Dual Function of an Amino-terminal Amphipatic Helix in Androgen Receptor-mediated Transactivation through Specific and Nonspecific Response Elements*

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Steroid receptors are transcription factors that, upon binding to their response elements, regulate the expression of several target genes via direct protein interactions with transcriptional coactivators. For the androgen receptor, additional interactions between the amino- and carboxyl-terminal regions have been reported. The first amino acids of the amino-terminal domain are necessary for this amino/carboxyl-terminal interaction. Deletion of a FQNLF core sequence in this region blunts the interaction, as does a G21E mutation. We investigated the effect of the aforementioned mutations in the context of the full size androgen receptor on a series of selective and nonselective androgen response elements. Strikingly, the FQNLF deletion strongly reduced the androgen receptor capacity to transactivate through nonspecific motifs but did not affect its activity on selective elements. Although the G21E mutation strongly impairs the amino/carboxyl-terminal interaction, it does not significantly influence androgen receptor activity on either selective or nonspecific elements. Surprisingly, this mutation leads to an increased binding of the amino-terminal domain to the glutamine-rich region of the steroid receptor coactivator-1 of the p160 family. Taken together, these data suggest that the amino-terminal amino acids of the androgen receptor play a key role in determining its transcriptional activity by modulating the interaction with the ligand-binding domain as well as interaction with p160 coactivators.

Androgens are involved in the differentiation of the male reproductive organs in the embryo and the development and maintenance of secondary sexual characteristics. The biological actions of androgens are mediated by the androgen receptor (AR). The AR is a ligand-activated transcription factor and belongs to the class I subgroup of the nuclear receptor superfamily. Two structural domains are well conserved among the nuclear receptors (NR): the DNA-binding domain (DBD), consisting of two zinc finger modules, and the ligand-binding domain (LBD), containing a transcription activation function (AF-2) (1, 2). The amino-terminal domain (NTD) and the hinge region are more divergent. Because of their highly conserved DBD, it is not surprising that the class I steroid receptors (AR, GR, progesterone receptor, and mineralocorticoid receptor) recognize the same palindromic (inverted) repeats of the 5′-TGT-TCT-3′ core sequence, spaced by three nucleotides. The mechanisms that lead to the in vivo steroid specificity of gene regulation are still not completely understood (3–6). Selective DNA binding by the AR could be one possible mechanism for hormone-specific gene regulation. It has been reported that the AR-DBD does not only recognize response elements organized as inverted repeats of 5′-TGT-TCT-3′ sequences with a three-nucleotide spacer but can also bind to direct repeats of 5′-TGT-TCT-3′-like sequences, which is proposed to contribute to the AR specificity of transcriptional responses (7–14).

The NTD of the AR is indispensable for AR transactivation and contains a strong transcription activation function (AF-1). Its activity is affected by coregulators that influence a number of functional properties of AR (15–18). Several putative coregulators (coactivators and corepressors) have already been described (19). The best characterized subgroup of receptor-interacting proteins is the family of 160-kDa NR coactivators, also called p160 proteins, comprising steroid receptor coactivator-1 (SRC-1) (20–23), the human and rat transcription intermediary factor 2 (24–26), and its mouse orthologue glucocorticoid receptor-interacting protein 1 (27), and receptor-associated coactivator 3 (28, 29), also known as activator of thyroid hormone receptor (ACTR) (30), thyroid receptor-associated molecule 1 (TRAM-1) (31), amplified in breast cancer (AIB) (32), p300/CBP co-integrator-associated protein (p/CIP) (33), and SRC-3 (34). They interact with the LBD via a centrally located NR-interacting domain containing three highly conserved α-helical LXXLL motifs or NR boxes (20, 28, 29, 35, 36). In addition, the glutamine-rich region of SRC-1 (Qr) can interact with the NTD of the AR in a ligand-independent manner (18, 37–39).

