Journal of Chromatography B, 878 (2010) 2760–2764

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

High-yield expression and purification of the Hsp90-associated p23, FKBP52, HOP and SGTα proteins

Zacariah L. Hildenbrand[a], Sudheer K. Molugu[a], Atanu Paul[b], Gustavo A. Avila[a], Nadia Herrera[a], Chuan Xiao[a,c], Marc B. Cox[b,c], Ricardo A. Bernal[a,c,*]

[a] Department of Chemistry, University of Texas at El Paso, 500 W. University Ave., El Paso, TX 79968, USA
[b] Department of Biology, University of Texas at El Paso, 500 W. University Ave., El Paso, TX 79968, USA
[c] Border Biomedical Research Center, University of Texas at El Paso, 500 W. University Ave., El Paso, TX 79968, USA

ARTICLE INFO

Article history:
Received 29 March 2010
Accepted 14 August 2010
Available online 21 August 2010

Keywords:
Affinity
Ion-exchange
Size-exclusion chromatography

ABSTRACT

Hsp90 is a ubiquitous molecular chaperone that plays a key role in the malignant development of hormone-dependent pathologies such as cancer. An important role for Hsp90 is to facilitate the stable binding of steroid hormones to their respective receptors enabling the ligand-based signal to be carried to the nucleus and ultimately resulting in the up-regulation of gene expression. Along with Hsp90, this dynamic and transient process also involves the recruitment of additional proteins and co-chaperones that add further stability to the mature receptor–chaperone complex. In the work presented here, we describe four new protocols for the bacterial over-expression and column chromatographic purification of the human p23, FKBP52, HOP and SGTα proteins. Each of these proteins plays a distinct role in the steroid hormone receptor regulatory cycle. Affinity, ion-exchange and size-exclusion techniques were used to produce target yields greater than 50 mg/L of cultured media, with each purified sample reaching near absolute sample homogeneity. These results reveal a reliable system for the production of p23, FKBP52, HOP and SGTα substrate proteins for use in the investigation of the Hsp90-associated protein interactions of the steroid hormone receptor cycle.

1. Introduction

Advances in the study of steroid hormone receptor regulation continue to highlight the scaffolding role Heat Shock Protein 90 (Hsp90) plays in the stabilization of steroid hormone receptors for efficient binding of their respective hormone ligands [1–5]. Furthermore, it is becoming increasingly clear that Hsp90 does not act alone but it is dependent on a cohort of co-chaperone proteins that transiently bind to Hsp90 at different stages of the receptor maturation cycle in an ATP-dependent manner [6]. Terminal receptor maturation, a state in which the receptor is able to bind hormone, is believed to involve an Hsp90 dimer that is further stabilized by the p23 co-chaperone and the large immunophilin FK506-binding protein FKBP52 [7–20].

The small, acidic co-chaperone p23 binds directly to Hsp90 in a nucleotide-dependent fashion [21]. It is the smallest component in the Hsp90 machinery (M_r 18,000–25,000) and was first discovered in complex with Hsp90 and the progesterone receptor [22,23]. The complete cellular function of p23 has not been fully elucidated, however in the context of steroid hormone receptor regulation, p23 binding to Hsp90 is believed to stabilize the ATP-bound state by obstructing the nucleotide-binding domains of Hsp90 [7,8]. A recent crystal structure of an $Hsp90_2$–$p23_2$ complex revealed this interaction in which p23 is seen bound to the amino-termini of Hsp90 through hydrophobic interactions [7]. As a result, p23 stabilizes the conformational changes induced by ATP binding which in turn triggers the subsequent binding of immunophilin proteins and the activation of the steroid hormone receptor to its high-affinity ligand-binding state [12,24–26].

