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The peptidyl–prolyl isomerase Pin1 regulates phospho-Ser$_{77}$ retinoic acid receptor α stability

Vincent Brondani*, Quirino Schefer, François Hamy, Thomas Klimkait

Institute of Medical Microbiology, University of Basel, Peterplatz 10, CH-4003, Basel, Switzerland

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Abstract

Peptidyl–prolyl isomerases (PPIase) facilitate the *cis–trans* interconversion of the peptidyl–prolyl bond and in such way affect protein folding. Pin1 is a PPIase, which specifically recognizes phosphorylated S/T-P bonds. The transcription factor TFIIH mediates phosphorylation of the retinoic acid receptor alpha (RARα) at position Ser$_{77}$. In the presence of retinoic acid ligand (RA), the Ser$_{77}$ non-phosphorylated receptor is suggested to undergo degradation through the proteasome pathway. Here we provide evidence that Pin1 is able to selectively destabilize RARα in a ligand independent-manner. We show that this is caused by RARα ubiquitination, which in turn is phosphorylation dependent. The single mutation Ser$_{77}$>A completely abolishes RARα degradation whereas the mutation Ser$_{77}$>E rescues this effect. In addition, we correlate RARα stability to Ser$_{77}$ phosphorylation required for the ligand independent transcriptional activity on *fgf8* promoter. Finally, we show that the ligand-independent Ser$_{77}$ phosphorylation requires the genuine ligand-binding domain.

Keywords: RARα; Pin1; Phosphorylation; Protein stability

Phosphorylation is one of the most widely utilized post-transcriptional modifications of proteins and occurs principally at serine, threonine, and tyrosine residues. Phosphorylation contributes to a modification of conformation, and hence the properties of proteins. It also creates the conditions for binding of signal transducers that contain suitable domains capable of recognizing the phosphorylated residue(s). A number of proteins belonging to unrelated families have been found to contain a common module, the WW domain, which was selected during evolution for its ability to recognize phosphorylated amino acids in a proline-rich context [1]. WW domain-containing proteins are either element of large complexes, to which the bound substrate is displayed upon binding, or they possess a catalytic function in a region distinct from the WW domain. Examples of the first type of WW domain-containing proteins are F-box proteins [2], whereas representative of the latter is the peptidyl–prolyl isomerase (PPIase) Pin1 [3].

Pin1 is expressed in all eukaryotes so far examined [4]. It displays a ubiquitous pattern of tissue expression [5], and its protein level does not vary during the cell division cycle [6]. The murine Pin1 is not an essential gene since deletion does not result in any apparent functional abnormalities [4]. Pin1 PPIase recognizes its substrates in a phosphorylation-dependent manner [7] and catalyzes the rotation of the peptide bond preceding a proline residue. Transition through the cell cycle is orchestrated by the subsequent activation of a subfamily of protein kinases, the cyclin-dependent kinases (Cdks). Within the Cdk subfamily, Cdk7 has emerged as an atypical member since it displays invariant activity throughout the cell division cycle [8]. Upon binding to its partners Cyclin H and the assembly factor MAT1,

* Corresponding author. Fax: +41 61 267 3283.
E-mail address: vincent.brondani@unibas.ch (V. Brondani).

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Cdk7 forms a complex (CAK kinase). Cdk7 has been shown to be a component of the general transcription factor TFIIH [9], able to phosphorylate the C-terminal heptad repeats of the large subunit of RNA polymerase II [10]. TFIIH has been shown to associate with the retinoic acid receptors (RARs), a transcriptional regulator belonging to the super-family of nuclear receptors [11]. RARα activity is regulated by phosphorylation and ubiquitination. Phosphorylation by the protein kinase A (PKA) at Ser_{369}, located in the ligand binding domain of RARα, promotes receptor heterodimerization and DNA binding [12], whereas protein kinase C (PKC) dependent phosphorylation in RARα at Ser_{157} in the "T-box" leads to destabilization of the heterodimer–DNA complex [13]. A third important site of phosphorylation in RARα is Ser_{77}. The phosphorylation at Ser_{77} is mediated by Cdk7 and results in a receptor stabilization in the presence of ligand [14,15]. In addition, we recently reported that the Ser_{77} is required for a ligand-independent transcriptional activity of RARα. This constitutive activity was characterized on the *fgf8* promoter [16], an important gene involved in development and cancer.

Given the effect of RARα phosphorylation by Cdk7 and considering that Ser_{77} is located in a proline-rich region, we postulated that phosphorylation by Cdk7 may represent a substrate for Pin1. In this study, we show that Pin1 is able to regulate down RARα activity on *fgf8* promoter by induction of RARα degradation. The RARα destabilization is associated with a ubiquitination process, and we were able to characterize a stable interaction between GST-Pin1 and RARα. In addition, mutant receptors (Ser_{77} to Ala or Glu) were generated to demonstrate the crucial requirement of a phosphorylated Ser_{77} residue for this stable interaction. In order to analyze the specificity of this regulatory mechanism, we investigated Pin1 activity on the RARγ receptor. Using domain swaps between RARα and RARγ receptors we were able to observe that not only Pin1 activity is specific for phosphorylated RARα but also that the RARα Ser_{77} phosphorylation is dependent on the presence of the cognate ligand binding domain (LBD) dependent.