The AR depends on a strong amino/carboxyl-terminal (N/C) interaction for its full activity (18, 40–43). These N/C interactions have also been demonstrated in the progesterone receptor (44) and the estrogen receptor α (45, 46), but in these receptors the implication in receptor activity is less pronounced. The AR N/C interaction involves LKDIIL and FQNLF core sequences, both present in predicted α-helical structures within the NTD (18, 41). The FQNLF motif is located within the thirty amino-terminal amino acids of the AR and has the ability to bind AF-2 of the carboxyl-terminal domain in an androgen-dependent...
manner. The LKDIL motif is located between amino acids 178 and 183 and is important for the transcriptional activation function AF-1. In this paper, we describe the involvement of the FQNLF motif and a flanking conserved Gly in the N/C interaction. In addition, we have analyzed the functional role of this interaction on isolated direct and indirect repeat AREs and on AR-specific versus nonspecific enhancers. Our data suggest that the interaction between the AR-NTD and -LBD may be a prerequisite for its transcriptional activity on nonspecific HREs, whereas it is dispensable on specific HREs.

MATERIALS AND METHODS

**Plasmid Constructs**—The expression vectors pSg5AR538, pSg5AR, pQnFL, and pAR529-435P were made by a PCR-based method. The construct pAR538 was made by using the following primers: 5′-GGATCCTCAAGGAGCTCAGAGC-3′ and 5′-GGATCCCTCAGTGCCCTCCGATGAG-3′. As template, the expression vector for the full-length human AR (pSV-AR), a kind gift of Dr. A. B. Brinkmann (Erasmus University of Nijmegen, The Netherlands) was used. The FQNLF deletion was generated with the oligonucleotides 5′-CTCTAGAGCTCAGAGC-3′ and 5′-CCGGAGACGTCTTCGCGG-3′, in which the mutated base is underlined. These PCR-generated AR-NTD fragments were inserted in frame with the Gal4 and the VP16 fusion constructs into the pGal-4BD-LBD plasmid (Promega). The constructs have been described previously (14). In brief, 5 μl of total extract was preincubated with 1 μl of poly(dI-dC) (1 μg/μl), 10 μl of D100 (20 ng Hepes, 5 μM MgCl2, 0.1 mM EDTA, 17% glycerol, 10 mM NaCl), 1 μl diithiothreitol (20 μM), 1 μl Triton X-100 (1%), and 1 μl of water. Subsequently, the probe is added and incubated for 20 min on ice. Bound probe was separated from the free by electrophoresis for 2 h at 120 V on a 5% polyacrylamide gel. To obtain supershifts, a rabbit antisemir against human AR was added prior to the probe (48). In Western blotting, equal amounts of protein were separated by SDS-PAGE on an 8% gel and blotted onto polyvinylidene difluoride membranes (Amersham BioSciences). The membranes were probed with a monoclonal M2 anti-FLAG antibody (Stratagene), and immunoreactive bands were detected by chemiluminescence (PerkinElmer Life Sciences). For the detection of VP16 fusion proteins, extracts were made in passive lysis buffer. Western blot analysis was performed as described above.

**RESULTS**

**Hormone-dependent Functional Interaction between the LBD and the Amino-terminal Domain**—Previous studies have shown that an FQNLF motif at position 25 in the amino-terminal domain of the human AR plays a key role in the androgen-dependent interaction between the NTD and the LBD. Another motif FQNLF was also postulated as a possible interaction site (14). We have studied the N/C interaction in a mammalian two-hybrid system using a luciferase reporter construct driven by the E1b promoter and containing a tandem repeat of the rat tyrosine aminotransferase-GRE (2×TAT-GRE/E1b-Luc). The first construct consists of a deletion of the first motif (Fig. 1A). The second construct has a point mutation immediately amino-terminally of the FQNLF motif; Gly25 is mutated into a Glu (AR529L435P). Wild type and mutant forms of the AR-NTD domain were fused to the VP16 transactivating domain and were coexpressed in the mammalian two-hybrid assay with pSG5AR538 and pAR529L435P, which encodes a DBD-LBD-containing fragment of the AR (Fig. 1C). Expression levels of the VP16 fusion constructs were similar (Fig. 1D). As expected, the AR529L435P fragment interacts hormone-dependently with AR538. The deletion of the FQNLF motif in AR529L435P completely abolished the R1881-dependent interaction. These findings are in agreement with previous reports (40, 41). Surprisingly, the G21E mutation in the NTD, outside the FQNLF motif, also markedly reduces N/C interaction. In contrast, no difference in the LBD interaction is observed for AR538. This finding is in agreement with previous reports (40, 41). Surprisingly, the G21E mutation in the NTD, outside the FQNLF motif, also markedly reduces N/C interaction. In contrast, no difference in the LBD interaction is observed for AR529L435P.
Role of the FQNLF Motif in Transactivation through Specific versus Nonspecific AREs