FKBP52 is a large peptidyl-proyl *cis–trans* isomerase (PPIase) immunophilin protein that carries the ability to bind immunosuppressive drugs. Structurally, FKBP52 consists of carboxy- and amino-termini that have distinct roles in steroid hormone receptor regulation. A tetratricopeptide repeat domain (TPR) at the carboxy-terminus characterizes the binding surface for the well-defined-EEVD sequence motif at the carboxy-terminus of Hsp90 [18,27–29]. The amino-terminus of FKBP52 is highlighted by the FK-1 and 2 domains. Of particular interest, the FK-1 domain is believed to be largely responsible for FKBP52-mediated potentiation of receptor efficiency by providing the binding surface for the receptor [17–19]. Mutagenesis studies have indicated this to be true for point mutations in and around the FK-1 domain which signifi-

* Corresponding author at: Department of Chemistry, University of Texas at El Paso, 500 W. University Ave., El Paso, TX 79968, USA. Tel.: +1 915 747 6918; fax: +1 915 747 5996.
E-mail address: rbernal@utep.edu (R.A. Bernal).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.08.016

cantly abrogate FKBP52-mediated potentiation of receptor activity and severely affect hormonal potency [17,18,30].

In addition to p23 and FKBP52, Hsp-organizing protein (HOP) and the Small Glutamine-rich Tetratricopeptide protein (SGTα) each play individual roles in steroid hormone receptor regulation by acting as intermediaries between Hsp70 and Hsp90. HOP recruits Hsp90 to interact with preexisting receptor–Hsp70 complexes, thus facilitating the well-articulated progression towards a fully mature receptor complex in which the receptor is primed for ligand-binding [1,3,4,26,31]. SGTα is believed to interact with Hsp70 and 90 in a similar fashion [32], however its role in the regulation of receptor activity remains to be elucidated. Like FKBP52, HOP and SGTα are both TPR domain-containing proteins and characteristically bind to Hsp90 in a very similar fashion. FKBP52 and HOP are believed to compete for the same Hsp90 binding site [4,33,34], while interacting at separate stages in the receptor cycle [35]. In the case of SGTα, it is unknown whether there is competition for Hsp90 binding with the other TPR domain-containing proteins.

The dynamic nature of the four Hsp90-associating proteins presented here makes their separation from the host cell milieu challenging due to their ability to potentially interact with the chaperone proteins of the host. Using Ni^{2+}-affinity, anion-exchange (AEX) and size-exclusion (SEC) chromatographic techniques, we reveal a series of reproducible methods for the high-yield production of purified p23, FKBP52, HOP and SGTα proteins. By design, these protocols will enable the downstream investigation of steroid hormone receptor regulation under highly purified conditions in addition to alleviating the need for crude mammalian lysates to conduct *in vitro* binding assays. Furthermore the products of the aforementioned chromatographic experiments may serve as excellent substrates in the elucidation of steroid receptor dynamics as they pertain to hormone-dependant pathologies such as prostate cancer.

2. Materials and methods

2.1. Chemicals and cells

All buffering components, antibiotics, inducing agents and lysis compounds were obtained from Sigma–Aldrich (St. Louis, USA). SDS-PAGE molecular weight standards were from BIO-RAD (Hercules, USA). The *Esherichia coli* BL21* (DE3) competent cells used in the bacterial transformation of the respective expression plasmids were from Invitrogen (Carlsbad, USA).

2.2. Equipment

Cells and cell lysates were harvested in the Beckman Coulter Allegra X-15R and Avanti J-E centrifuges (Fullerton, USA) respectively. All chromatography was executed on the AKTA Purifier HPLC system (GE Healthcare, Uppsala, Sweden) using the HisTrap Fast Flowthrough (FF) Crude, DEAE FF Crude, HiLoad Q-Sepharose 26/10 and HiPrep Sephacryl S-200 16/10 columns (GE Healthcare, Uppsala, Sweden). Target elutions were concentrated in 10,000 MWCO VIVASPIN6 centrifugation membrane tubes (Sartorius Biolabs, Goettingen, Germany) and quantified spectrophotometrically using the BCA protein assay (Pierce, Rockford, USA).