Materials and methods

Cell line, plasmids, and antibodies. Cell line used in this studies were COS cells. The cDNAs corresponding to RAR receptors (RARα and γ), mutants (S77E or S77A), and chimeric receptors (RARα LBDγ and RARα LBDγS77E) were cloned between the site *Eco*RI and *Bgl*II in the mammalian expression vector pSG5 (Stratagene). The cDNAs corresponding to Pin1 and mutant Pin1 (mutation of Arg68 and 69 to Ala) were cloned into pEVRF vectors. Pin1was subcloned in a pFast-Bac GST vector (Invitrogen) for baculovirus expression according to the manufacturer's instructions. The *fgf8* promoter construct is described elsewhere [16]. Polyclonal antibodies against RARα (rabbit polyclonal C-20: amino acid sequence mapping at the carboxyl terminus of RARα of human origin, termed Ab RARα) or against all isoforms (rabbit polyclonal M-454 reacts with RARα,β, and γ, termed Ab RAR) are commercially available (Santa Cruz). Antibodies against Pin1, GST and HA tag are also from commercial sources (Santa Cruz). Retinoic acid (RA) was purchased from Sigma and LnLL was from Calbiochem.

Transfection assays and protein extraction. Cotransfection assays were performed in COS cells using a 75 cm^2-dish format. 2×10^6 cells were allowed to attach to the plate for at least 1 h before transfection. Retinoic acid receptor expression vectors (pSG5 RARα or mutants, RARγ and chimeric receptors) were transiently transfected using lipofectamine (Invitrogen) with or without Pin1 expression vectors into COS cells along with the CAT-based reporter construct. pCMV βGal was used as an internal control for transfection efficiency. Cells were lysed and homogenized in 300 μL lysis buffer (Promega). Of the cell extracts, 120 μL was incubated in a final volume of 200 μL with 0.5 μCi [^{14}C]chloramphenicol (Amersham) and 20 μL of 4 mM acetyl-CoA for 2 h at 37 °C. The reactions were terminated by extracting converted chloramphenicol with 500 μL ethyl acetate. The acetylated products were collected, dried under vacuum, separated by thin layer chromatography (Whatman silicate gel) in chloroform/methanol (95/5), and detected by autoradiography. The protein extracts were analyzed by Western blot in order to characterize retinoic acid receptor expression. Normalized amounts of protein were used for subsequent analysis with a monoclonal antibody against β tubulin. The protein bands were visualized with ECL under conditions according to the manufacturer's instructions.

Retinoic acid receptor immunoprecipitation. The retinoic acid receptor α expression vector was co-transfected with Pin1 and ubiquitin HA-tagged expression vectors. The cells were treated or not with LLnL (50 μM). To examine protein ubiquitination, cells were lysed in 50 mM Tris–HCl, pH 7.5, 5 mM DTT, 1% SDS, and immediately boiled as previously described [17]. Samples were clarified by centrifugation for 10 min at 14,000*g* and diluted with four volumes of 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Triton, and protease inhibitor. Detection of RARα by Western blot analysis was performed following separation of proteins in a 10% SDS–acrylamide gel. Immunoprecipitations were carried out for 4 h on ice using equal amounts of protein and 2 μg of anti-RARα rabbit antibody. Antibodies were immobilized on protein A–Sepharose beads. The resin was washed three times with 10 volumes of same buffer. The beads content was subsequently analyzed by Western blot using a murine monoclonal antibody against the HA tag. The protein bands were visualized with ECL.

Protein expression, purification, and GST pull-down assays. PIN1 was cloned in the pFast-Bac GST expression plasmid (Invitrogen). GST and GST-Pin1 were expressed in SF9 cells according to the manufacturer's instructions. Three days after infection, cells were lysed in buffer L (Tris, HCl, pH 8, 20 mM; KCl 50 mM; DTT 0.1 mM; Triton X-100 0.1%, and antiprotease cocktail without EDTA, Roche) and incubated with glutathione–Sepharose 4B resin (Pharmacia). Recombinant proteins were washed using buffer L and stored at −80 °C in the same buffer supplemented with 10% glycerol. Binding assays were initiated by incubating cell extracts in lysis buffer (see transfection assays) with recombinant GST or GST-Pin1 fusion protein for 4 h at 4 °C. After three washes with 10 volumes of buffer L the bound proteins were subjected to SDS–PAGE, transferred to nitrocellulose membrane, and immunoblotted with RARα antibodies. The protein bands were visualized with ECL.

Results and discussion

Pin1 downregulates fgf8 promoter activity by RARα destabilization

We recently reported that the retinoic acid receptor RARα is able to activate the fibroblast growth factor

