— **Review** —

Vitamin A in Health

Noriko Takahashi*

Laboratory of Physiological Chemistry, Institute of Medicinal Chemistry, Hoshi University, 2–4–41, Ebara, Shinagawa, Tokyo 142–8501, Japan

(Received January 16, 2010)

The physiological roles of vitamin A have been elucidated based on a knowledge of the relatioships between vitamin A and health. However, this knowledge is not complete and it still remains to be studied what the actions of vitamin A are and the mechanisms behind its actions. Vitamin A, including β-carotene, is consumed from diet, are metabolized and transported within the body. Vitamin A is stored as retinyl ester in the liver, and vitamin A metabolites (retinal and retinoic acid) exert their actions in various target cells (organs). The mechanisms of action of these agents depend on nuclear retinoid receptors and protein modifications. This review provides a comprehensive survey of vitamin A and new insights into the latter's mechanisms of action.

Key words —— vitamin A, retinoylation, retinoic acid receptor, retinoid, retinoic acid, retinal

INTRODUCTION

Vitamin A plays an important role in many essential biological processes. The main function of vitamin A in nature is to serve as a chromophore in sight. Vitamin A deficiency and its over-abundance, markedly change the differentiation state of typical epithelial cells whithin the body. Vitamin A is known to be involved in fetal development and in the regulation of proliferation and differentiation of many types of cells throughout life. In late 1987, the mechanism of action of vitamin A in these processes was made clear by the discovery of nuclear receptors that are specific for retinoic acid, a vitamin A metabolite.[1–3] These receptors regulate gene expression by binding to DNA sequences upstream of target genes. However, there may still be other mechanisms of action and other functions of vitamin A that are not yet discovered. The aim of this review is to show the involvement of vitamin A in health and disease.

*To whom correspondence should be addressed: Laboratory of Physiological Chemistry, Institute of Medicinal Chemistry, Hoshi University, 2–4–41 Ebara, Shinagawa, Tokyo 142–8501, Japan. Tel. & Fax: +81-3-5498-5950; E-mail: t-noriko@hoshi.ac.jp

RETINOIDS AND METABOLISM

Vitamin A (retinol, ROH) and carotenoid are dietary requirements because of the inability to synthesize sufficient quantities in the body. Vegetables including, β-carotene, and lamprey, animal oil and cod liver oil, including ROH or retinyl ester (RE) *etc.* are consumed, and then β-carotene and ROH are absorbed in the small intestine (Fig. 1). In intestinal cells, ROH is generated through intermediate retinal (RAL), which is derived from β-carotene.

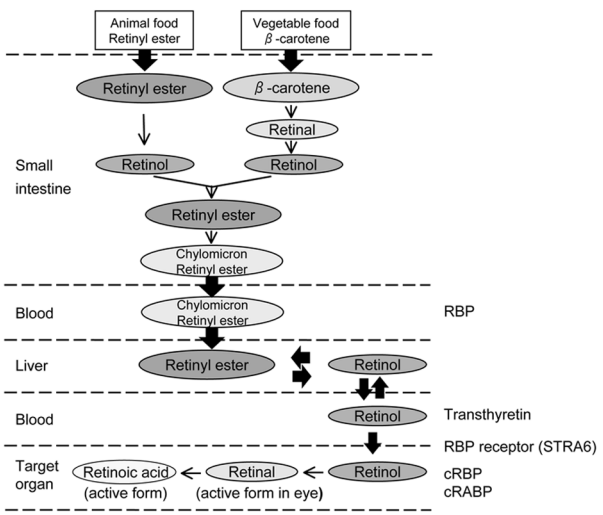

Fig. 1. Vitamin A Metabolism

RBP: retinol binding protein, cRBP: cellular retinol binding protein, cRABP: cellular retinoic acid binding protein.

ROH is then convert to RE. RE chylomicron, generated from RE, moves into the circulatory system *via* the lymphatic system. A chylomicron-RE remnant is formed, which is incorporated into the liver. After degradation of RE in the liver, ROH binds to ROH-binding protein (RBP) and the complex is released from the liver into the circulatory system. ROH-RBP bound to prealbumin or transthyretin, then reaches the target cells. ROH-RBP binds to RBP receptors on the surface of cells and ROH is incorporated into target cells.[4] In the cytoplasm, ROH is generated by oxidization of RAL to retinoic acid (RA).[5] RA is subsequently metabolized by cytochrome P-450s (CYP) including CYP26A1.[6]

RETINOID ACTIONS *IN VIVO*: CAROTENOID, ROH AND RAL

A major dietary source of vitamin A is the carotenoids from fruits and vegetables. These carotenoids have two functions; as antioxidants and as pro-vitamin A function. Oxidative stress is an important physiological reaction to various situations, including exposure to chemicals in air, infection and diseases, particularly cancer. β-Carotene is effective in neutralizing singlet oxygen ($^1O_2$) and inhibiting of oxidation by peroxides.[7] However, these effects are variable and depend on the level of oxygen and on the concentration of β-carotene.[8]

Among retinoids, ROH action is not as well understood. The existence of RBP receptors on RAL pigment epithelium (RPE) as well as other tissues and cells has been known (Fig. 1). The role of RBP receptors is to specifically bind to RBP and facilitate the uptake of ROH from ROH-RBP complexes. Recently, RBP receptors have been identified as the stimulated by retinoic acid 6 (STRA6), a widely expressed multi-transmembrane domain protein.[4,9] In addition, RBP receptors are expressed during embryonic development and exist in placenta, choroids plexus, testis, macropharge, and skin.[10,11]

