Differential Role of Homologous Positively Charged Amino Acid Residues for Ligand Binding in Retinoic Acid Receptor α Compared with Retinoic Acid Receptor β

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The diverse biological actions of retinoic acid (RA) are mediated by retinoic acid receptors (RARs) and retinoid X receptors. Although it has been suggested that the ligand binding domains (LBDs) of RARs share the same novel folding pattern, many RAR subtype-specific agonists and antagonists have been synthesized demonstrating that the LBD of each RAR subtype has unique features. We have examined the role of several positively charged amino acid residues located in the LBD of RARα in RA binding. These results are compared with previously published data for the homologous mutations in RARβ. Lys227 of RARα does not appear to be important for RA binding or RA-dependent transactivation, whereas the homologous residue in RARβ, Lys220, plays an important synergistic role with Arg269 in these two activities. In addition, Arg276 of RARα, like its homologous residue Arg269 of RARβ, was found to play an important role in the binding of RA most likely by interacting with the carboxylate group of RA. However, the orientation of and electronic environment associated with Arg276 in RARα appears to be different from that of Arg269 in RARβ, thus contributing to the uniqueness of the ligand binding pocket of each receptor.

Retinoic acid (RA), a vitamin A metabolite, is a potent regulator of a diverse group of biological processes, including growth, differentiation, and morphogenesis (for review, see Ref. 1). These actions of RA are mediated by a group of nuclear proteins, which belong to the multigene family of steroid and thyroid hormone receptors, termed retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (for review, see Ref 2). In dimeric form, the RARs and RXRs function as ligand-inducible transcriptional regulatory factors by binding to DNA sequences called retinoic acid-responsive elements (RAREs) and retinoid X-responsive elements, which are located in the promoter region of target genes. Three subtypes, termed α, β, and γ, of both RAR and RXR have been identified along with several isoforms of each subtype (3–11). In vitro binding assays have demonstrated that only 9-cis-RA is a ligand for the RXRs, whereas both all-trans-RA and 9-cis-RA have been shown to be ligands for the RARs (12, 13).

RARs, like other members of the steroid and thyroid hormone superfamily, have a modular structure consisting of six domains (A–F), each of which has been assigned specific functions (2). The C domain, which contains two zinc fingers, is important for both DNA binding and dimerization. The A and B domains have been demonstrated to have ligand-independent transcriptional transactivation activity (AF-1), whereas ligand-dependent transcriptional transactivation activity (AF-2) is associated with the E domain. The E domain, in addition, also contains all the information necessary for high affinity ligand binding and accessory dimerization sequences.

Recently, the x-ray crystal structures of the ligand binding domains of apo-RXRα and holo-RARα were reported (14, 15). Analysis of these two crystal structures has led to the suggestion that the novel folding pattern observed in these two receptors, an antiparallel α-helical sandwich, may be shared by all members of the steroid and thyroid hormone superfamily (16). In addition, RAR subtype-specific site-directed mutagenesis studies have identified amino acid residues that are functionally important for the binding of RA to a given RAR (17–22).

The similarities and differences between the ligand binding domains of the nuclear RARs have been the topic of many articles in the current literature. The experimentally determined differential retinoid specificity of the three RAR subtypes has been the driving force behind the successful efforts of several medicinal chemists to synthesize RAR subtype-selective agonists and antagonists (23–30). On the other hand, as described above, protein structural chemists suggest a common folding pattern for the ligand binding domains of all RAR subtypes (16). In an effort to help establish the structural requirements for ligand specificity of RAR subtypes, we have examined the relative importance of RA binding of several homologous, positively charged amino acid residues located within the ligand binding domains of RARα and RARβ. Lys227 of RARα, unlike its homologous residue Lys220 in RARβ, does not appear to be important for RA binding. On the other hand, Arg276 in RARα, like its homologous residue Arg269 in RARβ, was found to play an important role in the binding of RA most likely by interacting with the carboxylate group of RA. However, the orientation of and electronic environment associated with Arg276 in RARα appears to be different from that of Arg269 in RARβ, thus contributing to the uniqueness of the ligand binding site of each receptor.
Role of Positively Charged Amino Acids in RARs

MATERIALS AND METHODS

Plasmid Constructs and Site-directed Mutagenesis—Mutants were created according to the site-directed mutagenesis technique described by Higuchi et al. (31). pSG5-mouse RARα and pSG5-mouse RARβ2, generous gifts from Prof. Pierre Chambon (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France), were linearized with BamHI and XhoI, respectively, were used as templates for the preparation of the mutants. Both sense (s) and antisense (as) oligonucleotide primers were purchased from Ransom Hill Biotechnology (La Jolla, CA). The GCT codon and the CAG codon were used to encode the mutant Ala residues and mutant Gln residues, respectively, indicated in bold and underlined in the mutagenic primers.