2.3. Expression of human p23, FKBP52, HOP and SGTα in bacteria

The human p23 co-chaperone was expressed as an untagged construct in the pET23 vector. The human FKBP52 and HOP proteins were expressed as amino-terminal 6xHistidine-tagged constructs in the pET28b vector. Additionally the human SGTα protein was also expressed in the pET28b vector system as a carboxy-terminal 6xHistidine-tagged construct. All recombinant constructs were transformed into the *E. coli* BL21* competent cell system and were isolated on LB-agar plates containing the appropriate antibiotic. All transformants were grown at 37 °C in 1 L cultures of 2xTY media until reaching an optical density (A_{600nm}) of 0.8. Protein expression was induced with isopropyl-β-D-1-thiogalactoside (IPTG) to a final concentration of 1 mM. After a 5 h incubation at 30 °C, the cells were harvested by centrifugation (6000 × *g*, 60 min, 4 °C) and resuspended in a 50 mM Hepes, 10 mM EDTA, 1 mM PMSF lysis buffer (pH 7.5). Hen egg white lysozyme was added to the respective resuspensions at a 2.5 mg/ml final concentration prior to an initial freezing at −20 °C.

2.4. Cell disruption

The *E. coli* expression cells containing the respective p23, FKBP52, HOP and SGTα proteins were mechanically lysed through multiple freeze/thaw cycles. Cell/lysate emulsions were treated with $MgCl_2$ to a final solution concentration of 25 mM in conjunction with the addition of trace amounts of bovine pancreatic DNase I. Each mixture was rocked on ice for 60 min to facilitate DNA degradation prior to harvesting the cell lysate by centrifugation (20,000 × *g*, 60 min, 4 °C).

2.5. Chromatographic purification of the p23 co-chaperone

The human p23 protein was initially purified over a quadruple-stack of 5 ml DEAE FF anion-exchange columns that were equilibrated with 50 mM Hepes, 10 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 0.02% NaN_3 (pH 6.0). Prior to loading, the p23-containing lysate was diluted in the equilibration buffer (pH 6.0) to bring down the pH of the sample. The target protein was eluted over a 100 ml of 0–600 mM NaCl gradient with a 5 ml/min buffer flow rate. The target fractions were then passed through a HiLoad Q-Sepharose 26/10 AEX column that was equilibrated with 50 mM Hepes, 10 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 0.02% NaN_3 (pH 7.5). p23 was separated over a 400 ml of 0–600 mM NaCl gradient that was executed under a flow rate of 4 ml/min. The p23 sample was further purified by treatment with a HiPrep Sephacryl S-200 16/10 size-exclusion column that was equilibrated with 50 mM Hepes, 150 mM NaCl, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 0.02% NaN_3 (pH 7.5), with elution occurring at a 0.5 ml/min flow rate.

2.6. Chromatographic purification of the TPR domain-containing FKBP52, HOP and SGTα proteins

The 6xHistidine-tagged FKBP52, HOP and SGTα proteins were individually purified over a 5 ml nickel–nitriloacetic acid (Ni–NTA) HisTrap FF Crude column. Prior to column loading, the respective lysates were treated with Imidazole to a final concentration of 20 mM. The column was equilibrated and washed with 50 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 20 mM Imidazole, 1 mM PMSF, 0.02% NaN_3 (pH 7.5) and the respective proteins were eluted over sharp 20–500 mM Imidazole gradients with a 5 ml/min flow rate. In separate experiments the FKBP52, HOP and SGTα target fractions were further purified on a HiLoad Q-Sepharose 26/10 AEX column as previously described. Additionally the three TPR domain-containing proteins were subsequently passed through a HiPrep Sephacryl S-200 16/10 column as described previously.

All proteins were dialyzed extensively against 50 mM Hepes, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT (pH 7.4) prior to sample concentration and storage at −80 °C.

2.7. Automated electrophoresis analysis

The molecular weight of the p23, FKBP52, HOP and SGTα proteins and the relative degree of each sample's target homo-

Table 1
High-yield expression of the Hsp90-associating proteins.