It is well known that RAL, a ROH metabolite, exhibits visual effects.[12] Visual effects of RAL depend on a number of mechanisms including protein modification by RAL. This latter mechanism is completely different from RA action mediated by cellular RA binding protein (cRABP) and nuclear RA receptors. 11-*cis*-RAL covalently binds to Lysine (-CH=N-Lys) within opsin protein to form a Schiff base (Fig. 2). Protonated rhodopsin is generated, which is able to absorb light. In the resulting light receptor, rhodopsin, wherein opsin proteins bind to RAL covalently, can absorb three kinds of color (437 nm, 533 nm, and 564 nm), as protein structure of RAL binding site are different. Once rhodopsin absorbs light, isomerization of 11-*cis*-retinyl residue occurs, and rhodopsin structure changes, becomes unstable, and quickly decomposes to opsin and *all-trans*-RAL. Subsequently, *all-trans*-RAL is converted to 11-*cis*-RAL by enzymatic isomerization. In this process, visual effects appears to be triggered by conformational changes of rhodopsin proteins, wherein the 11-*cis*-retinyl bond in rhodopsin is converted to *all-trans*-retinyl by the absorption of light. Accompanied by this structural change of rhodopsin, is a chain reaction that includes activation of transducin (G protein), activation of phosphodiesterase, hydrolysis of guanosine 3′,5′-cyclic monophosphate (cGMP) molecule, closure of cGMP-dependent cation (Na^+) channel, and the occurrence of a hyperpolarized potential. This results in the transmission of signals to the optic nerve. As mentioned above, visual effects of RAL entail protein modification by RAL and subsequent reaction with light. Recently, it has been found that acidic glycerophospholipids (phosphatidylserine and phosphatidic acid) release tightly bound 11-*cis*-RAL (the initial compound for vision) from cellular RAL-binding proteins.[13]

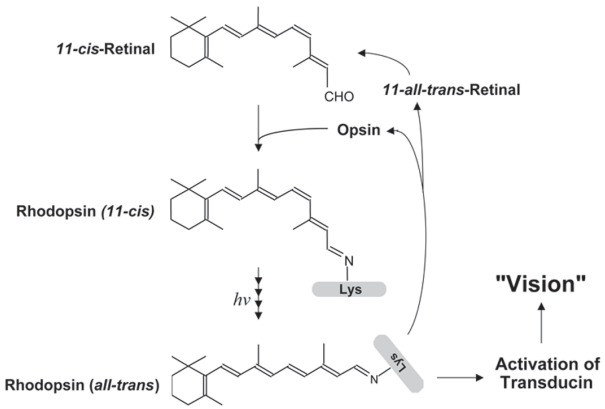

Fig. 2. Rhodopsin: Opsin Bound to Retinal Covalently
Lys: lysine (amino acid).

RA ACTIONS *IN VIVO*

RA is known as the active form of vitamin A. RA shows various effects, including promotion of mucocutaneous formation, growth and embryonic development, as well as induction of cell differentiation, immune regulation and anti-cancer ef-

fects.[6,14–17] In particular, RA's importance is exemplified by a strong differentiation-inducing capability on cells and its utility in the therapy of acute promyelocytic leukemia.[18]

RA effects are mediated by nuclear receptors that bind specifically to RA.[19] RA nuclear receptors (RARs: RA receptors, RXRs: retinoid X receptors) are members of steroid/thyroid multigene families that have specific high-affinity binding sites for RA and its metabolites. It is generally accepted that the action of RA in development and cell differentiation is mediated by these receptors, which directly activate transcription of target genes by binding to specific DNA sequences. While nuclear receptors have been extensively studied, there may still be additional mechanisms of RA action as well as other functions of RA that have not yet been discovered. Many phenomena cannot be explained by RA nuclear receptor-mediated mechanisms.[20] For examples, it has been shown that RARs cannot be involved in the RA-induced differentiation of F9 embryonal carcinoma cells. In this case, function is by mechanisms that do not involve direct interaction with DNA. Some effects of RA are nongenomic.[21] In a study dealing with cells of patients with acute non-lymphoid leukemia and another leukemia cell line [human promyelocytic leukemia cells (HL60) and HL60/RA-res cells], it was found that while RAR levels in cells that underwent induced differentiation by RA was increased slightly, a correlation between RARα and RA action was not observed. Therefore, other mechanisms in addition to RABP and RAR may be involved in RA action. One new mechanism of the RA action is termed retinoylation (protein modification by RA, where acylation of protein by RA) has been proposed.[22] In the following section, RA receptors and retinoylation are presented as RA mechanisms.

MECHANISMS OF RA ACTION

In cells, RA makes a complex with cRABP (Fig. 3), and the following sequence of events then occurs: RA-cRABP moves to the nucleus. Near the nucleus, RA is transferred from cRABP to the nuclear RA receptor (RAR and RXR), and this leads to the gene expression and ultimately changes in protein production. Alternatively, RA-cRABP moves to the microsomes, and retinoyl-CoA is formed from RA and transfered to proteins covalently (termed retinoylation). Retinoylated proteins relocate to the nucleus where they affect gene expression. The physiological functions of vitamin A including visual effects, and effects on skin- and mucous-formation, growth-promotion, cell differentiation-induction, immuno-regulation, and anti-tumor effects, *etc.*, are considered to be dependent on these mechanisms.