The effect of the deletion of the FQNLF motif (hARΔFQNLF) was analyzed in the context of the full size AR on a series of selective and nonselective AREs (Fig. 2). When using wild type AR, the induction factors are 16.5, 19.9, and 8.9 for the nonspecific AREs TAT-GRE, slp-HRE2, and C3 (1)-ARE, respectively, whereas hARΔFQNLF showed a markedly decreased induction of these reporter constructs (6.0, 8.7, and 3.1, respectively). However, for slp-HRE2 and sc-ARE1.2, which are androgen-specific HREs, no difference is seen between the wtAR and the ARΔFQNLF. In contrast, the FQNLF deletion does affect the transactivating abilities of the receptor when tested on the PB-ARE2, which is another known androgen-specific ARE (7, 49).

Two mutations were introduced within slp-HRE2 (slp-HRE2 mut-4T-A; -ARE1.2) and in sc-ARE1.2 (sc-ARE1.2 mut-4T-A; -2A-T), changing these elements into nonselective ones and leading to a functional loss of AR specificity (10). As shown in Fig. 3, this loss of specificity correlates with a strong decrease in transactivation by the ARΔFQNLF in comparison with wtAR activity. Although the activity of the ΔFQNLF mutant on the wild type slp-HRE2 and the sc-ARE1.2 does not significantly differ from that of wtAR, androgen-mediated induction factors of the mutated sc and slp response elements are two or three times lower for the ΔFQNLF construct compared with the wild type AR.

Role of the FQNLF Motif in Transactivation through Complex Enhancers—We tested four luciferase reporter constructs in transient transfections of COS-7 cells (Fig. 4). One construct is driven by the probasin proximal promoter (pPB-Luc). The three other constructs are driven by the thymidine kinase minimal promoter and contain either the C3 (1) intronic enhancer (pC3 (1)-TATA-Luc), the slp enhancer (pSLP-TATA-Luc), or the sc enhancer (pSC-TATA-Luc). Three of them (pPB-
Fig. 3. Transcriptional activation by the wtAR and AR\(^{\text{ΔFQNLF}}\) on slp-HRE2, sc-ARE1.2, and their mutated forms. Luciferase reporter constructs containing two copies of either the wild type or mutated slp-HRE2 and sc-ARE1.2 motifs, indicated on the left, were transiently transfected into COS-7 cells and cotransfected with 20 ng of empty vector, pSG5wtAR, or pSG5AR\(^{\text{ΔFQNLF}}\). The mutated nucleotides are underlined, and the repeats of 5'-TGTCTC-3' -like sequences are indicated by black arrows. The direction of each arrow indicates the orientation of the half-site. The experimental values are presented as in Fig. 2.

Fig. 4. Transcriptional activation of the wtAR and AR\(^{\text{ΔFQNLF}}\) on specific and nonspecific enhancers. COS-7 cells were transfected with 100 ng of the reporter constructs indicated on the left and cotransfected with 20 ng of empty vector, pSG5AR, or pSG5AR\(^{\text{ΔFQNLF}}\). The experimental values are presented as in Fig. 2.