For the preparation of R276a-RARα, two separate polymerase chain reaction fragments were prepared using the primer pairs RARα a (5′-GGAGGGGATCCATGGCCAGCAATAGCAG-3′) plus R276a-as (5′-CTCAGGCGTTAAGCCGTCGACG-3′) and RARα 3′-as (5′-GAGGG-AGGGATCCATCGAGGGTAGATTTGACTGTATGGAT-3′) and R276a-s (5′-CTCAGTCGCTTACAGCCTGAG-3′), respectively. The two polymerase chain reaction fragments were purified, annealed, and amplified in a second polymerase chain reaction using the RARα 5′-s and RARα 3′-as primers. Likewise, the K227a, R272a, R272q, and R276q mutants were constructed using the RARα 5′-s and RARα 3′-as primers and the following mutagenic primers: K227a-s (5′-CTCTGGGACATCTTGAGGGTAGATTGTACCTG-3′) and R272a-as (5′-GAGGGAGGGATCCATCGAGGGTAGATTTGACTGTATGGAT-3′) plus R276a-s (5′-CTCAGTCGCTTACAGCCTGAG-3′) and R272a-as (5′-GCTGACAGTACAGAACAGAGTACCTGACG-3′), respectively. The 52-base-pair SacI-BspEI fragment that contained the desired mutation was exchanged with that of pSG5-RARα wild type to create each of the mutant DNA constructs. For pSG5-K227aR272a, the 364-base pair PstI-EcoRV fragment of the pSG5-R276a single mutant was exchanged with that of pSG5-K227a. The pSG5-R276a RARβ mutant was created in the same fashion, except that the following primers were used: RARβ 5′-s (5′-GGAGGGGATCCATGGCCAGCAATAGCAG-3′) and RARβ 3′-as (5′-GAGGGAGGGATCCATCGAGGGTAGATTTGACTGTATGGAT-3′), RARβ 3′-as (5′-GAGGGAGGGATCCATCGAGGGTAGATTTGACTGTATGGAT-3′), RARβ 5′-s (5′-CTCAGTCGCTTACAGCCTGAG-3′), and R265q-as (5′-GTTCACTGAAATCAGCTGCTGAGATG-3′), and R265q-as (5′-GTTCACTGAAATCAGCTGCTGAGATG-3′), and the EcoRV-BstXI fragment was exchanged between the wild type and the mutant. In all cases, the presence of the specific mutation and the lack of random mutations were verified by DNA sequence analysis (32).

To make the pET-RARα prokaryotic expression constructs, the full-length RARα wild type coding region was synthesized by polymerase chain reaction using the primers RARα 5′-s and RARα 3′-as and cloned into the BamHI and HindIII restriction sites of pGem3. The various mutants were prepared by fragment exchange between the wild type construct in pGem3 and the given mutant via the PstI and BspEI sites. The entire sequence of the wild type and the site of each mutation were confirmed by DNA sequence analysis. Once constructed in pGem3, each RARα cDNA was subcloned in frame in the BamHI and HindIII restriction sites of pET-29a. For the pET-RARβ constructs, the Mscl-StuI fragment containing the desired mutation was exchanged with that of full-length wild type RARβ previously cloned in frame into the NotI restriction site of pET-29a (22).

Determination of EC50 and Kd Values—Transactivation assays were performed as described previously (17, 18, 33). The EC50 value represents the concentration of retinoid that resulted in 50% of the maximal relative CAT activity determined by extrapolation from the plotted points.

