THEMATIC REVIEW

Chronicle of a discovery: the retinoic acid receptor

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Abstract

The landmark 1987 discovery of the retinoic acid receptor (RAR) came as a surprise, uncovering a genomic kinship between the fields of vitamin A biology and steroid receptors. This stunning breakthrough triggered a cascade of studies to deconstruct the roles played by the RAR and its natural and synthetic ligands in embryonic development, skin, growth, physiology, vision, and disease as well as providing a template to elucidate the molecular mechanisms by which nuclear receptors regulate gene expression. In this review, written from historic and personal perspectives, we highlight the milestones that led to the discovery of the RAR and the subsequent studies that enriched our knowledge of the molecular mechanisms by which a low-abundant dietary compound could be so essential to the generation and maintenance of life itself.

Introduction

The precise control of gene expression is a fundamental molecular mechanism essential from the first cell division to the complete development of the organism and its maintenance throughout its lifespan. Cell-specific expression of genes is dependent on the action of transcription factors that transduce extra and intracellular signals into specific biological programs. Indeed, a purified fraction containing a receptor for the hormone cortisol (glucocorticoid receptor, GR) was among the first higher eukaryote transcription factors shown to recognize specific sites on a promoter (Payvar et al. 1981). Shortly thereafter, the GR would become the first full-length human transcription factor molecularly cloned and shown to be necessary and sufficient for the transcriptional activation of a reporter gene when re-introduced into a cell (Hollenberg et al. 1985, Giguère et al. 1986). The rapid subsequent identification of receptors for estradiol, thyroid hormones, aldosterone, progesterone, vitamin D3, and orphan receptors without known ligands, all sharing a common structure and functional domains, led to the surreal concept of a genomic superfamily of ligand-responsive transcription factors and the potential existence of previously unrecognized hormone/metabolites response systems (Evans 1988, Giguère et al. 1988).

Vitamin A, aka all-trans-retinol, was purified by McCollum and Davis (Wisconsin) and Osborne and Mendel (Yale) in 1913 (Semba 2012). They showed it to be a low-abundant dietary fat-soluble compound that is essential for vision, reproduction, embryonic development, immunity as well as the normal growth and preservation of a healthy organism. Vitamin A was shown very early to be essential for normal differentiation and preservation of epithelial...
tissues (Wolbach & Howe 1925). Nonetheless, it would take several decades of work and many detours along the way to finally answer the question as to how this small nutrient influences cell fate. Below is a brief history of vitamin A and of the unanticipated discovery that its receptor would be a member of the nuclear receptor family now referred to as the retinoic acid receptor (RAR) (Giguère et al. 1987). A companion article in this commemorative issue describes the contemporaneous identification of the RAR by the team of Pierre Chambon in Strasbourg (Petkovich et al. 1987).

Vitamin A

The concept that animals could thrive when fed suitable food like milk but not its separated components (proteins, fats, carbohydrates, salts, and water) led to the suggestion by Nicolai Lunin in 1881 that additional elements indispensable for nutrition must exist in foodstuffs (Lunin 1881). A few years later, Frederick Hopkins reiterated in a lecture in London that unsuspected dietary factors other than proteins, fat, carbohydrates, and minerals were present in food and intuited that the lack of these factors could be linked to ailments such as scurvy and rickets (Hopkins 1906). In a landmark study published in 1912, Hopkins then demonstrated that the growth of mice was indeed dependent on accessory factors present in milk in very small amounts, but the composition of these accessory factors was unknown (Hopkins 1912). The term ‘fat-soluble A’ was introduced in 1918 by Elmer McCollum to describe an accessory food factor necessary for general growth and preserving vision (McCollum et al. 1918). The name vitamin A was subsequently introduced by Jack Drummond at University College, London, as part of a new nomenclature to classify a growing list of dietary factors of different chemical compositions as vital factors, ergo ‘vitamins’ (Drummond 1920). Hopkins and Christiana Eijkman, who concurrently discovered the antineuric vitamin (now known as thiamin or vitamin B₁) while studying the cause of the diet-deficiency disease beriberi, were awarded the Nobel Prize in Physiology and Medicine in 1929 for ‘the discovery of the vitamins.’ Two more Nobel Prizes were later awarded for work related to vitamin A, one for determining the chemical structure of vitamin A to Paul Karrer (1937, Nobel Prize in Chemistry) and a second to George Wald who discovered that the vitamin A metabolite all-trans-retinal is a crucial component in rhodopsin and thus necessary for vision (1967, Nobel Prize in Physiology and Medicine). The development of the large-scale synthesis of vitamin A by chemists at Hoffman-La Roche in the 1940s allowed for the effective treatment and prevention of vitamin A deficiency-related diseases, including impaired immunity and hematopoiesis, xerophthalmia, and night blindness.