8 (*fgf8*) promoter by two pathways. The first element, a canonical DR2 sequence, acts as *cis*-element to promote a retinoic acid (RA) dependent transactivation. Another *fgf8* promoter region was characterized as a RARα unliganded response element. This second promoter region is involved in a ligand independent RARα transactivation activity, which requires a RARα Ser_{77} phosphorylated residue [16] located in the activating function 1 (AF1) of RARα (Fig. 1A). We first investigated by cotransfection whether Pin1 and a mutant Pin1, in which the peptidyl–prolyl isomerase activity is abolished (Pin1 $R_{68,69}$A, depicted as mPin1, Fig. 1A), were affecting the RARα ligand-independent transactivation activity. COS cells were cotransfected with the *fgf8* promoter reporter construct (p*fgf8*-CAT) together with the RARα expression vector (pSG5 RARα) and/or Pin1 expressing vectors (pEVRF Pin1 and mPin1). RARα ligand-independent transactivation was analyzed by scoring chloramphenicol acetyl transferase activity (CAT). Results, depicted in Fig. 1, show that a CAT activity is observed upon cotransfection of RARα receptor (lane 2) but not by cotransfection of Pin1 or mPin1 (lanes 5 and 6). In addition, the RARα transactivation is abolished only by cotransfection with the wild type Pin1 expression constructs (lane 3 versus 4). As a control, we investigated if the RARα protein expression level is stable in this cotransfection context. As already described by others [15,16], the unphosphorylated RARα receptor is destabilized under RA treatment. As a control experiment, the pattern of RARα protein level was therefore analyzed under both conditions: with or without RA treatment. Immunoblot analysis (Fig. 1C) showed that the receptor is detectable only in cells transfected by RARα (see antibody RARα, panel pSG5 versus pSG5 RARα). In the absence of RA, two bands are observed (lane 1) whereas the RA treated cells (lane 4) show only one band. According to the literature, the easiest explanation would be that the two bands reflect a post-translational modification such as protein phosphorylation. This modification should correspond to the Ser_{77} phosphorylation, which was described as a stabilization signal under RA treatment. In fact, only the upper band, corresponding to the modified protein, is observed in RA treated cell extract. In the case of cotransfection with Pin1 wild type, the RARα protein level is modified. The upper band corresponding to the phosphorylated receptor is not detectable (lane 2). For the RA treated cells (lane 4) RARα protein expression is completely abolished. Cotransfection of the mutant mPin1 did not affect RARα (lanes 3 and 6) under both conditions. As a control, the RARα RNA expression level was assessed by semi-quantitative RT-PCR, and no differences were observed between the samples (data not shown).

Pin1 interacts with RARα and induces its degradation

To address the question of how RARα is decreased upon cotransfection with Pin1, we examined the possible involvement of the ubiquitin-mediated pathway. To this aim, COS cells were cotransfected with pSG5

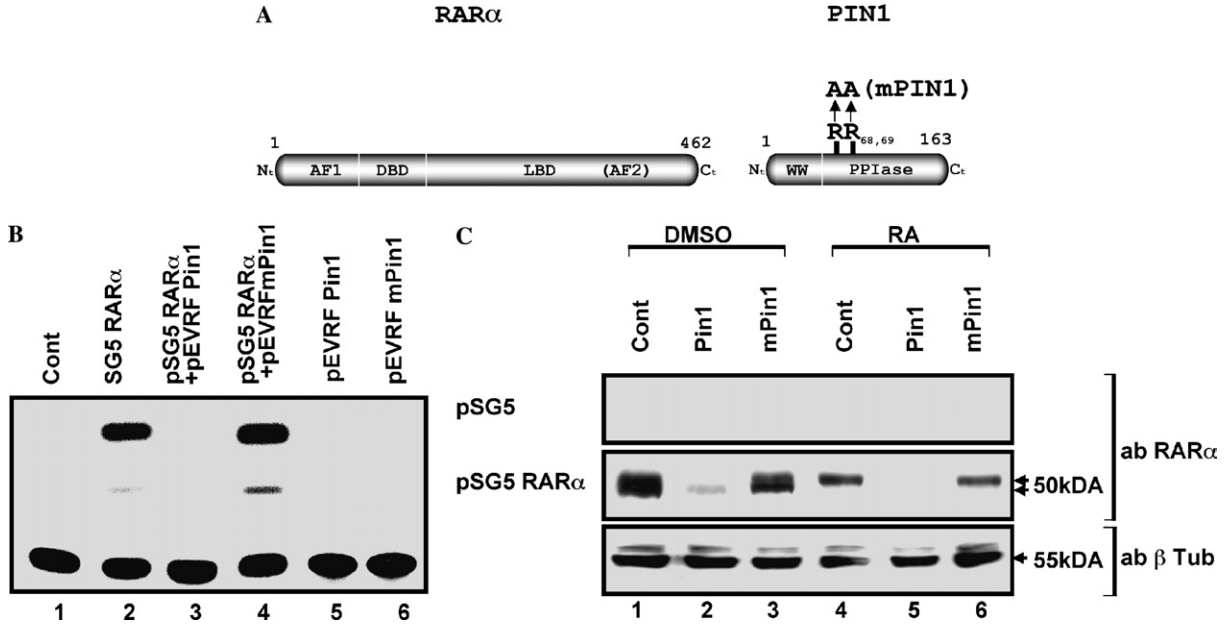

Fig. 1. Pin1 downregulates *fgf8* promoter activity by RARα destabilization. (A) Schematic representation of RARα and Pin1. Size of protein and functional domains are depicted. (B) Chloramphenicol acetyl transferase induction assay of RARα ligand-independent transcriptional activity on *fgf8* promoter. COS cells were transfected with a p*fgf8* CAT reporter construct along with pSG5 RARα expression vector with or without pEVRF Pin1 and mutant Pin1 (mPin1) expression vector. (C) RARα protein expression in transfected COS cells. COS cells were cotransfected with the pSG5 parental vector or pSG5 RARα together or not with pEVRF Pin1 wild type or mutant. The cells were treated or not with all *trans*-retinoic acid (+RA) or with DMSO.