Target protein	% of target in crude lysate	Concentration of target in crude[a] (ng/μl)	Expression Total yield (mg/L)
p23	45.4 ± 0.5	671.4 ± 50.0	83.9
FKBP52	20.4 ± 0.8	647.2 ± 35.2	80.9
HOP	26.7 ± 0.1	825.4 ± 104.3	103.5
SGTα	30.9 ± 0.1	650.0 ± 25.2	81.2

Each p23-, FKBP52-, HOP- and SGTα-containing bacterial crude lysate was analyzed using the Bio-Rad Experion microfluidic electrophoresis unit. For each protein, values included a percentage (%) of target protein occupancy in the respective crude lysate and a resulting concentration of the target in the crude lysate. The total expression yields for the four proteins were calculated from the resulting Experion values in conjunction with the respective 1:5 dilution factors and the resulting lysates volumes garnered upon bacterial resuspension (~25 ml).

[a] Each sample was diluted 1:5 in a 50 mM Hepes solution to maintain the expression yield of recombinant targets within the linear range of the Experion instrument (2.5–2000 ng/μl).

geneity was determined by 10% SDS-PAGE and the Experion™ Automated Microfluidic Electrophoresis station (BIO-RAD Laboratories). The Experion automated electrophoresis system utilizes LabChip microfluidic separation technology and fluorescent sample detection (Caliper Life Sciences) to perform automated analysis of multiple protein samples [36]. Protein concentrations were determined spectrophotometrically via BCA assay against a 2 mg/ml bovine serum albumen standard. All data analysis was done with the PRISM software suite.

3. Results and discussion

The bacterial BL21* system was used to express the human p23, FKBP52, HOP and SGTα proteins to extremely high yields (Table 1). In all four cases, the addition of 1 mM IPTG was considered optimal for the induction of target protein expression as higher concentrations of the inducing agent did not result in higher expression (data not shown). Additionally, an incubation of 5 h at 30 °C was determined to be ideal for producing large amounts of the respective proteins. Under these conditions, all four recombinant proteins were expressed at levels above 80 mg/L each making up at least 20% of the total protein profile within their respective bacterial lysates. Interestingly the HOP recombinant was expressed the most efficiently in which to total expression in the crude lysate was above 100 mg. It is also worth noting that the high level expression of the four target proteins and the recombinant addition of 6xHistidine tags to the FKBP52, HOP and SGTα proteins did not result in any traceable amount of protein aggregation nor did it affect their ability to bind to Hsp90.

We developed a simple and efficient three-step protocol for the purification of the p23, FKBP52, HOP and SGTα Hsp90-associated proteins based on their individual biochemical characteristics. FF AEX chromatography was first used to initially separate p23 from its respective crude lysate. p23 was expressed as an untagged construct due to the observation that affinity binding tags interfere with p23's ability to bind with Hsp90 (unpublished observations). The FKBP52, HOP and SGTα proteins were first passed through

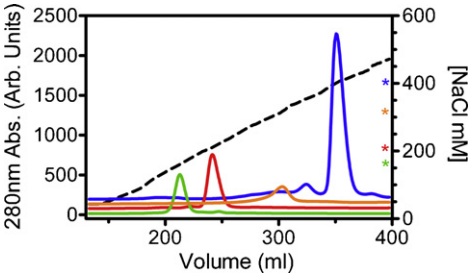

Fig. 1. Q-Sepharose anion-exchange elution profiles of the respective chromatography experiments. The p23 (blue), FKBP52 (red), HOP (green) and SGTα (orange) proteins characteristically eluted at different positions in the 0–600 mM NaCl gradient (black) as a result of their respective molecular charges at a pH 7.5 and the resulting binding affinities with $CH_2N^+(CH_3)_3$ column matrix. Colored stars represent the NaCl concentration at which each individual protein eluted from the NaCl gradient.