All-trans-retinoic acid

The pro-vitamin β-carotene and vitamin A, itself present in the diet, are inactive compounds that must be metabolized by the organism to all-trans-retinoic acid (at-RA) to sustain vision and cell differentiation, respectively. Indeed, while a role for vitamin A in promoting cellular differentiation was demonstrated early in the characterization of the surprisingly diverse biological properties of the vitamin (Wolbach & Howe 1925), several decades passed before at-RA was identified as the active metabolite of vitamin A responsible for observable changes in cell phenotype. In fact, at-RA was synthesized years before the determination of its unique properties on cell differentiation (Arens & Van Dorp 1946a). However, it was established at that time that at-RA could sustain normal growth but not reproduction and vision in rats fed a vitamin A-deficient diet and that at-RA could not be converted back into retinol by the liver (Arens & Van Dorp 1946b, Van Dorp & Arens 1946). These experiments thus established that at-RA was an active metabolite with biological activity independent of retinol. In 1967, the group of Hector DeLuca showed that at-RA was formed in vivo in rats injected with 14C-retinol (Emerick et al. 1967), and a few years later, the same team demonstrated that at-RA was indeed a natural metabolite of retinol (Ito et al. 1974). The next key advance in the field came when at-RA was shown as the most potent vitamin A metabolite to induce multiple phenotypic changes in F9 teratocarcinoma stem cells in culture (Strickland & Mahdavi 1978). The at-RA-induced differentiation of cultured F9 embryonal carcinoma cells into endoderm was accompanied by an increase in the synthesis of collagen-like proteins. This at-RA regulated cell system soon became a favored standard model to study the induction of protein synthesis (Chytil 1986). However, the initial observations on the effect of at-RA on cell differentiation made in F9 cells were quickly expanded to other cultured cell systems including neuroblastosomas, melanomas, fibroblasts as well as the HL60 promyelocytic leukemia (PML) cell line that could be induced to differentiate into granulocytes (Breitman et al. 1980, Schroder et al. 1982, Haussler et al. 1983, Lotan et al. 1983).

Subsequently, at-RA-inducible genes encoding various keratins were cloned (Eckert & Green 1984, Gilfix & Eckert 1985, Wang et al. 1985), consolidating the hypothesis put
forward earlier by Sporn & Roberts (1983) that the action of at-RA could be mediated via changes in gene expression. Several molecular mechanisms underlying the activity of at-RA as potential regulators of gene expression were being investigated at that time. Those included modification of membrane structure, sugar transfer reactions by means of the intermediate retinyl phosphate mannose, direct interactions with protein kinases, cooperation with growth factors through unknown mechanisms, and most prominently, control of gene expression by small cellular retinoic acid-binding proteins (CRABPs) (Chytíl & Ong 1979, Sporn & Roberts 1983, Chytíl 1986). CRABPI, a small cytoplasmic protein with a molecular weight of 14,500 daltons, was initially purified by Ong and Chytíl from rat testis for its ability to bind 14C-at-RA (Ong & Chytíl 1978). A complementary cDNA encoding CRABPI was cloned and its sequence confirmed the small size of the protein and incorrectly suggested its possible role as a transcriptional regulator (Shubeita et al. 1987). A second CRABP isoform, referred to as CRABPII, was subsequently identified by molecular cloning and its expression was found to be highly inducible by at-RA, consolidating a possible function for CRABPs in transmitting the at-RA signal (Giguère et al. 1990a). However, despite decades of ensuing investigation on the potential roles played by CRABPs in at-RA biological activities, the exact function of the two CRABP isoforms remains to be fully uncovered.