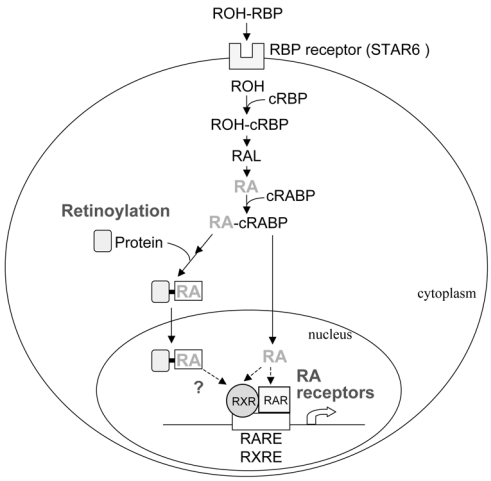

Fig. 3. Signal Transduction Pathway of Retinoic Acid

STRUCTURE AND FUNCTION OF RA RECEPTORS

In the late 1980s to 1990, RARs and RXRs genes were discovered.[1–3] For their ligands, RARs bind to *all-trans*-RA and 9-*cis*-RA. In contrast, RXRs bind to 9-*cis*-RA.[19] There are three subtypes (α, β, γ) for each RAR and RXR. Both of these nuclear receptors are members of the steroid/thyroid nuclear receptor multigene family, which are ligand-inducible transcription factors.[23]

These nuclear receptors have six regions (A–F region) with a common structure (Fig. 4a).[24] The percentages of amino acid sequence homology of each nuclear receptor in the region C and E/F of RARα structure have been calculated, and the features of each region have been defined. The C region contains a binding site for DNA and the E region binds with ligands. Subtypes of RARs (α, β, γ) show high homology. RARα is identical with approximately 97% for the C region with RARβ and RARγ and approximately 82% for RARβ and approximately 76% for RARγ in the E/F region. In addition, homologies between RARα and RXRα are approximately 61% for the C region and approximately 27% for the E/F region. The A/B region

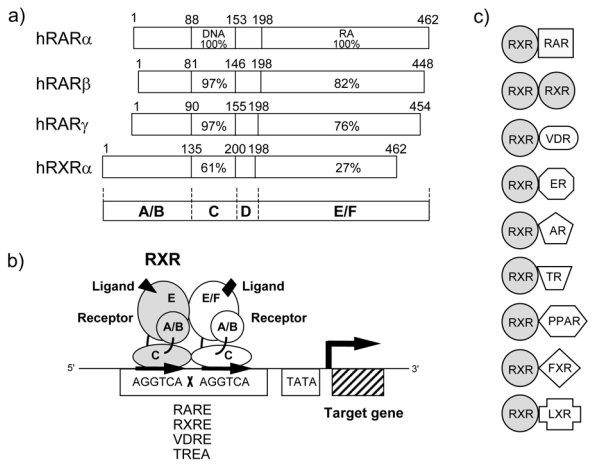

Fig. 4. RA Nuclear Receptors (RARs and RXRs) and Transcription

VDR: Vitamin D receptor, ER: Estrogen receptor, AR: Androgen receptor, TR: Thyroid hormone receptor, PPAR: Peroxisome proliferator-activated receptor, FXR: Farnesoid X receptor, LXR: Liver X receptor.

in the *N*-terminal of RARs is very short as compared to other receptors. The trans-activation function of this region (autonomous function-1, AF-1) shows constant activity with or without a ligand-binding capacity and its activation varies among cell types. The C region located in the center of the nuclear receptor is the site of direct binding to DNA. This exhibits a structure that includes eight molecules of cysteine with two zinc fingers that bind double-stranded structure of DNA. While the *N*-terminal zinc finger structure recognizes specific sequences of chromosomal DNA, the *C*-terminal zinc finger makes binding stronger. The E region in the *C*-terminal is a ligand-binding site. In addition, the E region exhibits dimer-forming ability. Its transcription-promoting function (AF-2) interacts with transcription factors, including co-activators, in regulating ligand-dependent gene expression. Both AF-1 and AF-2 show cell type specificity. Nuclear receptors bound to DNA do not promote transcription in the absence of ligand binding. Therefore, AF-1 activity is limited by AF-2. The F region is absent in RXRs.[23)]

TRANSCRIPTIONAL REGULATORY MECHANISMS OF RA RECEPTORS

Non-steroid nuclear receptors form homo- and hetero-dimers and bind to response elements located upstream of target genes. Recognition sequences are direct repeats of 5′-AGGTCA-3′ or similar sequences (direct repeat: DR), with hetero dimers binding to two tandemly-arrayed sequences (Fig. 4b and 4c). The numbers of bases in these motifs (X) define the pairing specificity of each receptor (1-2-3-4-5 rule). The RAR and RXR heterodimers and RXR homo-dimers bind to RA-responsive elements (RARE, RXRE) upstream of target genes. As gene target of RAR and RXR, the Hox-A1 gene plays a most important role in development of the body shape and somite in embryo development. In addition, genes controlling the expression of RARβ, cRBPI, cRBPII, cRABPII, Krox-20 (transcription regulator), laminin B1, ApoA1 (a lipid transport protein), and platelet-activating factor receptor have also been reported.[23)]

Regulation of transcription by nuclear receptors is similar to molecular mechanisms of DNA-bound transcription factors. TATA boxes form large complexes, and chromosomal DNA is significantly curved to create a better working environment for RNA polymerase (Fig. 4b). Transcription-coupled factors (co-activators and co-repressors) mediate ligand-responsive element interaction with TATA boxes. These are involved in the regulation of transcription without direct binding to DNA. In the case of RAR and RXR, SRC-1, AIB1, TIF2, CBP/p300, and RIP140 as co-activators, TIF1α, β, and SUG1 as co-integrators, and N-CoR, and SMRT as co-repressors have been reported.[23)] In addition, phosphorylation of RAR and RXR is also been known to change biochemical characteristics, such as DNA binding capacity.[23, 25–27)] These are considered to be important in the regulation of transcriptional activity.