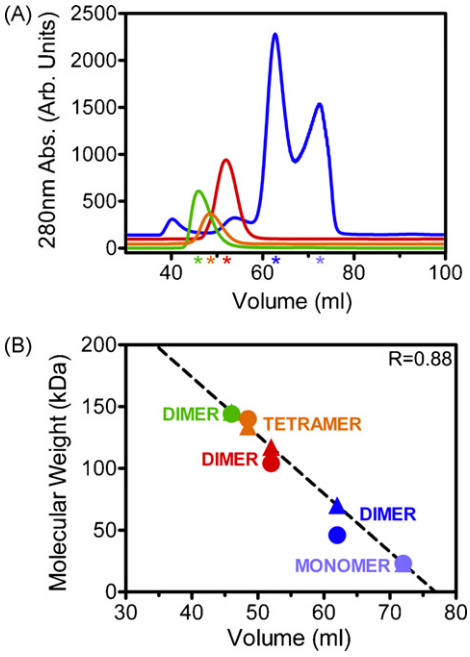

Fig. 2. The elution patterns of highly pure p23, FKBP52, HOP and SGTα protein samples during size-exclusion chromatography. (A) The p23 protein (blue) eluted as two distinct peaks, representative of dimer and monomer species. The FKBP52 (red), HOP (green) and SGTα (orange) TPR domain-containing proteins each eluted as single isolated peaks. Colored stars representative of each individual protein highlight the respective elution points relative to the column elution volume. The two p23 peaks are individually represented by a blue and a light blue star. (B) A linear regression was done on the respective elution positions of seven proteins with well-known molecular weights to interpolate the molecular weight of the target proteins from their elution positions. The colored triangles represent the interpolated molecular weight values derived from the linear regression ($R = 0.88$) while the colored circles denote similar values derived from primary sequence information. Each protein is labeled to highlight its assumed multimeric conformation.

Table 2
Statistical analysis of the step-wise purification of the p23, FKBP52, HOP and SGTα proteins.

Protein	1. Crude	2. DEAE FF	2. HisTrap FF	3. Q-Sepharose	4. S-200 SEC
p23	45.4 ± 0.5	58.9 ± 8.2		94.3 ± 0.1	96.3 ± 1.0
FKBP52	20.4 ± 0.8		87.3 ± 0.9	98.5 ± 0.5	99.8 ± 0.0
HOP	26.7 ± 0.1		74.9 ± 0.4	93.7 ± 1.6	98.6 ± 0.5
SGTα	30.9 ± 0.1		64.0 ± 2.0	94.4 ± 0.1	95.1 ± 0.2

The homogeneity (% purity) of each protein sample was analyzed on the Bio-Rad Experion unit after each step in the purification process to quantify the relative effect of each chromatographic separation.