**Discovery of the RAR**

Our discovery of the RAR (Giguère et al. 1987), later referred to as RARz, was enabled by a confluence of specialized expertise within the lab and the rapid evolution of our knowledge of nuclear receptors and how they work. During a 2-year window, this project evolved over seven brisk steps, each briefly summarized below.

**Cloning of the GR and its homology to v-erbA**

The recognition that the GR shared sequence homology and structural relationship with the RNA tumor virus v-erbA suggested an unforeseen kinship between a steroid receptor and a viral protooncogene (Hollenberg et al. 1985, Weinberger et al. 1985). These observations were validated a few months later by simultaneous publications reporting the cloning of the estrogen receptor (ER) by the teams of Pierre Chambon and Geoffrey Greene (Green et al. 1986, Greene et al. 1986). The oncogene v-erbA became the focus of both Weinberger and Bjorn Vennstrom, a world expert in RNA leukemia viruses. The successful discovery of the human and avian homolog of the verbA oncogenes led to its exciting discovery as the thyroid hormone (T3,R) (Sap et al. 1986, Weinberger et al. 1986). This set of experiments helped to launch a new era in receptor discovery and function with the mindful notion that newly discovered nuclear receptors need not necessarily have to bind or respond to steroids.

**The ‘co-transfection assay’**

The cloning of the GR led us to develop new techniques, the first of which was the ‘co-transfection assay.’ The goal behind the co-transfection assay was to reconstitute a functional hormonal response in cells to characterize the molecular mechanisms underlying nuclear receptor signaling. To address this, we transfected cells with two separate plasmids pairing an hGR cDNA expression plasmid with a glucocorticoid-responsive reporter gene (Giguère et al. 1986). The chosen reporter was the gene encoding the bacterial enzyme chloramphenicol acetyltransferase (CAT). The CAT assay had originally been developed to monitor the transcriptional activity of the long terminal repeat (LTR) of the Rous sarcoma virus (RSV) (Gorman et al. 1982). One of us (V G) had the opportunity to obtain the pRSV-CAT construct in 1984 directly from Dr. Gorman who was at that time at the same institution, the National Institute for Medical Research at Mill Hill, England. In London, I (V G) modified the pRSV-CAT construct by replacing the sequence of the RSV LTR with the promoter of the gene encoding the mouse Thy-1 antigen and used this construct to demonstrate that a GC-rich/TATAA box-less mammalian promoter could efficiently drive gene expression (Giguère et al. 1985). At the Salk Institute in La Jolla, the original pRSV-CAT construct was then modified to build two vectors to create the co-transfection assay. The first vector was used to express a transcription factor and the second, a reporter gene whose expression would be dependent on the transcription factor expressed by the first vector. To generate the first vector, the CAT gene in pRSV-CAT was replaced by the cDNA encoding the human GR to generate high levels of expression of the receptor. The second vector included the glucocorticoid-responsive regulatory region of mouse mammary tumor virus that had been previously shown to confer potent hormone responsiveness to a heterologous promoter (Chandler et al. 1983). The co-transfection of the two expression plasmids resulted in a very impressive hormone-dependent transcriptional response allowing for the rapid analysis of multiple aspects of the hormonal activation, for example, ligand potency and specificity, characterization of hormone response...
elements (HREs) within regulatory regions of genes, studies of structure–function relationship within nuclear receptors, and the interaction and dependency of nuclear receptors on co-regulatory proteins and the general transcription machinery. Indeed, the co-transfection assay was so adaptable as a cell-based platform to study gene transcription that it rapidly became (and remains) one of the most indispensable and widely used assays in molecular biology. It also rapidly became an indispensable screening tool in the pharmaceutical industry, underpinning the development of a panoply of new receptor-directed therapeutics (Evans & Mangelsdorf 2014).