To prepare recombinant protein for the Kd measurements, each pET-29a-RAR expression construct was transformed into Escherichia coli K12 strain BL21(DE3) cells (Novagen) (38). The expression of each S-tag RAR protein and the preparation of the receptor extracts was performed as described previously (22). The production of the recombinant S-tag wild type and mutant RAR fusion proteins in the receptor extracts was monitored using the S-Tag Western blot kit (Novagen). The Western blot analysis of the wild type and all mutant receptor extracts demonstrated a major band that migrated at the same position (approximate molecular mass, 55 kDa) along with several minor, smaller molecular mass bands, which were of similar size.

Retinoid binding assays were performed as described previously (22, 37) with the following exceptions: the total protein concentration in each assay was 6–12 μg, and both 9-cis-RA and all-trans-retinol binding were determined exactly as described for all-trans-RA using either [3H]9-cis-RA (1.74 TBq/mmol (47.2 Ci/mmol); Amersham Corp.) or [3H]all-trans-retinol (1.75 TBq/mmol (47.2 Ci/mmol); DuPont NEN).

Western Blot Analysis—The levels of wild type and mutant RAR protein were determined by Western blot analysis using CV-1 cells transfected with the indicated expression construct essentially as described previously (17, 39).

Electrophoretic Mobility Shift Assay (EMSA)—The wild type RARα, selected mutant RARα proteins, wild type RXRα, and β-galactosidase used in the EMSA were all recombinant S-tag fusion proteins prepared in BL21 cells as described above. BL21 cell extract (25 μg total protein) containing the indicated S-tag proteins were incubated in 25 mM Tris, pH 7.9, 125 mM NaCl, 2.5 mM EDTA, 25 mM dithiothreitol, 12.5 mM MgCl2, 12.5% sucrose, 12.5% glucose, 0.5% Nonidet P-40, and 2.6 mM salmon sperm DNA (Sigma) containing a 32P-labeled RARE probe. The RARE probe was obtained by annealing two complementary single-stranded oligonucleotides (5′-TGCAAGGGTGTCGGTACGAGGTGGCTCTGA-3′ and 5′-CGGTGCAGATCGCCGATCCATCGG-3′), which contain the RARE in the RARβ promoter (positions −63 to −33 relative to the start site of transcription) (42). The resulting double-stranded RARE DNA was filled in with Klenow polymerase (Promega) in the presence of [32P]dCTP (111 TBq/mmol (3000 Ci/mmol); DuPont NEN). Unlabeled cold RARE DNA was used in some assays as a com-
petitor at a 100-fold excess. The RAR-RXR complexes were resolved by electrophoresis through a 6% polyacrylamide gel containing 2.5% glycerol in 0.5 \( \times \) TBE (0.09 M Tris borate, pH 8.2, 0.002 M EDTA) at 200 V for 3 h. The gels were dried and exposed to Kodak XRP x-ray film at 270 °C.

**RESULTS**

**Effect of Site-specific Mutations of RAR-\( \alpha \) on RA-dependent Transactivation and RA Binding**—Initially we examined the roles of Lys 227 and Arg276 of RAR-\( \alpha \) in the binding of RA and RA-dependent transactivation. These are the homologous amino acids to Lys220 and Arg269 of RAR-\( \beta \) (see Fig. 1), which we have previously demonstrated to act together synergistically in the binding of RA (17, 18). Figs. 2 and 3 show representative transactivation assays and saturation binding curves, respectively, for wild type RAR-\( \alpha \) and selected RAR-\( \alpha \) mutants. Table I lists the EC\( _{50} \) values and the apparent \( K_d \) values for the wild type and all mutant proteins. K227A displayed an EC\( _{50} \) value for all-trans-RA and a \( K_d \) for both all-trans-RA and 9-cis-RA comparable to those of wild type RAR-\( \alpha \). On the other hand, R276A displayed low activity, with EC\( _{50} \) and \( K_d \) values for both isomers of RA that were elevated approximately 100- and 50-fold, respectively, when compared with those of wild type RAR-\( \alpha \).

Interestingly, mutation of Arg276 to an Ala (R276A) had a more dramatic effect than the corresponding mutation to Gln (R276Q), with R276A displaying an approximately 3-fold greater increase in both EC\( _{50} \) and \( K_d \) values compared with R276Q. Finally, although the K227A/R276A double mutant displayed very low activity in the RA-dependent transactivation assay, the EC\( _{50} \) value was only increased 3-fold when compared with that of the single R276A mutant.