RARα, pEVRF Pin1 and a vector expressing a HA tagged ubiquitin. Cells were treated or not with the proteasome inhibitor LLnL and proteins were extracted in a buffer containing 1% SDS and boiled in order to block degradation of ubiquitinated proteins. Extracts were subsequently analyzed by immunoblotting using antibody against RARα. Results depicted in Fig. 2A (left panel) show that the receptor is stabilized upon LLnL treatment (lanes 1 and 2, RARα protein at 50 kDa). Additional bands are observed in the LLnL treated cell extract (lane 2, arrows on the right side). The same extracts were immunoprecipitated using a polyclonal rabbit RARα specific antibody and analyzed using a mouse antibody against HA-tag. The obtained results (Fig. 2A, right panel) indicate that the immunoprecipitated receptor is ubiquitinated (lane 2). To further analyze if Pin1 is directly involved in this process, we tried to characterize a direct interaction between RARα and Pin1 by performing a GST pull-down assay using GST-Pin1. GST-Pin1 and GST were expressed in SF9 cells infected with baculovirus coding for each protein. The purified proteins were analyzed on a 12% SDS–acrylamide gel and detected by Coomassie blue staining (Fig. 2B, left panel). The same samples were subjected to immunoblot analysis using antibody against Pin1 (middle panel) and GST (right panel). The Pin1 specific antibody interacted only with the 45 kDa protein corresponding to GST-Pin1. As expected, the antibody against GST recognized both products (45 and 27 kDa corresponding, respectively, to GST-Pin1 and GST). The proteins associated to the glutathione 4B–Sepharose beads were then used to perform a GST pull-down assay (Fig. 2C). Protein extracts from mock transfected cells pSG5 (top panel) or from pSG5 RARα (bottom panel) transfected cells were incubated with GST or GST-Pin1 beads. The obtained pull-down samples were analyzed by immunoblotting and RARα was detected using a specific antibody. RARα was detected in the input lane (bottom panel, lane 1), and when associated with the GST-Pin1 protein. Only the upper band, corresponding to the post-translationally modified receptor, was found on the beads. These results suggest that the receptor requires a phosphorylation site for this molecular interaction.

Pin1 interacts with Ser_{77} phosphorylated RARα and induces its degradation

To access whether it is the phosphorylation event that is required in this molecular interaction, and knowing that the receptor stabilization required the Ser_{77} residue phosphorylation, we next compared protein stability and Pin1 interaction of engineered mutant receptors (Fig. 3A). The serine residue was either substituted by an alanine residue (S77A), mimicking an unphosphorylated receptor at this position, or by a glutamic acid (S77E), a side-chain which is isosteric to phosphorylated serine. Cotransfection experiments with p*fgf8* CAT promoter construct were performed. Results show a high

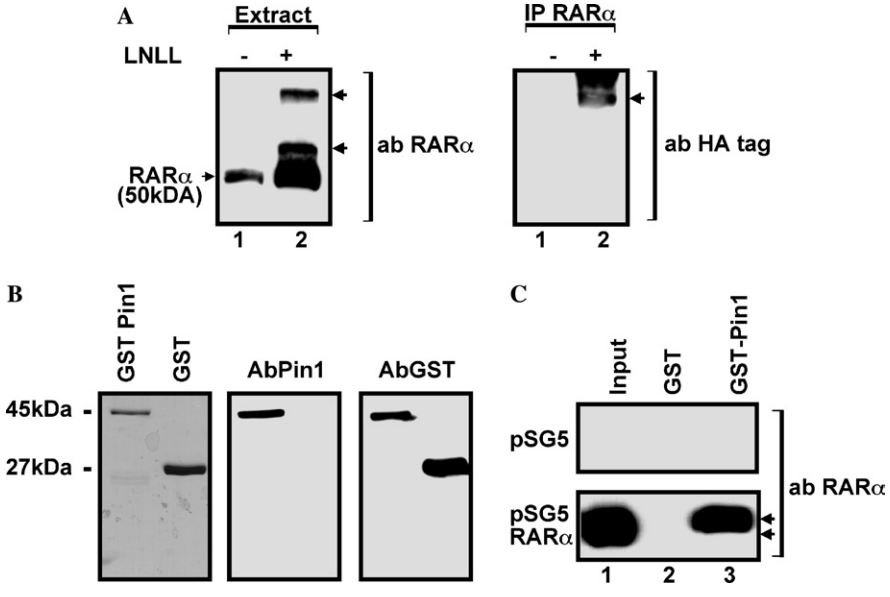

Fig. 2. Pin1 interacts with RARα and induces its degradation. (A) COS cells were cotransfected with pSG5 RARα, pEVRF Pin1, and pHAtag-ubiquitin expression vectors and treated or not with proteasome inhibitor (LNLL). Cell extracts were subsequently analyzed by immunoblot for RARα expression characterization (Ab RARα). The same samples were immunoprecipitated using Ab RARα and beads were suggested to immunoblot using Ab HA tag for detection of RARα ubiquitinated products. (B) Purification of GST and GST-Pin1, and immunoblot analysis of products with antibodies against Pin1 and GST. (C) GST pull-down assay using GST and GST-Pin1 purified protein and COS cell extracts from cells transfected by pSG5 parental vector or pSG5 RARα.