KNOCKOUT MICE OF RARS

In order to understand the importance of RAR, RAR gene knockout mice have been investigated. Deletions of RARα genes showed no obvious phenotypes. This is believed to be due to the fact that RAR subtypes (α, β, γ) genes are considered to control a functionally complementary actions. RARγ gene-defective mice and RAR gene-double-knockout mice have also been generated. These mice died shortly after birth in the embryo, or exhibited abnormalities in body organs.[28)] These results lead to recognition of the importance of RAR genes at nascent stages. In particular, abnormal skeletal formation has been observed due to abnormal os-

sification of cartilage in the cartilage replaced by bone. RA has been estimated to play an important role in the formation of chondrocytes from the cartilage primordium.[29)]

DISEASE AND RARS

Among RAR abnormalities, aberrant expression of RARβ by an insertional mutagen of type B hepatitis virus, as well as chromosomal abnormalities of RARα gene in acute promyelocytic leukemia (APL) have been reported. For the latter, the production of promyelocytic leukemia (PML)/RARα is not seen in other types of leukemia cells except APL and in APL patients in remission.[30)]

RETINOYLATION (ACYLATION OF PROTEIN BY RA): POSTTRANSLATIONAL MODIFICATION OF PROTEINS BY LOW-MOLECULAR COMPOUNDS, INCLUDING RA

Protein modifications by low-molecular compounds are well known and include phosphorylation, palmitoylation, mirystoylation, acetylation, lipoylation, ADP-ribosylation, glycosylation, isoprenylation, and arachidonoylation. Such protein modification by ubiquitous ligands, plays important roles in signal transduction processes involved with life. In contrast, RA exists only in trace amounts *in vivo* and is the bioactive substance. As a fatty acid, RA contains a terminal carboxylic acid (Fig. 5). RA is a long-chain fatty acid similar to palmitic acid and myristic acid. Accordingly, it is reasonable that RA exerts at least some of its actions through protein modification by RA, thereby changing the structure, nature, and function of the target protein. Accordingly, studies of protein modification by RA (termed "retinoylation") have been carried out using primarily cell differentiation models involving induction by RA. The objective of these studies has been to elucidate other mechanisms for RA action in addition to nuclear receptors.

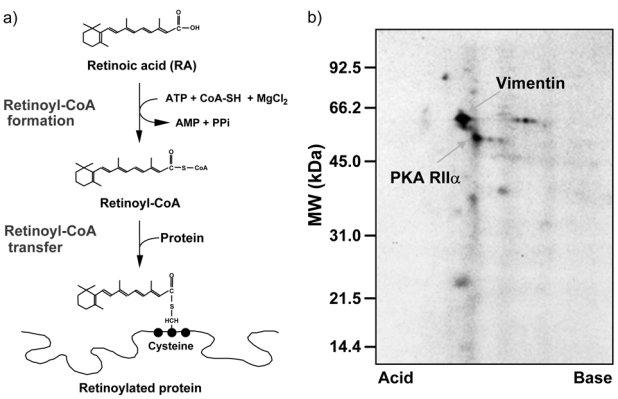

Fig. 5. Two-step Protein Modification by Retinoic Acid (Retinoylation) (a) and Two-dimensional Gel Pattern of Retinoylated Proteins in HL60 Cells (b)[34)]

The major difference between retinoylation and action through nuclear receptors involves the binding mode between RA and protein. While RA binds to the nuclear receptor primarily through hydrogen bonding, in retinoylation, RA bonds RA covalently to the protein. One metabolic pathway for retinoylation utilizes the formation of a retinoyl-CoA intermediate and the subsequent transfer and covalent binding of the retinoyl residue to the protein (Fig. 5a).[31,32)] RA is incorporated into proteins in the acute promyeloid leukemia cell line (HL60) and many other cell lines, *in vitro* and into proteins of cells in liver, kidney, testis, brain and skin, *in vivo*. Therefore, retinoylation is widespread, and the response to RA of different cell types *in vitro* and mammal organs *in vivo* may depend on retinoylation of specific proteins.

RETINOYLATION *IN VITRO*

As indicated abobe, retinoylation is seen in HL60 cells *in vitro* (Fig. 5b). RA links to protein through a thioester bond. The rate of retinoylation of HL60 proteins is dependent on the initial extracellular concentration of RA and it is saturable. The estimated ED_{50} value for retinoylation (300 nM) is close to the ED_{50} value for RA-induced differentiation of HL60 cells (100 nM). Purified nuclei contain most of the cellular retinoylated protein. The extent of retinoylation (mol bound/cell) is approximately five-fold higher in HL60/MRI cells, a mutant that is more sensitive to RA, as compared to HL60 cells.[33)] Retinoylation occurs to the same extent and at similar rates in HL60 cells and HL60/RA-res, a mutant that is resistant to differentiation by RA. However, while same major retinoylated protein (relative molecular mass (Mr) 55000) is the same in both cell lines, HL60/RA-res cells contain a high level of a protein with the same Mr but a more acidic Isoelectric Point (pI). Retinoylation in growing HL60 cultures occurs both on newly formed and preexist-

ing proteins in cells. Formation is very rapid initially and then continues at a linear rate for approximately 48 hr, indicating that it is primarily a modification of preformed proteins.[21] There are similarities between retinoylation and palmitoylation of proteins. Both RA and palmitic acid covalently bind to preformed proteins *via* thioester bonds. Some proteins are substrates for both retinoylation and palmitoylation.[34] In HL60 cells, retinoylated proteins have been identified as the regulatory subunits (RIα, RIIα) of adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent protein kinase (PKA),[35] as well as vimentin (Fig. 5b).[36] This work was performed using an older methodologies employing radiolabelled RA as well as by a new method using RA antibodies.[37]