**Functional domains of nuclear receptors**

The co-transfection assay proved to be crucial in localizing and characterizing the hGR functional domains. To address the question of domain structure, we created a series of plasmids harboring mutations scattered throughout the hGR sequence. These mutant GR plasmids were generated via insertion (or deletion) of three or four extraneous amino acids, an improvement on the approach previously exploited to identify functional regions in the transforming protein of Fujinami sarcoma virus (Stone et al. 1984). An in-depth analysis of these mutant proteins allowed us to assemble a detailed model of the GR and its functional domains (Giguère et al. 1986). The model revealed that the GR protein architecture is comprised of an ensemble of discrete functional domains responsible for ligand-binding, DNA-binding, and transcriptional activation. This model was quickly validated by studies on the human ER and rat GR (Kumar et al. 1987, Miesfeld et al. 1987). The independence of each functional domain within the receptor led to the proposal that functional domains could be switched between related proteins (Giguère et al. 1986). Swapping domains between receptors was then successively shown to generate functional hybrid nuclear receptors (Green & Chambon 1987) and to confer hormone-responsiveness, or a transcriptional switch, to otherwise constitutive transcription factors (Picard et al. 1988, Eilers et al. 1989). The distinctiveness, but at the same time, the interdependence of each nuclear receptor functional domain, was subsequently depicted in great detail by crystallographic and cryoelectron microscopy studies (Chandra et al. 2008, 2013, Yu et al. 2020).

**Multiple genetic loci related to nuclear receptors**

Low stringency hybridization studies using genomic DNA indicated the existence of multiple genetic loci that hybridized with the cDNA clones encoding the T3R, GR, and ER. It was thus realized that the close homology between the DNA sequences encoding different nuclear receptors could be exploited to clone additional receptors related to these sequences, especially receptors for other cholesterol-derived steroid hormones such as progesterone, androgens, aldosterone, and vitamin D3. Low stringency homology screening led in rapid succession to the cloning of the mineralocorticoid receptor (Artiza et al. 1987), a second T3R isoform (Tr3) (Thompson et al. 1987) and two clones encoding receptors with homology to the ER, originally named estrogen-related receptor 1 and 2 (ERR1 and 2) (Giguère et al. 1988). No known hormone or other small ligands could be shown to bind to the ERRs, which thus became the first ‘orphan nuclear receptors’ to be inducted into the superfamily. Members of the ERR subfamily of nuclear receptors are now known as master regulators of cellular energy metabolism but remain classified as orphan receptors 35 years later (Scholtes & Giguère 2022). We discuss ERR cloning above in 1988 but we have now to go backward to 1986 for the RAR/hepatitis B virus challenge described below.

**The hepatitis B virus integration site**

In addition to the discovery of multiple genetic loci with sequence similarity with nuclear receptors, Anne Dejean (Dejean 1986) reported that a hepatitis B virus (HBV) integration into human liver DNA placed the viral sequence next to a sequence with striking homology to both the oncogene v-erbA and the DNA-binding domain (DBD) of the human GR and ER. In early in 1986, several candidate steroid hormone receptors remained to be cloned (e.g. androgen, progesterone, and aldosterone). I (V G) decided to ‘throw my hat in the ring’ and capture the HBV associated as a possible new nuclear receptor. The race thus promptly started to clone the corresponding full-length cDNA and identify a hormone ligand associated with this putative receptor.

**An unexpected twist**

A strategy to clone the cDNA transcribed from the gene disrupted by the HBV integration was conceived. First, we synthesized a long oligonucleotide containing the sequence of the exon encoding a segment of the putative nuclear receptor. The oligonucleotide was then labeled and used as a probe to screen a number of human cDNA libraries. Several clones were obtained and the longest contained ~2900 base pairs that could be translated into an
open reading frame of 462 amino acids which could easily encode a full-length nuclear receptor (Giguère et al. 1987). Indeed, comparison of the amino acid sequence of this receptor ‘X’ with GR and both T3Rs showed that the highest degree of similarity was found in a cysteine-rich sequence of 66 amino acids encoding the DBD of nuclear receptors. However, the nucleotide sequence encoding this segment of the cDNA, while displaying strong homology with the published sequence of the HBV integration site (Dejean et al. 1986), was not a perfect match. In addition, the hybridization pattern of genomic DNA obtained with our cDNA clone was unrelated to the restriction enzyme map previously described by Dejean for the HBV integration site. By relaxing the stringency of the hybridization to genomic DNA, we then confirmed the existence of two or more loci related to this cDNA, including the original HBV integration site. We thus had cloned a full-length cDNA encoding a novel nuclear receptor but not the one we intended to obtain using the probe derived from the HBV integration site.

