We used a cellular system to elucidate the molecular determinants of the large immunophilin FK506-binding proteins (FKBP) 51 and 52 for their action on the glucocorticoid receptor in mammalian cells. Increasing the levels of FKBP51 reduced the transcriptional activity of the receptor, as reported. Elevated levels of FKBP52 per se showed no effect but mitigated the inhibition of the receptor induced by FKBP51. We discovered that nuclear translocation of the glucocorticoid receptor was delayed by FKBP51. This correlates with the reduced interaction of FKBP51 with the motor protein dynein compared with FKBP52. From mutational analyses, we concluded that three features of the immunophilins are required for efficient receptor signaling in mammalian cells: hsp90 interaction, dynein association, and peptidylprolyl isomerase (PPIase) enzyme activity. The relevance of dynein for receptor function was substantiated by several experiments: 1) coexpression of dynamitin, which disrupts the transport complex and reduces receptor activity; 2) coexpression of the PPIase domain fragment of FKBP52, which is known to disrupt interaction of the receptor to dynein and reduce glucocorticoid receptor function, in contrast to the corresponding fragment of FKBP51; and 3) swapping of the PPIase domains FKBP51 and FKBP52, which reverses the respective activity. We concluded from our results that the mechanisms of the regulatory system FKBP51/FKBP52 discovered in yeast also operate in mammals to modulate hormone binding of the receptor. In addition, differential regulation of dynein association and nuclear translocation contributes to the effects of the two immunophilins on the glucocorticoid receptor in mammals.

Corticosteroids regulate a variety of biological processes, including the metabolism of carbohydrates, lipids, and proteins, cell proliferation, development, and reproduction (1–3). The mineralocorticoid receptor and the glucocorticoid receptor (GR) are the two corticosteroid receptors that mediate the effects of corticosteroids. They belong to the family of ligand-dependent transcription factors (4, 5).

In the classic paradigm, GR resides in the cytoplasm in the absence of ligand. Several molecular chaperones assemble the aporeceptor in a stepwise and ATP-dependent fashion to a conformational state capable of binding to hormone with high affinity (3). The mature GR complex consists of an hsp90 dimer, p23, possibly hs70, and one of the tetratricopeptide repeat (TPR)-containing proteins, such as the immunophilin FK506-binding proteins (FKBP) 51 and 52 (7). Upon hormone binding, GR is translocated into the nucleus. It either activates transcription by binding to its cognate DNA elements or decreases transcription by interaction with other transcription factors (8–9).

Chaperones are not only required for proper folding of GR but may also be involved in the nuclear translocation of GR (10–12). For example, pharmacological inhibition of hsp90 (13) delays nuclear translocation of hormone-bound GR (14). Moreover, FKBP52 has been shown to interact not only with hsp90 and GR but also with the motor protein dynein (15), apparently via dynamitin (16). These findings led to the formulation of the concept of a transportosome (10), i.e. a protein heterocomplex that guides GR along cytoskeletal tracts to the nucleus. Without transportosome activity, GR reaches the nucleus with slower kinetics (11). This model is further supported by the observation that the inhibitory effect of geldanamycin on nuclear translocation depends on an intact cytoskeleton (11). Hsp90 and FKBP52 have recently been shown to be involved in nuclear transport of p53 (16). The important question has been raised as to whether the highly homologous FKBP51 also interacts with dynes (17).

The suggested role of immunophilins in GR signal transduction also implies novel potential ways to explain occasional GR malfunction associated with many diseases (18–20). It has been known for several years that malfunction of GR due to low affinity binding to hormone can result from mutations of the GR receptor (21) or from limited hsp90 function (22–24). More recently, it has been shown that cochaperones of hsp90 have the ability to modulate the binding affinity of GR to hormone. For example, increased levels of FKBP51 have been reported to be the common cause of glucocorticoid resistance in three New World primates (25).