Luc, plSLP-TATA-Luc, and pSC-TATA-Luc) are controlled by androgen-specific elements, whereas pC3 (1)-TATA-Luc is activated by all class I steroid receptors (12). In contrast to the isolated elements tested in Figs. 2 and 3, no difference in the effect of the FQNLF deletion is seen between specific and nonspecific enhancers, because for all reporter constructs, the FQNLF deletion reduces the potency of the AR -2-fold. The average induction factors are 15.0, 15.6, 35.0, and 49.8 for wtAR and 6.2, 4.6, 19.0, and 17.0 for AR\(^{\text{ΔFQNLF}}\) on pC3 (1)-TATA-Luc, pPB-Luc, pSLP-TATA-Luc, and pSC-TATA-Luc, respectively.

Analysis of Gly21 Flanking the FQNLF Core—The point mutation G21E shows a decreased AR N/C interaction similar to the deletion of the FQNLF core in a mammalian two-hybrid assay (Fig. 1C). We tested whether the transcriptional activity of the full size AR is affected by this mutation when specific or nonspecific AREs are used. Surprisingly, the transcriptional activation of the TAT-GRE construct is decreased only marginally when the G21E mutation is introduced in the AR, and no clear effect is seen when the androgen-selective slp-HRE2 was tested. When the nonselective mutant of slp-HRE2 was used, the G21E mutation again had no effect on the functionality of the AR (Fig. 5).

Effects of Mutations within the NTD on DNA Binding—To analyze the influence of the deletion of the FQNLF motif and the G21E and the L435P mutations on the in vitro interaction of the full size receptor with nonspecific and specific AREs, band shift assays using wtAR, AR\(^{\text{ΔFQNLF}}\), AR\(^{\text{G21E}}\), and AR\(^{\text{L435P}}\) were performed (Fig. 6A). COS-7 cells were transfected with expression vectors for wtAR, AR\(^{\text{ΔFQNLF}}\), AR\(^{\text{G21E}}\), and AR\(^{\text{L435P}}\) (1 \(\mu\)g). As a specific ARE, we used slp-HRE2, and as nonspecific elements, we used slp-HRE2 mut-4T-A+2A-T and the TAT-GRE. The gel shift assays showed very similar binding of wtAR and the mutant ARs to different response elements. Western blot analysis of the extracts was performed to assess equal expression levels of the different receptors (Fig. 6B).

Analysis of the AF-1 Activity of the Mutated AR-NTDs and Binding the Qr Region of SRC-1—The AR-NTD contains a strong ligand-independent activation function in the carboxy-terminal region of the AR-NTD (Tau-5). To analyze whether the aforementioned mutations influence this constitutively active Tau-5 function, wild type and mutant forms of the AR-NTD domains were fused to the Gal4-DBD and transfected into COS-7 cells. As reporter construct, we used (Gal4)\(^5\)-TATA-Luc (Fig. 7A). Deletion of FQNLF and the G21E mutation resulted in an activation comparable with that of wtAR-NTD, whereas the L435P mutation resulted in a decreased activation.

It has already been proposed that the efficient recruitment of coactivators by the native AR occurs primarily through the NTD (18, 37–39). SRC-1 is a member of the p160 coactivator family and contains a glutamine-rich region (amino acids 989–1240) called Qr, needed for interaction with the amino-terminal domain of the AR in a ligand-independent manner (18, 37–39). We tested whether the mutations AR\(^{\text{L529ΔFQNLF}}\), AR\(^{\text{L529G21E}}\), and AR\(^{\text{L529L435P}}\), fused to the VP16 activation domain, are still able to interact with the Qr of SRC-1, fused to the Gal4-DBD (Fig. 7B). A striking characteristic for the point mutation G21E is that, although the NTD shows a strongly reduced interaction with the AR-NTD, whereas the L435P mutation resulted in a decreased activation.
the net coactivation of AR\(\Delta FQNL\)F in the presence of SRC-1e is lower.

**DISCUSSION**

For the members of the steroid receptor superfamily such as estrogen receptor \(\alpha\), GR, and progesterone receptor, a ligand-induced conformational change has been clearly demonstrated to result in a hydrophobic recruitment surface (AF-2) for transcriptional coactivators. These coactivators interact with the LBD via three highly conserved \(-\)helical LXXLL signature motifs (35). The AR has been reported to be different from the other steroid hormone receptors in that the AF-2 activity in the LBD is quite low when tested in mammalian cells (2). However, the AR-LBD has the same overall three-dimensional structure as the LBDs of the other nuclear hormone receptors (50).