A eureka moment

As the ligand for the gene product of receptor X was unknown, we modified the co-transfection assay into a sensitive screening tool to reveal its identity. As mentioned above, the DBDs of the human GR and ER had been shown to be interchangeable, resulting in functional hybrid nuclear receptors (Green & Chambon 1987). This observation suggested a more general strategy that could be exploited to identify the potential ligand associated with the presumptive novel hormone receptor. We thus swapped the DBD of the gene product encoded by the cDNA described in the previous section with the DBD from the human GR, expecting that the hybrid receptor would induce the activity of the MMTV-CAT reporter gene in response to an appropriate ligand. The dual plasmid co-transfection assay enabled us to challenge the hybrid receptor with a battery of 12 candidate ligands that included at-RA (Fig. 1). In a dramatic eureka moment never to be forgotten on a beautiful Sunday morning in La Jolla, the result of a co-transfection assay revealed that only at-RA could elicit a powerful increase in CAT activity in the presence of the hybrid receptor (Giguère et al. 1987). All other natural and synthetic ligands included in the screen, namely aldosterone, dexamethasone, dihydrotestosterone, estrogen, progesterone, triiodothyronine (T3), thyroxine, vitamin D3, and 25-OH-cholesterol, did not elicit CAT activity. The identity of the novel gene product as the receptor for at-RA was then validated by its capacity to specifically bind to radiolabeled at-RA. We had suddenly discovered a receptor for at-RA that is now part of the family of steroid and thyroid hormone receptors. This sudden advance disrupted the field of vitamin A biology, opening entirely new directions in understanding how this simple vitamin can have such a vast impact on cellular function and body physiology.

A family of retinoic acid receptors

Over the next 2 years, two additional receptors responsive to at-RA were rapidly identified and referred to as RARβ and RARγ (Brand et al. 1988, Krust et al. 1989, Zelent et al. 1989). Several isoforms of the three RARs harboring distinct amino-terminal domains were subsequently identified in human and mouse (Giguère et al. 1990b, Kastner et al. 1990, Leroy et al. 1991, Zelent et al. 1991, Nagpal et al. 1992). Genes encoding evolutionarily conserved RARs were found in a variety of other species such as chicken (Smith & Eichele 1991), frog (Ellinger-Ziegelbauer & Dreyer 1991), and newt (Giguère et al. 1989, Ragsdale et al. 1989, Ragsdale et al. 1992) but not in invertebrates.

The sustained pursuit to associate ligands with newly cloned orphan nuclear receptors channeled the Evans team to the discovery of a second retinoid-responsive system. David Mangelsdorf (Mangelsdorf 1990) showed that the activity of an orphan receptor referred to as RXR could be induced by pharmacological concentrations of at-RA. In a genomic organization similar to that of the RARs, three distinct genes encoding RXR isoforms (α, β, γ) were identified in mouse and human (Hamada et al. 1989, Mangelsdorf et al. 1990, 1992, Fleischhauer et al. 1992, Leid et al. 1992). A search for a higher affinity retinoid for RXR led to the identification of the 9-cis isomer of RA (9-cis-RA) as a suitable ligand (Heyman et al. 1992, Levin et al. 1992). Given its low abundance in tissues and high affinity of the RARs, the exact role that endogenous 9-cis-RA plays as an RXR ligand in retinoid signaling in vivo has remained elusive. However, it has recently been reported that a retinoid related to 9-cis-RA present at high endogenous levels in mice, namely 9-cis-13-14-dihydroretinoic acid, binds and transactivates all three RXRs at physiological concentrations (Ruhl et al. 2015).

