known as retinoic acid receptors (RARs) or retinoid X receptors (RXRs) (referred to collectively as RA receptors), each family being composed of at least three different genes (Chambon, 1996). Much of the difficulty in understanding the interesting (and complicated) biological functions of these receptors arises from the fact that the two RA receptor families, which can homodimerize and heterodimerize not just with each other but also other nuclear hormone receptors, have different ligand-binding specificities: RXRs bind 9-*cis* RA with high affinity, while RARs bind both 9-*cis* and its stereoisomer all-*trans* RA with high affinity (Heyman *et al*, 1992; Levin *et al*, 1992). As detailed in two reviews in this issue of *Cell Death and Differentiation*, the recent development of RA receptor-specific retinoids is making it possible to clarify the roles of these receptors in different biological processes (Nagy *et al*, 1998; Szondy *et al*, 1998).

Like thyroid hormone and vitamin D_3 receptors, RA receptors bind to DNA response elements composed of two direct repeat (DR) half-sites with a consensus sequence of AGGTCA (Mangelsdorf *et al*, 1991; Umesono *et al*, 1991; Yu *et al*, 1991). Which receptors bind and mediate transactivation is determined by the spacing between the half-sites. RAR/RXR heterodimers bind to direct repeats separated by five nucleotides (DR5), while RXR/RXR homodimers bind to direct repeats separated by one nucleotide (DR1). Although RAR/RXR heterodimers also bind DR1 elements, they do not transactivate gene transcription at these sites, implying that RARs are inhibitory in this context. It appears that the polarity of protein binding to the half sites (that is, the position of each element of the RAR/RXR heterodimer on the DNA) is an important determinant of transactivation potential. In DR1-, but not DR5- (Kurokawa *et al*, 1993; Zechel *et al*, 1994), containing promoters, the RAR binds upstream of the RXR and allosterically prevents its RXR partner from binding ligand (Kurokawa *et al*, 1994).

An interesting and important feature of RA receptors is that in the unactivated (unliganded) state they bind to DNA and actively repress transcription of target genes. It has become apparent that they do not do so alone, but rather interact with distinct groups of corepressors (and coactivators, in the liganded state) to mediate their function. The basis for this lies in the exciting observation that chromatin conformation, which can profoundly affect the transcriptional process, is altered in biologically meaningful ways by acetylation (reviewed in Wade *et al*, 1997). In particular, the NH_2-terminal tails of core histones are exposed on the outside of nucleosomes, and their acetylation on lysine residues decreases their charge and therefore binding to DNA. The ensuing changes in nucleosomal packing and chromatin conformation results in increased access of transacting factors and components of the basal transcriptional machinery to the local DNA (Luger *et al*, 1997). RA receptors and their associated factors make use of reversible acetylation to regulate target genes. Among the factors (corepressors) that bind RA receptors in the absence of ligand are SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) or N-Cor (nuclear corepressor), mSin3A, and the histone deacetylase HDAC-1 (Heinzel *et al*, 1997; Nagy *et al*, 1997). Thus, unbound RA receptors recruit a deacetylating complex to their target genes, maintaining the local chromatin in an unfavorable conformation for transcription. Upon binding of RA, the inhibitory complex dissociates and a new set of factors (coactivators) bind. These include p300 and its homolog CBP (CREB-binding protein), P/CAF (p300/CBP-associated factor), and ACTR (and its related proteins SRC-1 and GRIP/TIF2). p300/CBP, P/CAF, and ACTR/SRC-1 have all been shown to have intrinsic histone acetyltransferase (HAT) activity (Bannister and Kouzarides, 1996; Chen *et al*, 1997; Ogryzko *et al*, 1996; Spencer *et al*, 1997; Yang *et al*, 1996). Thus, a major mechanism by which RA receptors regulate transcription of target genes is by controlling the reversible covalent modification of histones and thereby neighboring chromatin conformation. The fact that RA receptor coactivators and corepressors also associate with other nuclear factors offers a host of opportunities for functional interactions (cross-talk) between these transcriptional regulators.

Among the many biological properties of retinoids are their potent effects on cell differentiation and viability. One of several clinically important examples is acute promyelocytic leukemia (APL), for which RA is one of the cornerstones of therapy (Scheinberg *et al*, 1997). The

most common form of APL is caused by a reciprocal chromosomal translocation resulting in the production of a fusion protein composed of RARα and Pml, a nuclear phosphoprotein of unknown function (Chang *et al*, 1995). Transgenic mice that express the *PMLRAR*α fusion gene develop APL and, like their human counterparts, respond to therapy with RA (Brown *et al*, 1997; He *et al*, 1997). In this case, the therapeutic efficacy of RA is thought to be primarily due to its ability to promote differentiation of the promyelocytic cells. Based upon its 'anti-proliferative' effects, the use of RA as an adjunct in the treatment of a variety of other malignancies is being explored. The most commonly used form, all-*trans* RA, has limited usefulness in long-term therapy, however, because of its increased metabolic clearance over time with a resulting decrease in the plasma levels achieved (Adamson *et al*, 1993). It is likely that this problem can be overcome with synthetic retinoids that do not induce their own metabolism (Miller *et al*, 1997).

Two reviews in the current issue of *Cell Death and Differentiation* deal with a particularly efficient mechanism for controlling cell growth: apoptosis. RA, like glucocorticoids, have the distinction of both promoting and inhibiting apoptosis, the outcome depending upon the cell type, the specificity of the retinoid, and the presence or absence of other stimuli. RA prevents the activation-induced apoptosis of T cells and thymocytes, in the former case, at least, by inhibiting the upregulation of Fas ligand expression (Yang *et al*, 1995b). The finding that interactions between Fas and upregulated Fas ligand are responsible for the *ex-vivo* death of lymphocytes from HIV-infected individuals (Bäumler *et al*, 1996; Yang *et al*, 1997), and that this is inhibited by oral administration of RA (Yang *et al*, 1995a), may provide another potentially therapeutic role for RA. In the absence of activation, RA can also induce the death of thymocytes (Fesus *et al*, 1995). The biologic relevance of this is unclear, since at physiologic concentrations RA does not cause thymocyte apoptosis, and although RA does appear to enhance glucocorticoid-mediated thymocyte death the effect is, at best, modest (Fesus *et al*, 1995; Iwata *et al*, 1992). Fesus and colleagues detail this phenomenon, and using RA receptor-selective retinoids explore the different (antagonistic) roles played by RARα and RARγ (Szondy *et al*, 1998). Davies and coworkers review the mechanisms of RA action, both *in vitro* and *in vivo* (Nagy *et al*, 1998). A particularly interesting topic is the role of retinoids in the transcriptional regulation of tissue transglutaminase, an intracellular enzyme that cross-links proteins and which is upregulated during apoptosis.

The potent biological effects of retinoids have been known for many years. What is relatively new is the appreciation that different RAs, binding different members of a complex receptor superfamily, have such diverse and even antagonistic activities. Add to this the fact that coactivators and corepressors of RA receptor transcriptional activity are shared with other receptors/transactivation factors, and it is clear that the potential for fine-tuning biological responses are enormous. There is no doubt that dissecting and elucidating the means by which retinoids promote, and in some cases inhibit, apoptosis will provide fascinating insights into one of the most important decisions a cell must make: whether to live or to die.

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