Retinoylation occurs in other cell lines, including embryonal carcinoma (EC) cells, a normal canine kidney cell line (MDCK), and a human breast cancer tumor cell line (MCF-7).[33, 38] In normal human epidermal keratinocytes (NHEK) grown with RA, retinoylation of proteins in both undifferentiated and differentiated NHEK has been observed. Cytokeratins have been identified as retinoylated proteins in NHEK and the level of retinoylated cytokeratins is greater in differentiating cells than in undifferentiated cells.[39]

RETINOYLATION *IN VIVO*

While retinoylation has been demostrated *in vitro*, determination of whether or not retinoylation occurs *in vivo* was of interest. In vitamin A-deficient rats having intraperitoneally administered radiolabeled RA, proteins covalently bound to RA were detected in kidney, liver, and lung. The amount of retinoylated protein was highest in kidney, then liver and then lung. Retinoylation of protein was by means of ester bonds. The major retinoylated protein in liver and kidney had a molecular weight of approximately 17 K and an isoelectric point of approximately 6.[40]

Normal skin tissue in hairless mice administered RA topically, were found to contain eight RA-binding proteins as detected by new methodology using RA antibodies. The levels of these proteins increased following RA treatment. Three of the eight proteins were identified as cytokeratin 10, cytokeratin 16 and serum alubumin. Cytokeratin 16 is recognized as a stimulating cytokeratin that is expressed during hypergrowth in psoriasis and wound healing. Cytokeratins 10 is specific for keratinized squamous epithelium in abnormal keratinizing epitheliums generated by vitamin A deficiency. It is possible that RA-modification of cytokeratins *in vivo* may be involved in the effects of RA on keratinocytes in mouse skin.[41, 42]

METABOLIC PATHWAY FOR RETINOYLATION

Retinoylation is a two-step reaction, involving the intermediate formation of retinoyl-CoA and subsequent transfer and covalent binding of the retinoyl moiety to protein (Fig. 5a). *In vitro* microsomal preparations, the initial formation of retinoyl-CoA requires rat liver extract, RA, ATP, CoA, and $MgCl_2$.[31] No retinoyl-CoA is formed in the presence of boiled extract or in the absence of ATP, CoA, or $MgCl_2$ (a divalent cation). The amount of retinoyl-CoA formed in rat liver microsomal fractions is greater than in other subfractionations. Retinoyl-CoA is also formed in extracts prepared from rat testis, kidney, spleen and pancreas. The level of retinoylation in various tissue extracts is related directly to the amount of retinoyl-CoA formed. Vmax and Km values for RA in the formation of liver retinoyl-CoA are estuimated to be 1.0×10^{-4} μmol·min^{-1}·mg^{-1} and 24 nM, respectively. Synthesis of retinoyl-CoA is suppressed by fatty acids and fatty acyl-CoAs.[31] These data indicate ATP-dependent generation of retinoyl-CoA occurs in rat tissues, which may play a significant physiological role in RA actions mediated by retinoylation.

The second step of retinoylation involves the transfer of retinoyl-CoA onto protein. In rat tissues, retinoyl-CoA binds covalently to proteins in liver, kidney, testis and brain.[32] The levels of incorporation of retinoyl-CoA into proteins are higher in vitamin A deficient rats as compared to normal rats. The formation of retinoylated proteins is depend on the incubation time, and concentrations of retinoyl-CoA and homogenate. Retinoylation is suppressed by fatty acyl-CoAs and palmitic acid, but not by arachidonic acid. The Vmax and Km values for retinoyl-CoA in the formation of retinoylated proteins using crude liver extract are estimated to be 2.6 nmol/min/mg and 95 μM, respectively. Retinoylated proteins formed from retinoyl-CoA, including a 17 kDa protein exhibiting high radioactivity, disappear in the presence of 2-mercaptoethanol, in-

dicating that RA is linked to proteins through a thioester bond.[32] The above results demonstrate that retinoylation in rat tissues occurs *via* the ATP-dependent generation of retinoyl-CoA formed from RA. This process may play a significant physiological role on RA actions in cells.

SIMILARITY OF RETINOYLATION TO PALMITOYLATION

Retinoylation is more similar to palmitoylation rather than myristoylation.[34] Both RA and palmitic acid (PA) acylate existing proteins and covalently bind to protein *via* thioester bonds, while myristic acid (MA) binds to newly synthesized protein *via* amide bonds. These similarities raise the question whether the same proteins are substrates for both lipids. Palmitoylated proteins (up to 70 proteins) and myristoylated proteins (up to 140 proteins) are greater in number than retinoylated proteins (up to 20 proteins) (Fig. 5b) in HL60 cell. A major retinoylated protein, identified as vimentin, is modified by all three fatty acids. Palmitoylation is not reduced by the addition of RA, and retinoylation and myristoylation are not reduced by the addition of PA. These results indicate that RA, PA, and MA each bind to different amino acids on major retinoylated proteins. Certain proteins are substrates for retinoylation, palmitoylation and myristoylation in HL60 cells.[21,34]