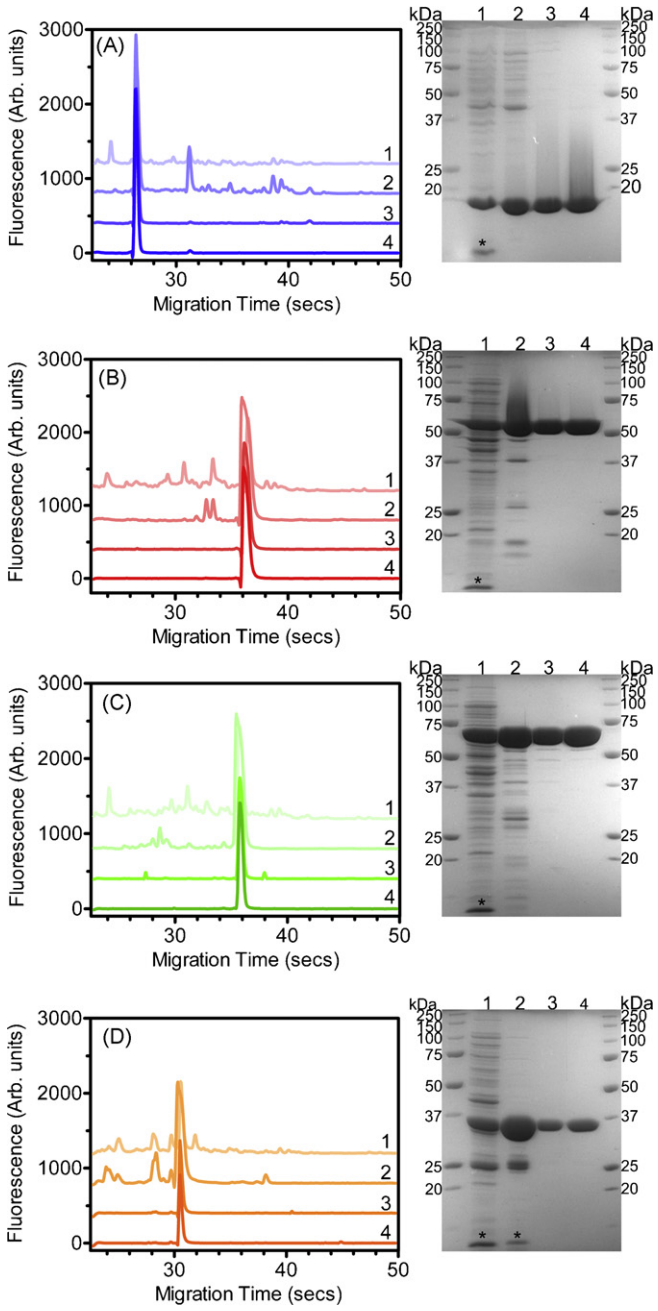

Fig. 3. Automated microfluidic electrophoresis analysis of the step-wise purification of the p23, FKBP52, HOP and SGTα proteins. (A–D) Laser excitation fluorescent detection chromatograms reveal the relative positions of the p23 (blue), FKBP52 (red), HOP (green) and SGTα (orange) peaks in relation to the microfluidic migration volume of the Pro260 chips. Four individual chromatograms increasing in contrast of the respective colors represent the step-wise purification of the substrate proteins (1. Crude-lightest, 2. DEAE/HisTrap-light, 3. Q-Sepharose-dark, 4. S-200-darkest). The noise from contaminating proteins decreases with each purification step and is essentially gone by the 4th step. Additionally SDS-PAGE results are shown to further illustrate the effectiveness of the step-wise purification protocols. Lane 1 in each of the SDS-PAGE gels contains a strong band (indicated by *) corresponding to the lysozyme added for bacterial cell lysis after protein expression.

Ni–NTA columns to take advantage of the selective immobilization of the target proteins as a result of the covalent binding between the histidine residues of the affinity tags and the Ni^{2+} of the stationary matrix [37].

As a second chromatographic measure, each protein was passed through an AEX column using a 0–600 mM NaCl gradient to separate the targets as a function of their molecular charge at a pH of 7.5 (Fig. 1). In separate runs the p23, FKBP52, HOP and SGTα proteins eluted from the Q-Sepharose AEX column at 390, 220, 180, and 320 mM NaCl respectively (Fig. 1). The relative elution positions of the four individual proteins within the NaCl gradient were characteristic of their respective isoelectric point (pI) values and corroborated previous experimental and theoretical results [22,38]. The HOP and FKBP52 proteins have the most basic pI values (6.40 and 5.35) and eluted the earliest in the NaCl gradient due to their weak binding to the $CH_2N^+(CH_3)_3$ column matrix. Conversely, the more acidic SGTα and p23 proteins (4.81 and 4.35) each eluted much later in the gradient. Of particular interest, all four resulting protein samples eluted from their respective AEX column separations as least 93% pure (Table 2). It is worth noting that Hepes–KOH was used as the buffering agent in all of the AEX separations with the Q-Sepharose anion-exchange column. This differs from the recommended use of Tris–HCl as the buffering agent as we found the use of Hepes–KOH yielded a higher degree of protein separation likely due to a more selective counter-ion.

In the third stage of this novel purification protocol, size-exclusion chromatography was applied to purify the substrate proteins to near absolute homogeneity. All four proteins eluted as multimeric species as indicated by their elution positions in relation to that of well-known molecular weight standards (Fig. 2). p23 eluted at 62 and 72 ml indicating the presence of both dimer and monomer species. As expected both of the p23 conformations eluted with at least 96% homogeneity. FKBP52 and HOP both eluted as single peaks representative of their individual dimer species, while SGTα eluted in a tetrameric conformation.