The AR-NTD not only harbors a strong activation function (AF-1) (16) but also displays a high affinity for the liganded LBD (18, 40–43). LXXLL-related sequences in the AR-NTD have been investigated for their interaction with the LBD. We
already reported the involvement of one LXXLL-like motif, 170LKDIL188 (18), the mutation of which (I182A/L183A) impairs the N/C interaction and the activity of the AR. Two other candidate motifs have been described: 170FQNLF207 and 430WHHTLF436 (40, 41). In this study, we performed a functional analysis of these latter motifs. Deleting the FQNLF motif destroys the ability of the AR-NTD to interact with the AR-LBD. The mutation of Gly21 flanking the FQNLF core to Glu strongly attenuates the N/C interaction. In contrast to another report (40), LBD binding by the L435P mutated NTD is comparable with that observed for the wtAR-NTD (Fig. 1C).

To investigate the role of the N/C interaction in AR functionality in more detail, we analyzed the activity of the mutated receptors on different responsive constructs. Next to the classical nonselective class I elements, there is an additional group of AREs that are only recognized by the AR. Examples of such androgen-specific elements are the PB-ARE2 from the rat pros- basin promoter (7, 49, 51), the SC-ARE present in the first exon of the human secretory component (SC) gene (13, 52), the sc-ARE1.2 in the far upstream enhancer of the human SC gene (8, 10), the slp-HRE2 from the upstream enhancer of the mouse sex-limited protein (53), and the Pem ARE-1 and ARE-2 in the proximal promoter of the murine pem gene (54). Elsewhere, we hypothesized that an alternative dimerization mechanism of the DNA-binding domain would be responsible for the androgen specificity (11). We demonstrated indeed that the AR is the only steroid receptor which binds direct repeats of 5′-TGTTCTT
3′-like sequences (9–11, 13), indicating that the AR could bind the specific elements in a head-to-tail configuration and not in the conventional head-to-head configuration. In this study, we compared the transcriptional activity of the constructs AR-FQNLF, ARG21E, and ARL435P on specific versus nonspecific response elements and enhancers (Figs. 3–5). For the mutation L435P in the WHTLF motif and the mutation I182A/L183A in the LKDIL motif, no difference on selective versus nonspecific elements was seen. In fact, the L435P mutation did not affect the transcriptional activation by any of the tested motifs (data not shown).

We observed a decrease in the transcription activating capacity of AR-FQNLF compared with wtAR on nonspecific HREs, whereas no changes were observed on the specific slp-HRE2 and sc-ARE1.2 elements. However, when these AREs are mutated in their left and/or right half-sites (slp-HRE2 mut-4T-A;+2A-T and sc-ARE1.2 mut-4T-A;–2A-T) to become palindromic repeats, AR transcription was significantly affected by the FQNLF deletion. A possible explanation for why no decrease in AR transcriptional activity is observed on the two specific AREs is that AR dimers, in which the DBD is dimerized in a head-to-tail configuration, might be less dependent on the N/C interaction for transcriptional activation. Whether other factors or cofactors play a role in these differences remains to be elucidated.

Nonspecific HREs seem to be more dependent on the N/C interaction compared with the direct repeat AREs (Figs. 2 and 3). One exception is the AR-specific PB-ARE2, although this might be explained by the fact that PB-ARE2 (5′-GGTCTTT-nnaAGTACT-3′) can be considered as a direct as well as a palindromic repeat. It is clear from Fig. 6A that there are no obvious differences between the wild type and the mutant receptors in their binding characteristics for the different DNA elements.