We next examined the role of Arg272 of RARs in RA binding and RA-dependent transactivation, because its homologous amino acid residue in RAR-\( \beta \) (Fig. 1) has been implicated from its x-ray crystal structure to be one of the positively charged amino acid residues forming the electrostatic field gradient in the ligand binding pocket (15). Mutation of Arg272 to either Ala or Gln had a negligible effect on the \( K_d \) for RA when compared with that of the wild type receptor. On the other hand, the EC\( _{50} \) value of R272A and R272Q in RA-dependent transactivation assays was increased approximately 25-fold when compared with that of the wild type receptor. Since we had observed this lack of correlation between RA binding and RA-dependent transactivation activity when Arg272 of RAR-\( \alpha \) was mutated, we examined the homologous residue in RAR-\( \beta \) (Fig. 1). Interestingly, R265Q RAR-\( \beta \) displayed near wild type activity in both RA-dependent transactivation assays and RA binding studies.

**Effect of Mutation of Arg276 of RARs on Retinol-dependent Transactivation and Retinol Binding**—Since R269Q RAR-\( \beta \) displays high affinity for and transactivation activity with retinol (Ref. 18; see Table II), we examined the activity of R276Q RARs in similar assays. Table II shows that, as expected, wild type RARs do not bind retinol within the limits of the binding assay and has low activity in transactivation assays with retinol (EC\( _{50} \), 1 \( \mu \)M). Unlike R269Q RAR-\( \beta \), R276Q RAR-\( \alpha \) displayed
no detectable binding of retinol or measurable activity in the retinol-dependent transactivation assays. As a positive control for the retinol binding assays, we measured the $K_d$ for retinol of recombinant R269Q RAR$\beta$ and obtained a value of 28 nM, which is quite comparable to our previously measured 18 nM using nuclear extracts prepared from COS cells transfected with R269Q DNA (18). Interestingly, R276Q displayed at least 10-fold lower activity in the retinol-dependent transactivation assays when compared with that of wild type RAR$\alpha$. The small amount of activity observed in the retinol-dependent transactivation assays is likely due to low levels of RA formed within the cells because of the oxidation of retinol. If the RA formed from the oxidation of retinol is indeed responsible for the observed activity, it is not unexpected that R276Q would display a higher $EC_{50}$ value than that of the wild type in retinol-dependent transactivation assays, because R276Q has an approximately 40-fold higher $EC_{50}$ value with RA compared with that of the wild type receptor (300 compared with 8 nM).

Western Blot Analysis and EMSA of Wild Type and Mutant RARs—Fig. 4 is a Western blot showing wild type and selected RAR$\alpha$ mutant protein levels in nuclear extracts isolated from transfected CV-1 cells. A similar level of RAR protein was detected in the nuclear extracts of cells transfected with wild type and all the mutant DNAs. Furthermore, Fig. 5 shows an EMSA using wild type and selected RAR$\alpha$ mutant S-Tag recombinant proteins. All mutant RAR$\alpha$ proteins dimerized with RXRs and bound a RARE, resulting in a gel shift pattern comparable to that of wild type RAR$\alpha$. This demonstrates that the differences between the $EC_{50}$ and $K_d$ values of the wild type RAR$\alpha$s and the mutant receptors (R272A, R272Q, R276A, R276Q, and R227A/R276A) are not likely to be due to any gross conformational changes in these receptors, since they behave normally with respect to dimerization, DNA binding, and expression pattern in transfected CV-1 cells.

| RAR               | $EC_{50}$-all-trans-RA | $K_d$-all-trans-RA | $EC_{50}$-9-cis-RA | $K_d$-9-cis-RA |
|-------------------|------------------------|---------------------|-------------------|----------------|
| Wild type         | 8                      | 1.7 ± 0.3           | 2.6 ± 0.6         |                |
| R272A             | 10                     | 2.6 ± 0.5           | 3.6 ± 0.5         |                |
| R272A             | 175                    | 4.2 ± 0.6           | 5.7 ± 0.4         |                |
| R272Q             | 200                    | 2.0 ± 0.5           | ND                |                |
| R276A             | 900                    | 86.2 ± 5.0          | 92.1 ± 3.0        |                |
| R276Q             | 300                    | 34.5 ± 3.5          | 36.8 ± 0.6        |                |
| R227A/R276A       | 3000                   | >1000              | >1000             |                |
| Wild type         | 8                      | 0.6 ± 0.1           | ND                |                |
| R265Q             | 25                     | 1.2 ± 0.2           | ND                |                |

| Values calculated from Fig. 2. | Mean ± S.E. calculated from Fig. 3. | ND, not determined. | Receptor did not saturate at concentrations of [3H]all-trans-RA or [3H]9-cis-RA up to 250 nM. |