Gene regulation by the RARs

Another outcome of the discovery of the RAR was the recognition that HREs, initially found to be configured as inverted repeats (i.e. palindromes), can, for a new class of receptors, be composed of tandem response elements
termed direct repeats. The characterization of the first at-RA response element (referred to as RARE) was present in the promoter of the RARβ receptor itself. Specifically, it contained two tandem repeats of the sequence PuGGTCA separated by five base pairs (de Thé et al. 1990b, Hoffman et al. 1990, Sucov et al. 1990, Näär et al. 1991). This finding led to a re-examination of the configuration of HREs implicated in the response to T₃ and vitamin D₃ (Näär et al. 1991, Umesono et al. 1991). Detailed mutational analyses of a wide variety of HREs by Kazuhiko Umesono (Umesono 1991) demonstrated that spacing of direct repeats (DRs) is receptor-specific. Thus, tandem repeats spaced by three, four, or five base pairs were selective HREs for the T₃Rs, vitamin D3 receptor, and RAR, respectively. This organizational scheme for the HREs is referred to as the ‘3-4-5 rule.’ The functional relevance of the RARE was quickly shown by the observation that this response element could direct specific spatial and temporal expression of an indicator transgene during mouse embryogenesis, mimicking the expression of the RARβ itself (Rossant et al. 1991). Remarkably, the transgene was not only expressed in a specific anterior–posterior domain, but the observed specific expression in the embryo was completely obliterated by treatment of pregnant mice with teratogenic doses of at-RA. These results demonstrated that teratogenesis induced by retinoids were due to ectopic activation of at-RA-responsive genes outside of the normal domains of action of at-RA during development. This mouse model soon became the reference to study the effects of manipulating the at-RA response pathway in vivo on embryonic development via genetic alterations or pharmacological interventions with natural and synthetic retinoids. The list of genes directly regulated by the RARs expanded rapidly thereafter, and molecular and functional

Figure 1
Schematic representation of the strategy leading to the discovery of the retinoic receptor (RAR). Top: schematic representation of the construction of the hybrid receptor. The main functional domains of the receptors are indicated by DBD (DNA-binding) and LBD (ligand-binding). The LBDs also indicate the identity of the natural ligand of each parent receptor. The cDNAs encoding each receptor were mutated to introduce novel restriction enzyme sites (NotI and XhoI) bordering the DBD. The mutations did not modify the amino acid sequence of each parent receptor. The DBD of the RAR was then switch for the DBD of the glucocorticoid receptor (GR), generating a hybrid receptor (RGR) now able to recognize the glucocorticoid response element (GRE) located within the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). The reporter plasmid consisted in the MMTV-LTR driving the expression of the bacterial gene encoding the enzyme chloramphenicol acetyl transferase (CAT). The hybrid RGR construct as well as the two parent receptors (GR and RAR as positive and negative controls, respectively) were then independently co-transfected into CV-1 cells together with the MMTV-CAT reporter plasmid. Bottom: schematic representation of the result of the original CAT assay performed with extracts obtained from co-transfected cells challenged with a series of putative ligands. The assay revealed in a singular eureka moment that at-RA (RA) was the only ligand eliciting a powerful increase in CAT activity in the presence of the hybrid receptor RGR. A receptor for at-RA had been discovered. A, aldosterone; AC, acetyl chloramphenicol; C, chloramphenicol; C₉C, 25-hydroxy-cholesterol; D, dihydrotestosterone; DEX, dexamethasone; E₂, estradiol; P, progesterone; RA, all-trans-retinoic acid; T₃, triiodothyronine; T₄, thyroxine; VD₃, vitamin D₃.
characterization of multiple RAREs showed RARs could additionally bind to a variety of half-site configurations, including DR-1, DR-2, and DR-5 response elements (reviewed in Giguère 1994).

A second unanticipated outcome of the discovery of the RARs and RXRs was that the transcriptional activity of members of the two distinct retinoid receptor subfamilies was mechanistically linked. The presence of RXR was found to be absolutely essential for high-affinity binding of RAR to DNA and induction of transactivation by RAR (Yu et al. 1991, Buggé et al. 1992, Kliwer et al. 1992, Leid et al. 1992, Marks et al. 1992, Zhang et al. 1992). The binding of the RXR/RAR heterodimer was then shown to be strictly ordered when bound to direct repeats, RXR occupying the 5′-upstream half-site and RAR the 3′-downstream half-site on both DR-2 and DR-5 RAREs (Kurokawa et al. 1993, Perlmann et al. 1993, Predki et al. 1994). The three RXRs were then shown to not only operate as accessory factors for the RARs but, remarkably, also function as partners for a large contingent of the superfamily of nuclear receptors, particularly for adopted orphan receptors (reviewed in Evans & Mangelsdorf 2014).