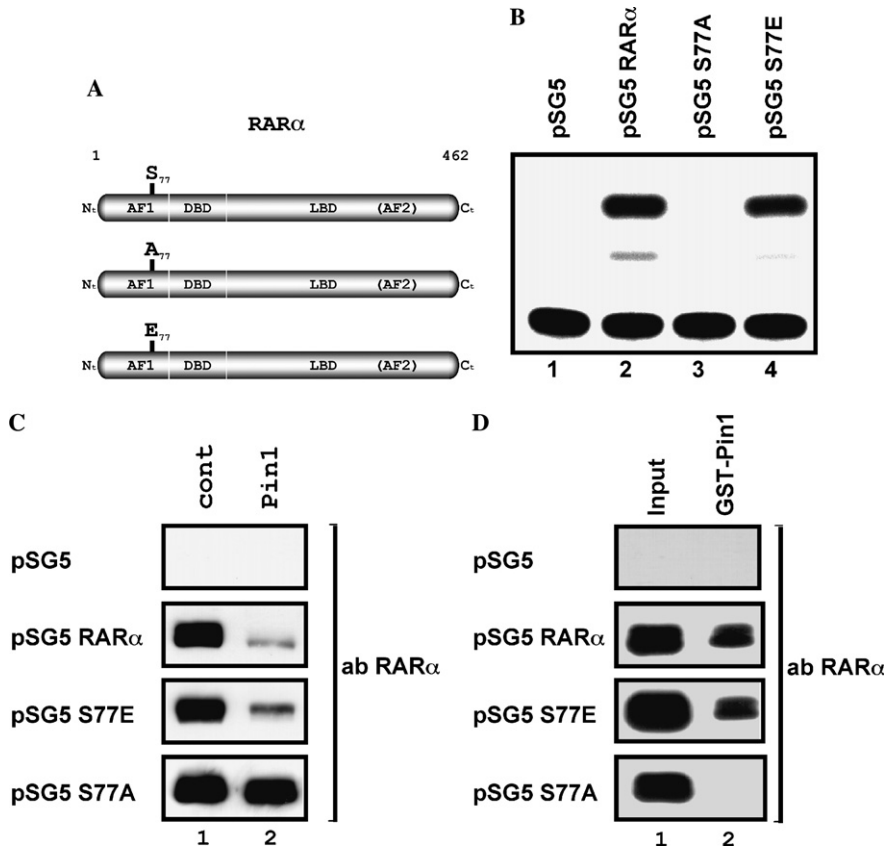

Fig. 3. Pin1 interacts with Ser_{77} phosphorylated RARα and induces its degradation. (A) Schematic representation of RARα and Ser_{77} mutated in alanine (S77A) or in glutamic acid (S77E). (B) Chloramphenicol acetyl transferase induction assay of RARα ligand independent activity on *fgf8* promoter. COS cells were transfected with p*fgf8* CAT reporter construct along with pSG5 RARα or RARα mutants (S77A and S77E) expression vectors. (C) RARα protein expression in COS transfected cells. COS cells were cotransfected by pSG5 parental vector or pSG5 RARα or RARα mutants (S77A and S77E) together or not with pEVRF Pin1. (D) GST pull-down assay using GST and GST-Pin1 purified protein and COS cell extracts from cells transfected by pSG5 parental vector or pSG5 RARα or RARα mutants (S77A and S77E).

CAT activity by cotransfection with RARα wild type or S77E mutant (Fig. 3B, lanes 2 and 4), whereas the S77A mutation completely abolished the constitutive activation by RARα (lane 3). This enlightened the crucial role of the serine residue at position 77 in this RARα ligand-independent transactivation activity. In order to analyze the protein stability, COS cells were transfected with wild type, S77A or S77E mutated RARα expression vectors. Each transfection was analyzed alone (Cont) or after cotransfection with pEVRF Pin1 (Fig. 3C). The immunoblot analysis of protein extracts shows that the receptors RARα and S77E are destabilized by Pin1 cotransfection (lane 1 versus 2 for the panel pSG5 RARα and S77E). In contrast, Pin1 had no destabilizing effect on the expressed S77A mutant (panel pSG5 S77A). These results were then correlated to GST-Pin1 pull-down assay using the same extracts (Fig. 3D). In fact, an interaction between GST-Pin1 and RARα or mutant receptor was observed only with the wild type and the S77E receptors (panel pSG5 RARα and S77E, lane 1 versus 2). The mutant S77A receptor, which is stable upon pEVRF Pin1 cotransfection, did not interact with GST-Pin1 (panel pSG5 S77A).

Here we demonstrated that only the phosphorylated RARα receptor is destabilized upon Pin1 cotransfection. This process is associated to the ubiquitin-mediated pathway and requires a phospho-serine at the position 77. This phospho-serine residue itself is required for the interaction between RARα and Pin1.