Although the exact mechanistic contribution of the large immunophilins for GR function remains to be elucidated, important insight into the functional role has been provided (26–27). Overexpression of FKBP51 in COS cells reduces the binding affinity of GR and, therefore, decreases the transcriptional activity of GR after hormone exposure (28). In yeast, overexpression of FKBP51 has no effect on GR, but FKBP52 enhances GR-dependent transcription (27). This enhancement is dependent on interaction with hs70 and the peptidylprolyl isomerase (PPIase) activity of FKBP52 (27). The observation that...
FKBP52 displayed a similar enhancement of GR in dyein-deficient yeast (27) cells into question the relevance of the described interaction of FKBP52 and other TPR proteins with dynemin for GR signaling. Alternatively, it is possible that yeast, which lacks steroid receptors and FKBP52, does not reflect all of the aspects of GR signaling that have developed in higher organisms to ensure optimal steroid signaling.

In this study, we investigated the requirements and mechanisms of the differential regulation of GR activity by FKBP51 and FKBP52 in mammalian cells. We show that virtually all features of this regulatory system found in yeast (27) are also functional in mammals, e.g. hsp90 binding of the immunophilins is essential, and the PPIase activity of FKBP52 is required for modulating GR hormone binding and, thus, transcriptional activity. Moreover, we discovered that in mammalian cells nuclear translocation of GR is also regulated by the immunophilins. We detected differential binding of FKBP52 and FKBP51 to dynemin, which corresponds to their differential effect on nuclear translocation. Thus, in mammals, FKBP51 and FKBP52 differentially regulate GR on two levels: hormone binding (28) and nuclear translocation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Transfection, and Assays for Luciferase and β-Galactosidase—**Cultivation and transfection of human neuroblastoma SK-N-MC cells (American Type Culture Collection catalog number HTB-10). HeLa cells, HEK, and H4-II-E-C3 cells were as described previously (29, 30). Two days before transfection, cells were seeded in medium containing charcoal-stripped serum. Unless indicated otherwise, the amounts of transfected plasmids~107 cells were 1.5 μg of steroid-responsive luciferase reporter plasmid MTVLuc, 3 μg of β-galactosidase expression vector pCMVβ-Gal (Stratagene) as control plasmid, various amounts of FKBP expression vector as indicated, and 0.75 μg of plasmid pRK7 (31). In all transfections, the total amount of plasmid was equaled for each condition by supplementing "empty" expression vector. After electroporation, the cells were seeded again in medium containing charcoal-stripped serum complemented with either hormone or the respective solvent.

Luciferase and β-galactosidase assays were performed as described by Schmidt et al. (29). Luciferase data are presented as % stimulation (i.e., after correction of the data by galactosidase activities, fold stimulation was calculated by comparing cells without hormone to cells with hormone). The stimulation in the absence of cotransfected FKBP52 was set to 100%.

**Tyrosine Aminotransferase (TAT) Assays and Reverse Transcription (RT)/PCR—**For the measurement of native GR target genes in glucocorticoid-responsive cell lines, H4-II-E-C3 and HeLa cells were used. First, the cortisol responsivity of the TAT promoter in H4-II-E-C3 cells was measured using ExGen (Fermentas) as recommended by the manufacturer. 0.25 μg of GFP receptor and 0.75 μg of FKBP plasmid were used for each well. The cells were stimulated 24 h after transfection and then fixed with 4% paraformaldehyde. After 20 min, the cells were washed 2 times with incubation buffer (1× Tris-buffered saline/Tween buffer, 5% fetal calf serum, 1% bovine serum albumin, 1% Triton X-100) and then embedded in ProTaqs Mount Fluor (Biocyc GmbH & Co KG, Luckenwalde, Germany). For time-resolved analysis of nuclear translocation, we followed the protocol by Galigniana et al. (34). The cells were analyzed using fluorescence microscopy (Axiophot, Zeiss, Jena, Germany). To score the cytoplasmic/nuclear distribution of the fluorescent receptors, >100 cells were evaluated by a non-involved investigator according to a method adapted from the literature (11). 0 was assigned to a cell with a balanced distribution, +1 for a cell with enhanced nuclear (–1 with enhanced cytoplasmic) fluorescence, and +2 for a cell with exclusively nuclear (–2 with exclusively cytoplasmic) fluorescence.