DISEASE AND RETINOYLATION: THE EFFECTS OF RETINOYLATION ON PKA DURING CELL DIFFERENTIATION

Retinoylation is a potential mechanism for induction of differentiation by RA in leukemia cells. In particular, retionoylatin occurs on the RIIα unit of PKA, which catalyzes the cAMP-dependent phosphorylation of proteins (Fig. 6). Retinoylation occurs very quickly after RA treatment in HL60 cells. After five minutes, retinoylation occurs on the RIIα subunit, but not on vimentin, a major retinoylated protein.[21] The RIIα subunit of PKA type II is modified by retinoylation at a high molar ratio of RA bound per mole of protein (1.5 mole RA/mole protein). In addition, retinoylated RIIα is located in nuclei. Therefore, retinoylation may play a role in the translocation of PKA to the nuclus. This translocation places PKA near nuclear proteins that serve as substrates. The rate of phosphorylation of these proteins is increased markedly by Prostaglandin E_2 (PGE_2) (cAMP-elevating agents), which increases the intracellular concentration of cAMP, the activator of PKA.[20] Combining RA with PGE_2 synergistically induces differentiation of HL60 cells.[43,44] High levels of PKA RIIα retinoylation may be the basis for the synergistic induction of differentiation seen by combining RA and cAMP-elevating agents, including PGE_2. Marked reductions are observed in the amount of each agent needed in combination to achieve the same effect as single agents. The dosage of RA needed in combination therapy is unlikely to lead to RA resistance. Such resistance is a limitation of RA therapy against APL. Retinoylation studies have suggested that combinations of RA with PGE_2 may have utility in the clinic for differentiation therapy against APL, and possibly other malignancies.

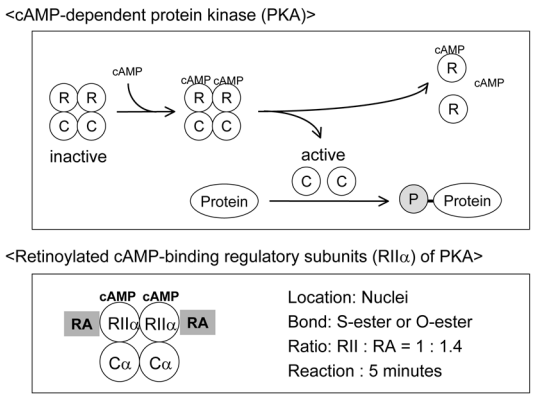

Fig. 6. Retinoylation on cAMP-binding Regulatory Subunits of cAMP-dependent Protein Kinase in HL60 Cells

C: catalytic subunit of PKA, R: regulatory subunit of PKA, P: phospate group.

The retinoylation pattern in leukemia cells from patients is similar to the pattern seen with HL60 cells. It is possible that monitoring retinoylation may be predictive of a patient's response to RA.[45]

FUTURE VIEW

RAL and RA exhibit dramatic actions in the body. RAL is an essential agent for vision and RA is both a potent inducer and repressor of cell differentiation. When RA is used as a sole agent, it can induce complete remission in patients with APL. While one mechanism for the effects of RA involves RA nuclear receptors, retinoylation is an alternate non-genomic mechanism by which RA acts on cells that occurs in a variety of cell types *in vitro* and in

tissues *in vivo*. A variety of evidence suggests that RAL and RA bind to proteins covalently to produce their actions. An early event in RA-induced differentiation may be retinoylation of the RIIα subunits of PKA. Once retinoylated, RIIα units are translocated to the nucleus where phosphorylation of nuclear proteins occurs and cell differentiation is induced. One metabolic pathway for retinoylation involves the intermediate formation of retinoyl-CoA and the transfer and covalent binding of the retinoyl moiety to proteins. It has been found that retinoylation occurs *via* the ATP-dependent generation of retinoyl-CoA formed from RA. This process may play a significant physiological role in the maintenance of healthy physiology. Identification of retinoylated proteins may provide insights into new protein networks that could serve as new targets for therapeutic development.[41, 42, 46–50)]

Acknowledgements I thank Dr. Terrence Burke, Jr. for helpful comments. A part of this review was supported in part by Sankyo Foundation of Life Science, the Ministry of Education, Culture, Sports, Science and Technology, Japan and the Open Research Center Project.