Automated electrophoresis was used to analyze each target protein on the basis of molecular weight as a function of microfluidic channel migration on a microchip. Laser excitation chromatograms and SDS-PAGE revealed the relative efficiency of the purification of the p23, FKBP52, HOP SGTα samples in a step-wise fashion (Fig. 3). As the result of our three-step purification process, each target protein surpassed the level of 95% homogeneity, with FKBP52 and HOP reaching at least 98% homogeneity (Table 2). In addition to the high level of purity attained for each purified sample, the proteins were produced and recovered in yields greater than 50 mg/L of cultured media.

In summary, our expression and purification systems reveal a highly proficient method for the production of purified Hsp90-associating proteins. Collectively these results represent a more efficient system for the mass production of purified p23, FKBP52, HOP and SGTα protein samples when compared to previous results. Furthermore, these methods can be utilized in the biochemical elucidation of the steroid hormone receptor cycle as well as supporting structure/function investigations of the three TPR domain-containing proteins.

Acknowledgements

Funding for this research was provided to Ricardo A. Bernal by the Welch Foundation (Grant number AH-1649). Support was also provided to Zacariah L. Hildenbrand by NSF Grant #083295. This publication was made possible by Grant Number 2G12RR008124-16A1 (to the Border Biomedical Research Center (BBRC)/University of Texas at El Paso) from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NCRR or NIH.

References

[1] K.D. Dittmar, K.A. Hutchison, J.K. Owens-Grillo, W.B. Pratt, J. Biol. Chem. 271 (1996) 12833.
[2] M.P. Hernandez, A. Chadli, D.O. Toft, J. Biol. Chem. 277 (2002) 11873.