RARα Ser_{77} phosphorylation is ligand-binding domain dependent but not Pin1 destabilizing activity

Members of retinoic acid receptor family are highly conserved, and their activity and stability are regulated by phosphorylation. We therefore examined whether other members of this family have a similar capacity for such regulation. Retinoic acid receptor gamma (RARγ) activity was then analyzed on the *fgf8* promoter by cotransfection of p*fgf8*-CAT and pSG5 RARγ. The obtained results demonstrate that the constitutive transactivation is RARα specific (Fig. 4B, lane 2 versus 3). In fact, cotransfection of a RARγ expressing vector did not at all increase CAT activity. Also stability upon Pin1 cotransfection was analyzed by immunoblotting using the antibody against all RAR isoforms. The results

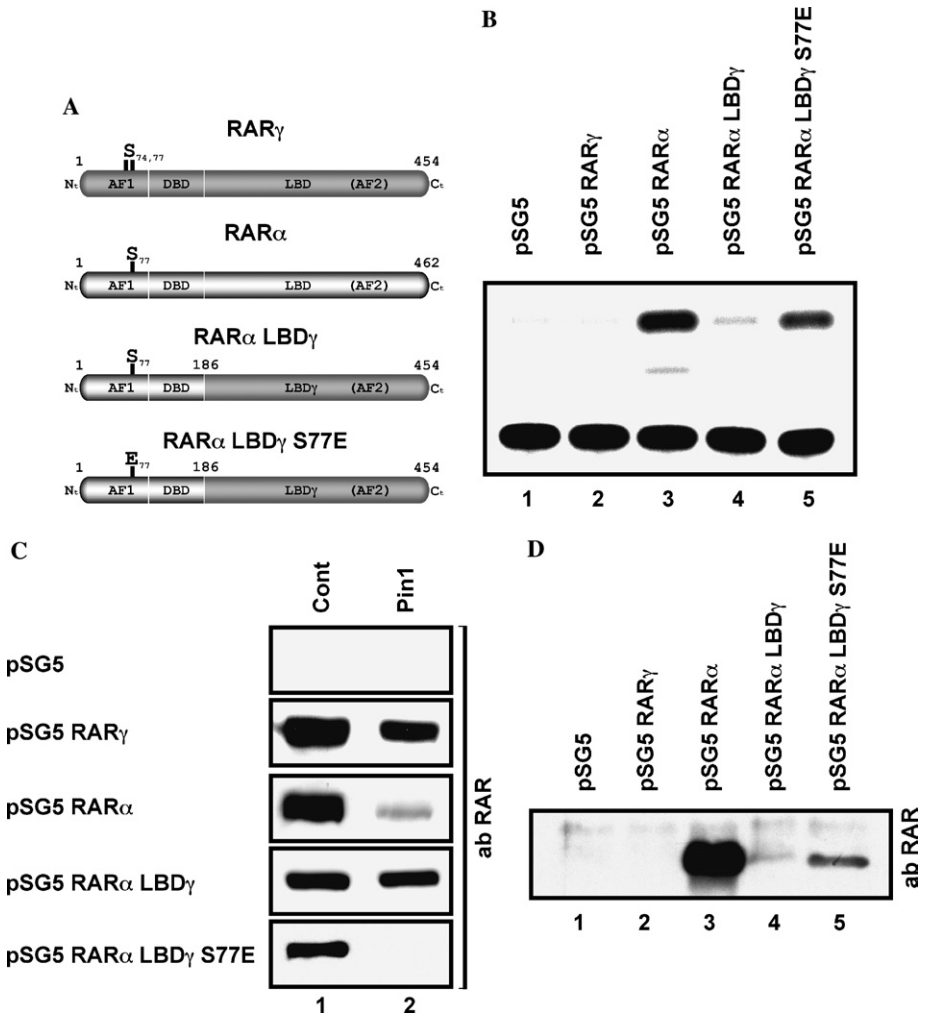

Fig. 4. RARα Ser_{77} phosphorylation is ligand-binding domain dependent. (A) Schematic representation of RARα, RARγ and chimeric receptors (RARαLBDγ and RARαLBDγS77E). (B) Chloramphenicol acetyl transferase induction assay of RARα ligand independent activity on *fgf8* promoter. COS cells were transfected with p*fgf8* CAT reporter construct along with pSG5 RARα, RARγ, RARαLBDγ or RARαLBDγS77E expression vectors. (C) RARα protein expression in COS transfected cells. COS cells were cotransfected by pSG5 parental vector or pSG5 RARα, RARγ, RARαLBDγ or RARαLBDγS77E expression vectors with or without pEVRF Pin1. (D) GST-pull-down assay using GST and GST-Pin1 purified protein and COS cell extracts from cells transfected by pSG5 parental vector or pSG5 RARα, RARγ, RARαLBDγ or RARαLBDγS77E expression vectors.

(Fig. 4C, lane 1 versus 2 panel pSG5 RARα and RARγ) indicated that the RARγ protein stability is not affected by Pin1 over-expression. Finally, a GST-Pin1 pull-down experiment confirmed that RARγ did not interact with Pin1. In fact, using an input corresponding to lane 1 of Fig. 4C, no receptor RARγ is detected (Fig. 4D, lane 2) whereas the RARα protein is observed (lane 3).