REFERENCES

1) Giguere, V., Ong, E. S., Segui, P. and Evans, R. M. (1987) Identification of a receptor for the morphogen retinoic acid. *Nature*, **330**, 624–629.
2) De The, H., Marchio, A., Tiollais, P. and Dejean, A. (1987) A novel steroid thyroid hormone receptor-related gene inappropriately expressed in human hepatocellular carcinoma. *Nature*, **330**, 667–670.
3) Petkovich, M., Brand, N. J., Krust, A. and Chambon, P. (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature*, **330**, 444–450.
4) Kawaguchi, R., Yu, J., Honda, J., Hu, J., Whitelegge, J., Ping, P., Wiita, P., Bok, D. and Sun, H. (2007) A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. *Science*, **315**, 820–825.
5) Pares, X., Farres, J., Kedishvili, N. and Duester, G. (2008) Medium- and short-chain dehydrogenase/reductase gene and protein families: Medium-chain and short-chain dehydrogenases/reductases in retinoid metabolism. *Cell. Mol. Life Sci.*, **65**, 3936–3949.
6) Thatcher, J. E. and Isoherranen, N. (2009) The role of CYP26 enzymes in retinoic acid clearance. *Expert opinion on drug metabolism & toxicology*, **5**, 875–886.
7) Tourniaire, F., Gouranton, E., von Lintig, J., Keijer, J., Luisa Bonet, M., Amengual, J., Lietz, G. and Landrier, J. F. (2009) beta-Carotene conversion products and their effects on adipose tissue. *Genes & Nutrition*, **4**, 179–187.
8) Zhang, P. and Omaye, S. T. (2001) Antioxidant and prooxidant roles for beta-carotene, alpha-tocopherol and ascorbic acid in human lung cells. *Toxicol. In Vitro*, **15**, 13–24.
9) Noble, B. R., Babiuk, R. P., Clugston, R. D., Underhill, T. M., Sun, H., Kawaguchi, R., Walfish, P. G., Blomhoff, R., Gundersen, T. E. and Greer, J. J. (2007) Mechanisms of action of the congenital diaphragmatic hernia-inducing teratogen nitrofen. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **293**, L1079–L1087.
10) Kawaguchi, R., Yu, J., Wiita, P., Honda, J. and Sun, H. (2008) An essential ligand-binding domain in the membrane receptor for retinol-binding protein revealed by large-scale mutagenesis and a human polymorphism. *J. Biol. Chem.*, **283**, 15160–15168.
11) Kawaguchi, R., Yu, J., Wiita, P., Ter-Stepanian, M. and Sun, H. (2008) Mapping the membrane topology and extracellular ligand binding domains of the retinol binding protein receptor. *Biochemistry*, **47**, 5387–5395.
12) Travis, G. H., Golczak, M., Moise, A. R. and Palczewski, K. (2007) Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. *Annu. Rev. Pharmacol. Toxicol.*, **47**, 469–512.
13) Saari, J. C., Nawrot, M., Stenkamp, R. E., Teller, D. C. and Garwin, G. G. (2009) Release of 11-cis-retinal from cellular retinaldehyde-binding protein by acidic lipids. *Mol. Vis.*, **15**, 844–854.
14) O'Reilly, K., Bailey, S. J. and Lane, M. A. (2008) Retinoid-mediated regulation of mood: possible cellular mechanisms. *Exp. Biol. Med. (Maywood)*, **233**, 251–258.
15) Bremner, J. D. and McCaffery, P. (2008) The neurobiology of retinoic acid in affective disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **32**, 315–331.
16) Bowles, J., Feng, C. W., Knight, D., Smith, C. A., Roeszler, K. N., Bagheri-Fam, S., Harley, V. R., Sinclair, A. H. and Koopman, P. (2009) Male-specific expression of Aldh1a1 in mouse and chicken fetal testes: implications for retinoid balance in gonad development. *Dev. Dyn.*, **238**, 2073–2080.

17) Blaner, W. S., O'Byrne, S. M., Wongsiriroj, N., Kluwe, J., D'Ambrosio, D. M., Jiang, H., Schwabe, R. F., Hillman, E. M., Piantedosi, R. and Libien, J. (2009) Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. *Biochim. Biophys. Acta*, **1791**, 467–473.
18) Huang, M. E., Ye, Y. C., Chen, S. R., Chai, J. R., Lu, J. X., Zhoa, L., Gu, L. J. and Wang, Z. Y. (1988) Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood*, **72**, 567–572.
19) Allenby, G., Bocquel, M. T., Saunders, M., Kazmer, S., Speck, J., Rosenberger, M., Lovey, A., Kastner, P., Grippo, J. F., Chambon, P. and Levin, A. A. (1993) Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 30–34.
20) Breitman, T. R., Chen, Z. X. and Takahashi, N. (1994) Potential applications of cytodifferentiation therapy in hematologic malignancies. *Semin. Hematol.*, **31**, 18–25.
21) Breitman, T. R. and Takahashi, N. (1996) Retinoylation of proteins in mammalian cells. *Biochem. Soc. Trans.*, **24**, 723–727.
22) Takahashi, N. and Breitman, T. R. (1989) Retinoic acid acylation (retinoylation) of a nuclear protein in the human acute myeloid leukemia cell line HL60. *J. Biol. Chem.*, **264**, 5159–5163.
23) Rochette-Egly, C. and Germain, P. (2009) Dynamic and combinatorial control of gene expression by nuclear retinoic acid receptors (RARs). *Nuclear Receptor Signaling*, **7**, e005.
24) Mangelsdorf, D. J., Umesono, K. and Evans, R. M. (1994) The retinoid receptors. In *The Retinoids: Biology, Chemistry, and Medicine* (Sporn, M. B., Roberts, A. B. and Goodman, D. S., Eds.), Raven Press, New York, pp. 573–595.
25) Wang, A., Alimova, I. N., Luo, P., Jong, A., Triche, T. J. and Wu, L. (2009) Loss of CAK phosphorylation of RAR{alpha} mediates transcriptional control of retinoid-induced cancer cell differentiation. *FASEB J.*, **16**, 16.
26) Gaillard, E., Bruck, N., Brelivet, Y., Bour, G., Lalevee, S., Bauer, A., Poch, O., Moras, D. and Rochette-Egly, C. (2006) Phosphorylation by PKA potentiates retinoic acid receptor alpha activity by means of increasing interaction with and phosphorylation by cyclin H/cdk7. *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 9548–9553.
27) Dejean, A. (2002) [Retinoic acid nuclear receptors: a common molecular basis for liver cancer and acute promyelocytic leukemia]. *Bull. Acad. Natl. Med.*, **186**, 751–757.
28) Lohnes, D., Mark, M., Mendelsohn, C., Dolle, P., Dierich, A., Gorry, P., Gansmuller, A. and Chambon, P. (1994) Function of the retinoic acid receptors (RARs) during development (I). Craniofacial and skeletal abnormalities in RAR double mutants. *Development*, **120**, 2723–2748.
29) Yamaguchi, M., Nakamoto, M., Honda, H., Nakagawa, T., Fujita, H., Nakamura, T., Hirai, H., Narumiya, S. and Kakizuka, A. (1998) Retardation of skeletal development and cervical abnormalities in transgenic mice expressing a dominant-negative retinoic acid receptor in chondrogenic cells. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 7491–7496.
30) Cornic, M. and Chomienne, C. (1995) Induction of retinoid resistance by all-trans retinoic acid in acute promyelocytic leukemia after remission. *Leuk. Lymphoma*, **18**, 249–257.
31) Wada, M., Fukui, T., Kubo, Y. and Takahashi, N. (2001) Formation of retinoyl-CoA in rat tissues. *J. Biochem.* (Tokyo), **130**, 457–463.
32) Kubo, Y., Wada, M., Ohba, T. and Takahashi, N. (2005) Formation of retinoylated proteins from retinoyl-CoA in rat tissues. *J. Biochem.* (Tokyo), **138**, 493–500.
33) Takahashi, N. and Breitman, T. R. (1991) Retinoylation of proteins in leukemia, embryonal carcinoma, and normal kidney cell lines: differences associated with differential responses to retinoic acid. *Arch. Biochem. Biophys.*, **285**, 105–110.
34) Takahashi, N. and Breitman, T. R. (1990) Retinoylation of HL-60 proteins. Comparison to labeling by palmitic and myristic acids. *J. Biol. Chem.*, **265**, 19158–19162.
35) Takahashi, N., Liapi, C., Anderson, W. B. and Breitman, T. R. (1991) Retinoylation of the cAMP-binding regulatory subunits of type I and type II cAMP-dependent protein kinases in HL60 cells. *Arch. Biochem. Biophys.*, **290**, 293–302.
36) Takahashi, N. and Breitman, T. R. (1994) Retinoylation of vimentin in the human myeloid leukemia cell line HL60. *J. Biol. Chem.*, **269**, 5913–5917.
37) Kubo, Y., Ohba, T. and Takahashi, N. (2008) Proteins in human myeloid leukemia cell line HL60 reacting with retinoic acid monoclonal antibodies. *J. Biochem.* (Tokyo), **144**, 349–355.
38) Takahashi, N. and Breitman, T. R. (1992) The covalent labeling of proteins by 17 beta-estradiol, retinoic acid, and progesterone in the human breast cancer cell lines MCF-7 and MCF-7/AdrR. *J. Steroid Biochem. Mol. Biol.*, **43**, 489–497.
39) Takahashi, N., Jetten, A. M. and Breitman, T. R. (1991) Retinoylation of cytokeratins in normal human epidermal keratinocytes. *Biochem. Biophys. Res. Commun.*, **180**, 393–400.