**RARs and cancer**

As discussed above, vitamin A and retinoids were known to be essential for normal development and the maintenance of a healthy organism. In particular, retinoids were well known to have the ability to suppress the development of the malignant phenotype in vitro (Sporn & Roberts 1983). The finding that disruption of the RARβ gene by integration of HBV in its loci could be a factor in the development of human hepatocellular carcinoma provided the first evidence potentially linking a mutation in a RAR gene and cancer (Dejean et al. 1986, Brand et al. 1988). However, the most stunning breakthrough came from the discovery that the characteristic t(15:17) translocation breakpoint observed in acute promyelocytic leukemia was within the locus encoding RARα (Borrow et al. 1990, de Thé et al. 1990a, Longo et al. 1990, Alcalay et al. 1991). The reciprocal translocation resulted in the generation of the expression of novel chimeric proteins comprised of the fusion of PML to RARα (de Thé et al. 1991, Kakizuka et al. 1991). These findings occurred just after the remarkable clinical observation that complete remission of acute promyelocytic leukemia patients could often be achieved by treatment with high doses of at-RA (Huang et al. 1988, Castaigne et al. 1990, Degos et al. 1990). Despite the expectation that at-RA could be used to induce the differentiation of cancer cells in cultured cells, the success of treating acute promyelocytic leukemia with at-RA did not immediately translate to other types of cancer (Singletary et al. 2002, Chiesa et al. 2007) but as discussed below multiple new cancer studies look very promising. For a short time, concerns arose, as retinoids potentiated tumor growth in certain mouse models and human patients (Ommen et al. 1996, Mikkelsen et al. 1998, Albright et al. 2004, Mollersen et al. 2004). However, the widespread use of oral at-RA (isotretinoin) shows very high safety.

As discussed above, 9-cis-RA, an active metabolite of vitamin A, was discovered by the Evans and Levin labs to be a high-affinity ligand for both RXR and RAR (Heyman et al. 1992, Levin et al. 1992). It is approved for use in T-cell lymphoma in people who are refractory to at least one prior systemic therapy (oral) and for the topical treatment of cutaneous lesions in patients with cutaneous T-cell lymphoma (CTCL) who have refractory or persistent disease after other therapies or who have not tolerated other therapies (topical). It is also used ‘off label’ for non-small cell lung cancer and breast cancer (Esteva et al. 2003, Dragnev et al. 2007). In addition, a recent phase 1 clinical trial has shown that at-RA is a potent stromal targeting agent in pancreatic cancer and is now entering phase 2 status (Kocher et al. 2020, Mere Del Aguila et al. 2022).
Epilogue

The discovery 35 years ago of the RAR by Giguère and Evans (Fig. 2) was the conclusion of a very long scientific odyssey that originated with the study of diseases and high mortality rate afflicting poorly fed infants in Paris in the early part of the 19th century (Semba 2012). Incremental work over decades led to the discovery of the vitamins, their synthesis, and eventually their mechanism of action. The discovery of the RAR occupies a singular place in this journey. First, because it solves an 8-decade mystery of how vitamin A achieves its vital impact in the body. Secondly, it is rare that a discovery can so expeditiously contribute to therapeutic applications from acne to a deadly disease such as acute promyelocytic leukemia. Thirdly, the three RARs soon became primary conduits of new science, not solely on vitamin A as an essential nutrient for life but in fields as diverse as embryonic development, reproduction, neurobiology, immunology, skin diseases, pharmacology, drug design, and cancer biology. Finally, ending on a promising note, the highly successful drug combination used to treat PML in patients (at-RA+arsenic trioxide) has shown great potential in the treatment of pancreatic cancer (Koikawa et al. 2021). We are thus convinced that an unexpected discovery made 30 years ago has a strong future ahead.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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