The G21E mutation strongly reduces N/C interaction (Fig. 1C). It is therefore clear that not only the core FQNLF motif but also some flanking residues contribute. This correlates with the stronger conservation of this region among the AR of different species (Fig. 1B) (41, 55). Surprisingly, the differences seen on AR transcriptional activity at specific versus nonspecific elements by the deletion of FQNLF were not observed with this point mutation. This might be explained by the residual AR-NTD/G21E/AR-LBD interaction, observed in Fig. 1C, which could be sufficient for AR activity on the nonspecific HREs.

The differences we observed for AR-FQNLF transcriptional activity at specific versus nonspecific elements are limited to isolated response elements because no effect is seen on specific and nonspecific enhancers. This could be due to the fact that hormone response elements form part of more complex enhancers, whose activity is also tightly controlled by additional transcription factors (12). The slp enhancer, for example, contains three HREs: HRE-1, HRE-2, and HRE-3, and other transcription factors binding to the slp-ARU were identified as NFκB, octamer transcription factor, and AML3/CFB-f1 (56).

A second interesting feature for the G21E mutation is an almost 3-fold increase in the affinity for the Qr of SRC-1 as measured in double-hybrid assays (Fig. 7B). This increase in Qr binding is not seen for the AR-NTD/FQNLF nor for the L435P mutated AR-NTD. It should be noted that deletion of the FQNLF core and the G21E mutation does not affect the intrinsic activity of the NTD, in contrast to L435P mutation, which reduces the activity 2-fold (Fig. 1C). Possibly, the lowered affinity of the NTDG21E for the LBD and its effect on transactiva-
tion by the AR is compensated by the increased affinity of the NTD for the Qr of SRC-1. SRC-1 coexpression seems to rescue the ARG21E activity to the same level as wtAR and ARL435P (Fig. 7C).

Our data do not exclude the possibility of a stabilization by the NTD of the conformation of the LBD, which would lead to an enhanced recruitment of coactivators as postulated earlier (57). However, the effects of the G21E mutation on Qr recruitment by the isolated NTD (Fig. 7B) leads us to propose a more direct role in the p160 recruitment.

In summary, our findings demonstrate a dual role of the first amino acids of the amino-terminal domain of the AR, which are essential for the N/C interaction and which seem to have an additional function in interaction with Qr of SRC-1. These results contribute to the unraveling of the mechanisms involved in the AR-N/C interactions and the mechanisms of SRC-1 recruitment. Recently, it has been described that the relative contribution of the two interaction sites of SRC-1 with the AR, the LXXLL motifs and the Qr region, depend on the nature of the enhancers (39). In addition, we provide the first indication that transactivation by the AR through some selective AREs (slp-HRE2 and sc-ARE1.2) is structurally different from transactivation through other classical AREs.

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REFERENCES

1. Evans, R. M. (1988) Science 240, 889–895
2. Molianen, A., Rouleau, N., Ikonen, T., Palvino, J. J., and Janne, O. A. (1997) FEBS Lett. 412, 355–358
3. Ponder, J. W. (1995) Science 269, 1122–1133
4. Cleutjens, K. B. J. M., Steketere, K., van Eekelen, C. C. E. M., van der Kort H. A. G. M., Brinkmann, A. O., and Trapman, J. (1997) Endocrinology 138, 5258–5300
5. Beato, M., Herrlich, P., and Schutz, G. (1995) Cell 83, 851–857
6. Truss, M., and Beato, M. (1993) Endocr. Rev. 14, 439–479
7. Claessens, P., Aelen, P., Devoos, A., Peeters, B., Verhoeven, G., and Rombaums, W. (1996) J. Biol. Chem. 271, 19013–19016
8. Verrijdt, G., Schoenmakers, E., Aelen, P., Haclens, A., Peeters, B., Rombaums, W., and Claessens, F. (1999) Mol. Endocrinol. 13, 1558–1570
9. Schoenmakers, E., Aelen, P., Verrijdt, G., Peeters, B., Verhoeven, G., Rombaums, W., and Claessens, F. (2000) J. Biol. Chem. 275, 12298–12305
10. Schoenmakers, E., Verrijdt, G., Peeters, B., Verhoeven, G., Rombaums, W., and Claessens, F. (2000) J. Biol. Chem. 275, 12290–12297
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