One particular difference between these two receptors is the phosphorylation site at position 77. The receptor RARγ is phosphorylated at two neighboring positions (Fig. 4A, residue 74 and 77). In order to further define if the LBD influenced the constitutive RARα receptor activity, we designed the vector expressing a chimeric receptor. The chimeric receptor (Fig. 4A) corresponds to the fusion of the 5′ extremity of RARα (amino acid 1–186) which includes the activating function 1 (AF1) and DNA binding domain (DBD), with the LBD of RARγ (amino acid 187–454). Transactivation activity of chimeric receptor was analyzed by cotransfection with the p*fgf8*-CAT reporter construct. The observed activity demonstrates that the AF1-DBD region of RARα fused to the RARγ LBD (corresponding to the vector pSG5 RARα LBDγ) is not able to induce RARα ligand-independent transactivating activity (Fig. 4B, lane 4). These results suggest that RARα transactivation activity requires the genuine RARα LBD. As described before, transactivation required Ser_{77} phosphorylation. A chimeric control receptor including the mutation of Ser_{77} to glutamic acid (vector pSG5 RARα LBDγS77E) was constructed in order to assess whether this loss of activity was associated with a constitutive dephosphorylation of residue Ser_{77}. The transactivation activity

obtained by p*fgf8*-CAT cotransfection shows that receptor activity is recovered by this single mutation. Taken together, these data suggest that Ser_{77} phosphorylation is crucial for the RARα transactivation. In addition, the genuine RARα LBD domain is required for the Ser_{77} phosphorylation.

To further confirm these observations, the destabilization by Pin1 was analyzed as previously. As expected, the chimeric receptor is stable upon Pin1 over-expression, whereas the mutant S77E is completely destabilized (Fig. 4C, panel pSG5 RARα LBDγ and RARα LBDγS77E, lane 1 versus 2). In addition, the pull-down assay shows that the interaction between Pin1 and the chimeric receptor is only observed with the mutant S77E (Fig. 4D, lanes 4 and 5 for the chimeric receptor RARα LBDγ and RARα LBDγS77E, respectively). The Pin1 destabilization activity is only associated with Ser_{77} and did not require an authentic RARα LBD.

RARα stability and degradation through the proteasome pathway

Altogether, our results suggest that RARα protein stability is regulated by several mechanisms (Fig. 5). As already observed and well documented by others, the non-phosphorylated Ser_{77} receptor RARα is destabilized in the presence of ligand RA. These results support the idea that the LBD domain is essential for efficient RARα ubiquitination and degradation through the proteasome pathway. The receptor RARα is stabilized upon phosphorylation of the Ser_{77} by the general transcription factor TFIIH. The phosphorylation reaction catalyzed by TFIIH requires the LBD domain but not RA binding. In addition, the interaction between RARα and TFIIH is stable.

Here we reported an additional level of regulation of RARα protein stability. The RARα receptor is destabilized upon Pin1 over-expression in the absence or under RA treatment. The mechanism involved in this process requires a phospho-serine at position 77, and only this receptor is able to interact with Pin1. This observation is attributed to the fact that Pin1 recognizes its substrates in a phosphorylation-dependent manner. The unphosphorylated RARα receptor is only partially affected by Pin1 over-expression. This could reflect the turnover of TFIIH in the phosphorylation process. In fact, TFIIH stably interacts with RARα [15] and is probably released after degradation of the phosphorylated receptor. Additionally, the RARα receptor requires the genuine LBD for destabilization because only the wild type receptor is phosphorylated. Finally, these mechanisms are specific to RARα and were not observed with the RARγ receptor.