**RESULTS**

**FKBP51 and FKBP52 Act Differentially on GR in Mammalian Cells—**To set up a mammalian model system to investigate the effect of FKBP51 and FKBP52 on GR, we used the neuroblastoma cell line SK-N-MC, HEK cells, and HeLa cells in a GR-responsive reporter assay. In all of these cell lines, we observed a dose-dependent inhibition of GR-mediated transcription by FKBP51 as reported for COS cells (28), whereas FKBP52 had no effect (Fig. 1A).

Overexpression of the carboxy terminus of Hsc70-interacting protein, another TPR domain protein, leads to degradation of GR through the proteasome machinery (35). In yeast, overexpression of FKBP52 or FKBP51 increased the levels of GR. In our mammalian cells, overexpression of FKBP51 or FKBP52 did not change the level of GR (Fig. 1A). This excludes degradation of GR as an explanation for the inhibitory effect of FKBP51. We also observed that, in the presence of FKBP51,
higher concentrations of cortisol were necessary to elicit a GR response (Fig. 1B). This reflects the change in $K_d$ of GR hormone binding observed by others (28). We noted that, at saturating levels of cortisol, FKBP51 still slightly but significantly decreased GR activity, indicating an additional level of action of FKBP51 (i.e. additive to the $K_d$ effect). GR activities were the same for the vector control and FKBP52 coexpression (not shown).

It was important to test whether the same pattern of activity of FKBP51 and FKBP52 can be observed on an endogenous GR-regulated gene as in our transient reporter system. We used the glucocorticoid-responsive cell lines HeLa and H4-II-E-C3 to measure the influence of additional FKBP51 or FKBP52 on the established GR target genes MT (metallothionein) and TAT (tyrosine aminotransferase), respectively (36, 37). We observed that the FKBP51 abolished the enhancement of MT RNA after the addition of hormone, whereas FKBP52 had no significant effect (Fig. 1C and data not shown). Likewise, TAT activity was significantly reduced by FKBP51 but not by FKBP52 (Fig. 1C).

Thus, the inhibitory action of FKBP51 is independent of the cell line used and, importantly, also independent of the promoter and its chromosomal context. Although FKBP52 enhanced GR signaling in yeast (27), the environment provided for GR activity is obviously different in mammals and yeast. Both WT and FLAG-tagged proteins produced indistinguishable results (not shown).

The Inhibitory Activity of FKBP51 Requires Interaction with Hsp90 and Is Competed by FKBP52—Hsp90 dependence has been demonstrated for the FKBP52-induced enhancement of GR in yeast (27). However, GR and FKBP51/52 evolved only in higher organisms, and the immunophilins also exhibit hsp90-independent features (15, 38, 39). To test the hsp90 dependence of the FKBP51-inhibitory effect on GR in our mammalian reporter system, we replaced the positively charged amino acids lysine 352 and arginine 356 with alanines in the hsp90-interacting TPR repeat motif, (see Fig. 6 for domain structure). The corresponding mutation in the TPR domain of protein phosphatase 5 has been shown to disrupt binding to hsp90 (40). The TPR-defective FKBP51 did not inhibit GR-dependent tran-