**Table II**

| RAR          | $EC_{50}$ | $K_d$ | RAR          | $EC_{50}$ | $K_d$ |
|--------------|-----------|-------|--------------|-----------|-------|
| Wild type    | 8000      | NB    | Wild type    | 70        | NB    |

| Relative to the maximum relative activity displayed by wild type RAR$\alpha$ at 10^{-6} M RA. | Relative to the maximum relative activity displayed by wild type RAR$\beta$ at 10^{-6} M RA. | No specific binding up to 200 nM [3H]all-trans-retinol. | From Tairis et al. (18). |

**DISCUSSION**

In this report we have examined the effect of mutation of several positively charged amino acid residues of RAR$\alpha$ on retinol binding and retinol-dependent transactivation activity. Arg$^{276}$ was found to play a major role in RA binding and RA-dependent transactivation, whereas Lys$^{227}$ does not appear to be important for either of these two activities in RAR$\alpha$. In addition, Arg$^{272}$ does not appear to be important for RA binding but may be important in the determination of the final active conformation of holo-RAR$\alpha$, since the R272A mutant displayed a significant reduction in RA-dependent transactivation activity. It is unlikely that global conformational changes are responsible for the reduced activity in the RA binding and RA-dependent transactivation assays observed with these RAR$\alpha$ mutants, since all the RARs mutants examined displayed similar levels of expression in transfected CV-1 cells and similar activity in the EMSA.

Table III presents a comparison of the -fold increase in $EC_{50}$ and $K_d$ values for all-trans-RA of several of the RAR$\alpha$ mutants described in this report compared with the homologous RAR$\beta$ mutants that have previously been reported (17, 18). In both RARs and RAR$\beta$, the homologous Arg (Arg$^{276}$ and Arg$^{269}$, respectively) plays an important role in the binding of RA most likely by interacting with the carboxylate group of RA. Site-specific mutation of this Arg in both receptors results in a significant reduction in RA binding and RA-dependent transactivation activity. This is consistent with the RAR$\gamma$ crystal structure, in which the homologous amino acid residue, Arg$^{278}$, has been shown to form a salt bridge with the carboxylate O22 of RA (15). However, we observed several important differences in the response of Arg$^{276}$ in RARs compared with that of Arg$^{269}$ in RAR$\beta$ when mutated to either Ala or Gln. Mutation of this Arg to Ala causes a significant reduction in RA binding and RA-dependent transactivation activity in RARs and has a minor effect in RAR$\beta$ (approximately 75- versus 8-fold). On the other hand, mutation of this Arg to Gln results in a much less dramatic reduction in RA binding and transactivation activity of RARs than that of RAR$\beta$ (approximately 30- versus 1000-fold). Finally, R269Q RAR$\beta$ is a very efficient retinol receptor, whereas R276Q RAR$\alpha$ has no detectable retinol binding or retinol-dependent transactivation activity (Table II). Taken together these data suggest that the orientation of Arg$^{276}$ and Arg$^{269}$ in the ligand binding site and the electronic environment associated with this Arg is different in RAR$\alpha$s and RAR$\beta$, contributing to the uniqueness of the ligand binding site of each receptor.

In RAR$\beta$, Lys$^{220}$ was found to act synergistically with Arg$^{269}$ in the binding of RA, since the simultaneous mutation of both residues to Ala resulted in an effect much larger than the additive effect of each single mutation both in RA binding and RA-dependent transactivation activity. This synergistic effect was not observed with the K227A/R276A RAR$\alpha$ mutant, suggesting that Lys$^{227}$ in RAR$\alpha$ does not appear to be involved directly in the binding of RA; however, it may function as part of the electronic guidance force, proposed to guide RA into the
binding site, described in the crystal structure of RARγ (15). Since the single mutation of Arg276 to an Ala in RARα has such a dramatic effect on RA binding and RA-dependent transactivation activity, it is possible that this single amino acid residue may act more independently in RA binding in RARα than the homologous Arg in RARβ. It is interesting to note that the crystal structure of RARγ shows that Lys226 and Arg278 directly interact with the carboxylate group of RA, whereas Lys329 does not appear to be sufficiently close to the carboxylate group of RA to be directly involved in its binding. It is possible that different positively charged amino acid residues may act synergistically with the RARα Arg276 homologous position in each of the three RAR subtypes, further contributing to the unique nature of each of these three retinoid binding sites.