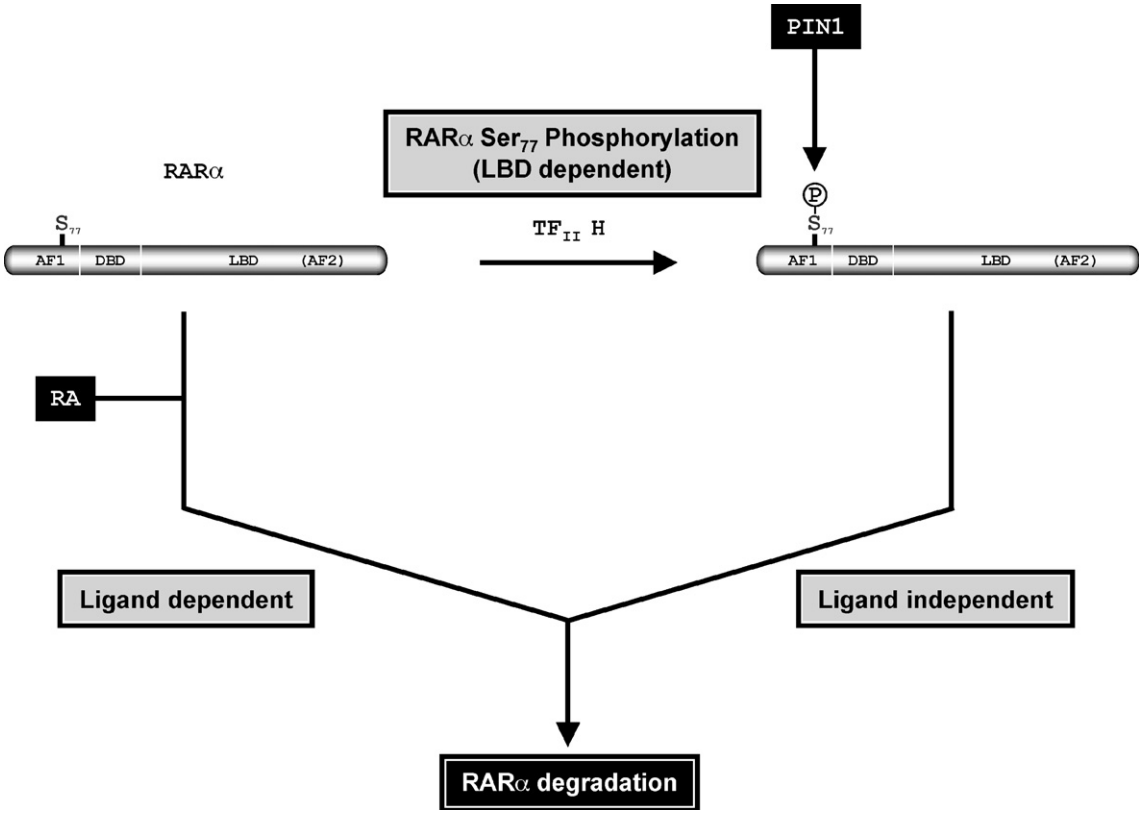

Fig. 5. Model of regulation of RARα stability.

We have previously reported on the analysis of the RARα receptor on *fgf8* gene promoter activity. As an revealing finding, we report here on the regulation of the ligand independent RARα receptor activity. Transactivation of the human *fgf8* promoter was only observed if the RARα receptor is phosphorylated and stabilized. The unliganded RARα activity could be downregulated by Pin1 destabilization.

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References

[1] B.K. Kay, M.P. Williamson, M. Sudol, The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains, FASEB J. 14 (2000) 231–241.
[2] E.T. Kipreos, M. Pagano, The F-box protein family, Genome Biol. 1 (2000) 3002.1–3002.7.
[3] K.P. Lu, Phosphorylation-dependent prolyl isomerization: a novel cell cycle regulatory mechanism, Prog. Cell Cycle Res. 4 (2000) 83–96.
[4] S.D. Hanes, P.R. Shank, K.A. Bostian, Sequence and mutational analysis of ESS1, a gene essential for growth in *Saccharomyces cerevisiae*, Yeast 5 (1989) 55–72.
[5] F. Fujimori, K. takahashi, C. Uchida, T. Uchida, Mice lacking Pin1 develop normally, but are defective in entering cell cycle from G(0) arrest, Biochem. Biophys. Res. Commun. 265 (1999) 658–663.
[6] M. Shen, P.T. Stukenberg, M.W. Kirschner, K.P. Lu, The essential mitotic peptidyl–prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins, Genes Dev. 12 (1998) 706–720.
[7] R. Ranganathan, K.P. Lu, T. Hunter, J.P. Noel, Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent, Cell 89 (1997) 875–886.
[8] R.P. Fisher, D.O. Morgan, A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase, Cell 78 (1994) 7113–7124.
[9] R. Roy, J.P. Adamczewski, T. Seroz, W. Vermeulen, J.P. Tassan, L. Schaeffer, E.A. Nigg, J.H. Hoeijmakers, J.M. Egly, The MO15 cell cycle kinase is associated with the TFIIH transcription-DNA repair factor, Cell 79 (1994) 1103–1109.
[10] W.J. Feaver, J.Q. svejstrup, N.L. Henry, R.D. Kornberg, Relationship of CDK-activating kinase and RNA polymerase II CTD kinase TFIIH/TFIIK, Cell 79 (1994) 1103–1109.
[11] P. Chambon, A decade of molecular biology of retinoic acid receptors, FASEB J. 10 (1996) 940–954.
[12] C. Rochette-Egly, M. Oulad-Abdelghani, A. Staub, V. Pfister, I. Scheuer, P. Chambon, M.P. Gaub, Phosphorylation of the retinoic acid receptor-alpha by protein kinase A, Mol. Endocrinol. 9 (1995) 860–871.
[13] M.H. Delmotte, A. Tahayato, P. Formstecher, P. Lefebvre, Serine 157, a retinoic acid receptor alpha residue phosphorylated by protein kinase C in vitro, is involved in RXR-RAR alpha heterodimerization and transcriptional activity, J. Biol. Chem. 274 (1999) 38225–38231.
[14] C. Rochette-Egly, S. Adam, M. Rossignol, J.M. Egly, P. Chambon, Stimulation of RAR alpha activation function AF-1 through binding to the general transcription factor TFIIH and phosphorylation by CDK7, Cell 90 (1997) 97–107.
[15] E. Kopf, J.L. Plassat, V. Vivat, H. de The, P. Chambon, C. Rochette-Egly, Dimerization with retinoid X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors alpha and gamma through the ubiquitin–proteasome pathway, J. Biol. Chem. 275 (2000) 33280–33288.
[16] V. Brondani, T. Klimkait, J.M. Egly, F. Hamy, Promoter of FGF8 reveals a unique regulation by unliganded RAR alpha, J. Mol. Biol. 319 (2002) 715–728.
[17] S. Charrasse, I. Carena, V. Brondani, K.H. Klempnauer, S. Ferrari, Degradation of B-Myb by ubiquitin-mediated proteolysis: involvement of the Cdc34-SCF(p45Skp2) pathway, Oncogene 19 (2000) 2986–2995.