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**Fig. 1. FKBP51 inhibits GR.** HEK (A) or SK-N-MC (B) cells were transfected with the reporter plasmid MTVLuc, the $\beta$-galactosidase control plasmid, a GR-expressing plasmid, and a FKBP51 or, alternatively, a FKBP52 expression plasmid. After transfection, the cells were cultivated for 16 h in the presence of 10 nM cortisol (A), or as indicated (B). Lower panel of A, representative Western blot of the FLAG-tagged immunophilins and hemagglutinin-tagged GR in the cell extracts used to determine luciferase activity. ND, not determined. Luciferase data were normalized by the $\beta$-galactosidase activities and are presented as relative stimulation ± S.E. of four (A) or three (B) independent experiments performed in duplicate. C, HeLa cells for MT RT/PCR (upper panel) or H4-II-E-C3 cells for TAT assays (lower panel) were transfected with 1 μg empty vector or FKBP expression plasmid and stimulated with cortisol (30 or 20 nM, respectively). Contr., cells transfected with cloning plasmid. Upper panel, representative agarose gel of PCR products of MT and GAPDH that was used for normalization (not shown). TAT activities were normalized by protein content. As in A and B, TAT data are presented as relative stimulation ± S.E. of three independent experiments performed in duplicates.
FKBP51 and FKBP52 on GR

**Fig. 2. Inhibition of GR by FKBP51 requires interaction with hsp90 and is mitigated by FKBP52.** A, SK-N-MC cells were transfected with the reporter plasmid MTVLuc, increasing amounts of a plasmid expressing FKBP51 with a double point mutation in the TPR domain (K352A/R356A), the β-galactosidase plasmid, and the GR plasmid pRK7GR. After transfection, the cells were cultivated for 16 h in the presence of 10 nM cortisol. **Upper panel,** luciferase data were normalized by the β-galactosidase activities and are presented as relative activities ± S.E. of five independent experiments performed in duplicate. **Middle panel,** Western blot of cell extracts probed with an anti-FLAG antibody, demonstrating increasing expression of the mutant Efficiency of the precipitation and with an Hsp90 antibody. **B, HEK cells** were transfected with the reporter plasmid MTVLuc, the GR plasmid pRK7GR, the β-galactosidase plasmid, and increasing amounts of the FKBP51 plasmid. The cells were then cultivated for 16 h in the presence of 10 nM cortisol. **Upper panel,** luciferase data were normalized by the β-galactosidase activities and are presented as relative activities ± S.E. of three independent experiments performed in duplicate. **Lower panel,** Western blot detection of untagged FKBP51 and FLAG-tagged FKBP52.

FKBP51 and FKBP52 compete with each other for binding to the GR heterocomplex. In other words, the activity of GR is dependent on the relative levels of the two immunophilins. To test this concept in mammalian cells, we transfected FKBP51 at a constant level, whereas the amount of FKBP52 was increased stepwise (Fig. 2B). This led to an abrogation of the inhibitory effect, without decreasing the level of expressed FKBP52 (Fig. 2B lower panels). Again, HEK and SK-N-MC cells yielded the same results. Thus, in mammalian cells, the beneficial role of FKBP52 is detected only in the presence of additional FKBP51.

**FKBP51 Inhibits Nuclear Translocation of GR—**There is evidence in the literature that FKBP52 binds to dynein and is part of a transport machinery that guides GR along cytoskeletal tracts to the nuclear pores (11, 15, 42, 43). Therefore, higher levels of FKBP51 that expel FKBP52 from the GR heterocomplex may either be unable to interact with the transport machinery or may interact unproductively. To explore this possibility, we followed the nuclear translocation of GR upon expression of FKBP51. HeLa cells were transfected with a plasmid expressing GR fused to GFP and either a FKBP52-expressing plasmid or a FKBP51-expressing plasmid. The cells were incubated with or without saturating levels of hormone of 1 μM dexamethasone (DEX), and subcellular localization was monitored under a fluorescence microscope. We rated >100 cells/condition for their cytoplasmic/nuclear distribution of GR for each of the three independent experiments. We adapted the procedure from the literature (11) and scored +2 for cells with exclusively nuclear (~2 for cytoplasmic) fluorescence, +1 for cells with enhanced nuclear (~1 for cytoplasmic) fluorescence, and 0 for cells with balanced distribution.