Based on the crystal structure of RARγ, Renaud et al. (15) have reported that there are 24 amino acid residues distributed over eight structural elements in the ligand binding domain that are positioned within 4.5 Å of RA and therefore delineate the ligand binding pocket. These eight structural elements include H1, H3, H5, β-turn, loop 6-7, H11, loop 11–12, and H12. Furthermore, Wurtz et al. (16) have suggested that the ligand binding pockets of all nuclear receptor holo-ligand binding domains have a similar architecture involving these structural elements. When the amino acid sequences of the three RAR subtypes are compared, only 3 of these 24 amino acid residues lining the ligand binding pocket are variable (Ser232, Ile270, and Val395 in RARα). All three of these divergent residues in RARγ are associated with α-helices, which form the hydrophobic portion of the ligand binding pocket and interact with the β-ionone ring and/or the isoprenoid side chain of RA. These three divergent residues have been suggested to play a role in the determination of the ligand specificity of the three RAR subtypes (15, 16).

Our data demonstrate that Lys227 and Arg276 of RARα, when mutated to Ala and Gln, behave differently in both retinoid binding assays and retinoid-dependent transactivation assays when compared with the homologous mutations in RARβ. These data suggest that there are other structural features of the ligand binding pocket besides the three divergent residues described above that are unique to each receptor subtype. Of the eight structural elements demonstrated to form the ligand binding pocket of RARγ, four of these (H1, H3, C-terminal

TABLE III
Comparison of the -fold increase in EC₅₀ and Kᵄ values for all-trans-retinoic acid displayed by the homologous mutants of RARα and RARβ

|        | RARα  | RARβ |
|--------|-------|------|
|        | EC₅₀   | Kᵄ   | EC₅₀   | Kᵄ    |
| Wild type | 1     | 1   | Wild type | 1     |
| K227A   | 1.3   | 2   | K220A   | 3     |
| R272Q   | 25    | 1   | R265Q   | 3     |
| R276A   | 110   | 50  | R269A   | 7     |
| R276Q   | 38    | 21  | R269Q   | >1000 |
| K227A/R276A | 375  | >60 | K220A/R269A | 500  |

a Fold increases relative to wild type RARα calculated from Table 1.
b Fold increases relative to wild type RARβ calculated from Table 1 and Tairis et al. (17, 18).
portion of H5 including Arg\textsuperscript{278}, and β turn contribute to defining that portion of the pocket involved in the interaction with the carboxylate group of RA. All of the amino acid residues of these four structural elements demonstrated to be within 4.5 Å of RA in RAR\textsubscript{γ} are conserved among the three RAR subtypes. However, when one considers the conservation of all amino acids that constitute these four structural elements of RAR\textsubscript{α} and RAR\textsubscript{γ}, only H1 and H3 contain residues that are not conserved. Six of the 18 residues in H1 (Ala\textsuperscript{187}, Val\textsuperscript{184}, Gly\textsuperscript{185}, Glu\textsuperscript{186}, Ile\textsuperscript{188}, and Val\textsuperscript{190} of RAR\textsubscript{α}) and 4 of the 23 residues in H3 (Ile\textsuperscript{222}, Asp\textsuperscript{223}, Ser\textsuperscript{232}, and Thr\textsuperscript{237} of RAR\textsubscript{α}) differ between RAR\textsubscript{α} and RAR\textsubscript{β}. In addition, 3 of the 5 positively charged amino acids reported to be part of the positively charged electrostatic guidance field in RAR\textsubscript{γ} are located on H3, whereas the other 2 are located on H1. Therefore, it is likely that these divergent residues in H1 and H3 and their effect on the positioning of the positively charged amino acid residues in H3 and H5 may be useful in the construction of subtype-selective retinoids.

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