[3] H. Kosano, B. Stensgard, M.C. Charlesworth, N. McMahon, D. Toft, J. Biol. Chem. 273 (1998) 32973.
[4] S. Chen, D.F. Smith, J. Biol. Chem. 273 (1998) 35194.
[5] D.F. Smith, W.P. Sullivan, T.N. Marion, K. Zaitsu, B. Madden, D.J. McCormick, D.O. Toft, Mol. Cell. Biol. 13 (1993) 869.
[6] W.M. Obermann, H. Sondermann, A.A. Russo, N.P. Pavletich, F.U. Hartl, J. Cell Biol. 143 (1998) 901.
[7] M.M. Ali, S.M. Roe, C.K. Vaughan, P. Meyer, B. Panaretou, P.W. Piper, C. Prodromou, L.H. Pearl, Nature 440 (2006) 1013.
[8] K.D. Dittmar, D.R. Demady, L.F. Stancato, P. Krishna, W.B. Pratt, J. Biol. Chem. 272 (1997) 21213.
[9] C.K. Vaughan, U. Gohlke, F. Sobott, V.M. Good, M.M. Ali, C. Prodromou, C.V. Robinson, H.R. Saibil, L.H. Pearl, Mol. Cell 23 (2006) 697.
[10] B. Panaretou, C. Prodromou, S.M. Roe, R. O'Brien, J.E. Ladbury, P.W. Piper, L.H. Pearl, EMBO J. 17 (1998) 4829.
[11] B. Panaretou, G. Siligardi, P. Meyer, A. Maloney, J.K. Sullivan, S. Singh, S.H. Millson, P.A. Clarke, S. Naaby-Hansen, R. Stein, R. Cramer, M. Mollapour, P. Workman, P.W. Piper, L.H. Pearl, C. Prodromou, Mol. Cell 10 (2002) 1307.
[12] C. Prodromou, G. Siligardi, R. O'Brien, D.N. Woolfson, L. Regan, B. Panaretou, J.E. Ladbury, P.W. Piper, L.H. Pearl, EMBO J. 18 (1999) 754.
[13] G. Siligardi, B. Panaretou, P. Meyer, S. Singh, D.N. Woolfson, P.W. Piper, L.H. Pearl, C. Prodromou, J. Biol. Chem. 277 (2002) 20151.
[14] S.H. McLaughlin, H.W. Smith, S.E. Jackson, J. Mol. Biol. 315 (2002) 787.
[15] K. Richter, S. Walter, J. Buchner, J. Mol. Biol. 342 (2004) 1403.
[16] P. Meyer, C. Prodromou, B. Hu, C. Vaughan, S.M. Roe, B. Panaretou, P.W. Piper, L.H. Pearl, Mol. Cell 11 (2003) 647.
[17] M.B. Cox, D.L. Riggs, M. Hessling, F. Schumacher, J. Buchner, D.F. Smith, Mol. Endocrinol. 21 (2007) 2956.
[18] D.L. Riggs, P.J. Roberts, S.C. Chirillo, J. Cheung-Flynn, V. Prapapanich, T. Ratajczak, R. Gaber, D. Picard, D.F. Smith, EMBO J. 22 (2003) 1158.
[19] D.L. Riggs, M.B. Cox, H.L. Tardif, M. Hessling, J. Buchner, D.F. Smith, Mol. Cell. Biol. 27 (2007) 8658.
[20] J. Cheung-Flynn, V. Prapapanich, M.B. Cox, D.L. Riggs, C. Suarez-Quian, D.F. Smith, Mol. Endocrinol. 19 (2005) 1654.
[21] J.L. Johnson, D.O. Toft, Mol. Endocrinol. 9 (1995) 670.
[22] T. Weikl, K. Abelmann, J. Buchner, J. Mol. Biol. 293 (1999) 685.
[23] J.L. Johnson, T.G. Beito, C.J. Krco, D.O. Toft, Mol. Cell. Biol. 14 (1994) 1956.
[24] C. Prodromou, B. Panaretou, S. Chohan, G. Siligardi, R. O'Brien, J.E. Ladbury, S.M. Roe, P.W. Piper, L.H. Pearl, EMBO J. 19 (2000) 4383.
[25] J.L. Johnson, A. Halas, G. Flom, Mol. Cell. Biol. 27 (2007) 768.
[26] D.F. Smith, D.O. Toft, Mol. Endocrinol. 7 (1993) 4.
[27] B. Wu, P. Li, Y. Liu, Z. Lou, Y. Ding, C. Shu, S. Ye, M. Bartlam, B. Shen, Z. Rao, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 8348.
[28] C. Scheufler, A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F.U. Hartl, I. Moarefi, Cell 101 (2000) 199.
[29] J.K. Owens-Grillo, M.J. Czar, K.A. Hutchison, K. Hoffmann, G.H. Perdew, W.B. Pratt, J. Biol. Chem. 271 (1996) 13468.
[30] Y. Miyata, B. Chambraud, C. Radanyi, J. Leclerc, M.C. Lebeau, J.M. Renoir, R. Shirai, M.G. Catelli, I. Yahara, E.E. Baulieu, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 14500.
[31] P.E. Carrigan, G.M. Nelson, P.J. Roberts, J. Stoffer, D.L. Riggs, D.F. Smith, J. Biol. Chem. 279 (2004) 16185.
[32] S.T. Liou, C. Wang, Arch. Biochem. Biophys. 435 (2005) 253.
[33] J.K. Owens-Grillo, K. Hoffmann, K.A. Hutchison, A.W. Yem, M.R. Deibel Jr., R.E. Handschumacher, W.B. Pratt, J. Biol. Chem. 270 (1995) 20479.
[34] S.C. Nair, R.A. Rimerman, E.J. Toran, S. Chen, V. Prapapanich, R.N. Butts, D.F. Smith, Mol. Cell. Biol. 17 (1997) 594.
[35] S.C. Onuoha, E.T. Coulstock, J.G. Grossmann, S.E. Jackson, J. Mol. Biol. 379 (2008) 732.
[36] O.T. Chan, D.A. Herold, Clin. Chem. 52 (2006) 2141.
[37] E.K. Ueda, P.W. Gout, L. Morganti, J. Chromatogr. A 922 (2001) 165.
[38] B.D. Halligan, Methods Mol. Biol. 527 (2009) 283, ix.