Nuclear translocation of GR was significantly reduced in cells transfected with FKBP51 up to 40 min after the addition of hormone compared with cells transfected with FKBP52 (Fig. 3). Nuclear translocation of GR in FKBP52-transfected cells was indistinguishable from the one in control vector-transfected cells (not shown).

The use of saturating hormone concentrations was necessary, because at limiting levels of hormone, a reduction of nuclear translocation could be explained by the known effect of FKBP51 on the Kd of GR (28). Indeed, at 10 nM cortisol, we observed a strong inhibition of nuclear translocation (not shown). The transcriptional readout at this concentration reflects the net result of both FKBP51 actions, i.e. on the Kd and on nuclear translocation. Under saturating hormone conditions, only the Kd-independent actions are detectable (Fig. 3).

**FKBP51 Disrupts Interaction of the GR Heterocomplex with Dynein—**As it has been reported that FKBP52 interacts with dynein (15, 26, 34, 42), an explanation for the delayed nuclear translocation of GR in the presence of FKBP51 may be its inability to interact with dynein. We performed coimmunoprecipitation experiments with either FLAG-tagged FKBP51 or FLAG-tagged FKBP52. As shown in Fig. 4A, dynein was coprecipitated with FKBP52 but not with FKBP51. Both immunophilins were precipitated to comparable levels. Moreover, both FKBP51 and FKBP52 were coprecipitated with hsp90 to similar extents, excluding denaturation of FKBP51 as a remotely possible explanation for the lack of coprecipitation with dynein (Fig. 4A). To prove that this differential interaction leads to disruption of the interaction of the GR heterocomplex with dynein, we immunoprecipitated GR in a different setup. We coexpressed FLAG-tagged GR with either untagged FKBP52 or FKBP51. As demonstrated in Fig. 4B, FKBP51 (but

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*Fig. 3. FKBP51 reduces nuclear translocation of GR.** HeLa cells were transfected with 0.25 μg of a plasmid expressing GR fused to GFP and with 0.75 μg of either the FKBP51 or the FKBP52-expressing plasmid. The cells were cultivated for another 24 h and then placed on ice, 1 μM of DEX was added, and then the cells were shifted after 1 h to 37 °C. The cells were fixed after the indicated times (t). The cytosol (Cyt.)/nucleus (Nucl.) distributions were scored for >100 cells for each condition in three independent experiments.
HEK cells were transfected with 10 μg of either FKBP51, FKBP52WT, or PPIase-mutated FKBP52 plasmid. After 24 h, the cells were harvested, lysed, and the FLAG-tagged immunophilins were immunoprecipitated. Shown are immunoprecipitated (IP) GR detected by a GR antibody and coprecipitated (Co-IP) dynein on the same blot. After 24 h, the cells were harvested, lysed, and the FLAG-tagged immunophilins were immunoprecipitated. Displayed are precipitated immunophilins (IP) and coprecipitated dynein (Co-IP). B, SK-N-MC or HEK cells were transfected with the reporter plasmid MTVLuc, the GR plasmid pRK7GR, β-galactosidase plasmid, and 3 μg of either control vector or wild type or PPIase mutated F67D/D68V FKBP51 or FKBP52, respectively. Luciferase data were normalized by the β-galactosidase activities and are presented as relative activities ± S.E. of three independent experiments performed in duplicate.

FIG. 5. PPIase activity of FKBP51 is not important for its activity on GR, in contrast to FKBP52. A, HEK cells were transfected with 10 μg of either FKBP51, FKBP52WT, or PPIase-mutated FKBP52 plasmid. After 24 h, the cells were harvested, lysed, and the FLAG-tagged immunophilins were immunoprecipitated. Displayed are precipitated immunophilins (IP) and coprecipitated dynein (Co-IP). B, SK-N-MC or HEK cells were transfected with the reporter plasmid MTVLuc, the GR plasmid pRK7GR, β-galactosidase plasmid, and 3 μg of either control vector or wild type or PPIase mutated F67D/D68V FKBP51 or FKBP52, respectively. Luciferase data were normalized by the β-galactosidase activities and are presented as relative activities ± S.E. of three independent experiments performed in duplicate.