40) Myhre, A. M., Takahashi, N., Blomhoff, R., Breitman, T. R. and Norum, K. R. (1996) Retinoylation of proteins in rat liver, kidney, and lung in vivo. *J. Lipid Res.*, **37**, 1971–1977.
41) Takahashi, N. and Fujiu, Y. (2009) Cytokeratins 16 and 10 bind to retinoic acid covalently in skin tissue of mice. *Br. J. Dermatol.*, Epub ahead of print.
42) Takahashi, N. and Fujiu, Y. (2009) Effects of the aminophenol analogue, *p*-dodecylaminophenol on mouse skin. *J. Invest. Dermatol.*, Dec. 24 Epub ahead of print.
43) Olsson, I. L., Breitman, T. R. and Gallo, R. C. (1982) Priming of human myeloid leukemic cell lines HL-60 and U-937 with retinoic acid for differentiation effects of cyclic adenosine 3′:5′-monophosphate-inducing agents and a T-lymphocyte-derived differentiation factor. *Cancer Res.*, **42**, 3928–3933.
44) Ishiguro, S., Takahashi, N., Nemoto, K., Negishi, M. and Ichikawa, A. (1998) Potentiation of retinoic acid-induced differentiation of HL-60 cells by prostaglandin EP2 receptor. *Prostaglandins Other Lipid Mediat.*, **56**, 145–153.
45) Takahashi, N. and Breitman, T. R. (1994). Retinoylation of proteins in mammalian cells. *Vitamin A in Health and Disease* (Blomhoff, R., Ed.), Marcel Dekker, New York, pp. 257–273.
46) Takahashi, N., Honda, T. and Ohba, T. (2006) Anticancer and superoxide scavenging activities of *p*-alkylaminophenols having various length alkyl chains. *Bioorg. Med. Chem.* **14**, 409–417.
47) Takahashi, N., Ohba, T., Yamauchi, T. and Higashiyama, K. (2006) Antioxidant and anticancer activities of novel *p*-alkylaminophenols and *p*-acylaminophenols (aminophenol analogues). *Bioorg. Med. Chem.*, **14**, 6089–6096.
48) Ohba, T., Yamauch, T., Higashiyama, K. and Takahashi, N. (2007) Potent anticancer activities of novel aminophenol analogues against various cancer cell lines. *Bioorg. Med. Chem.*, **15**, 847–853.
49) Takahashi, N., Watanabe, Y., Maitani, Y., Yamauchi, T., Higashiyama, K., and Ohba, T. (2008) *p*-Dodecylaminophenol derived from the synthetic retinoid, fenretinide: antitumor efficacy in vitro and in vivo against human prostate cancer and mechanism of action. *Int. J. Cancer*, **122**, 689–698.
50) Wasserfall, C. and Atkinson, M. A. (2009) Taking a daily vitamin to prevent type 1 diabetes? *Diabetes*, **58**, 24–25.