The mutant FKBP51 still had the ability to reduce GR-dependent gene transcription (Fig. 5B). The corresponding PPIase-mutated FKBP52 drastically decreased GR-dependent transactivation (Fig. 5B). We concluded that the PPIase activity is not required for the inhibitory action of FKBP51. However, it is essential to maintain the positive function of FKBP52 on GR. The severity of the inhibition of this mutant implies that the PPIase activity is required for hormone binding of GR, as suggested in the yeast system (27).

Relevance of the Differential Ability of the FKBP51 and FKBP52 PPIase Domains to Bind to Dynein—Among the four domains of the two FKBP52s (Fig. 6A), the PPIase domain has been shown to mediate the interaction of FKBP52 with dynnein (15, 34, 42). In an effort to corroborate the importance of dynnein binding, we swapped the N-terminal PPIase domain between the highly homologous FKBP51 and FKBP52 proteins. Replacing the PPIase domain in FKBP52 by that of FKBP51 (Fig. 6B, Ch1 (chimera 1)) led to a GR inhibitory protein (Fig. 6C, compare Ch1 with 52WT) with reduced dynnein interaction (Fig. 6D). Conversely, replacing the PPIase domain of FKBP51 by that of FKBP52 (chimera 2) almost completely abolished the inhibitory effect of FKBP51 on GR and also reconstituted dynnein interaction (Fig. 6D). These data further emphasize our conclusion that one mode of action for FKBP51 is disrupting the interaction of the GR heterocomplex with the motor protein dynnein and the ensuing reduction of receptor translocation. Moreover, the differential interaction of the PPIase domain of FKBP51 and FKBP52 with dynnein is important in explaining the differential effect of these highly homologous proteins on GR.

The PPIase Domain Fragment of FKBP51 Is Unable to Interfere with Nuclear Translocation of GR—The PPIase do-
cells were cultivated for another 24 h and then placed on ice, and 1 μM Dex was added, and then the cells were shifted after 1 h to 37 °C. The cells were fixed after the indicated times (t). The cytosol (Cyt.) and nucleus (Nuc.) distributions were scored for >100 cells for each condition in three independent experiments. B, HEK cells were transfected with MTV-Luc, β-Gal, pRK7GR, together with either the control vector or increasing amounts of a dynamitin expressing plasmid. Luciferase data were normalized by the β-galactosidase activities and are presented as relative activities ± S.E. of three independent experiments performed in duplicate.

**Fig. 7.** The PP1ase domain fragments interfere differentially with GR nuclear translocation. A, HeLa cells were transfected with 0.15 μg of a plasmid expressing GR fused to GFP and with 1 μg of FKBP51/FKBP52 PP1ase domain fragment-expressing plasmid. The cells were cultivated for another 24 h and then placed on ice, and 1 μM of DEX was added, and then the cells were shifted after 1 h to 37 °C. The cells were fixed after the indicated times (t). The cytosol (Cyt.) and nucleus (Nuc.) distributions were scored for >100 cells for each condition in three independent experiments. B, HEK cells were transfected with MTV-Luc, β-Gal, pRK7GR, together with either the control vector or the plasmids indicated. Luciferase data were normalized by the β-galactosidase activities and are presented as relative activities ± S.E. of 3 independent experiments performed in duplicate.

We demonstrated that, in mammalian cells, the mechanism for the inhibitory action of FKBP51 on GR involves impairment of nuclear translocation in addition to the reported reduction of hormone binding (28). This conclusion is supported by several different results: discriminative binding of FKBP51 and FKBP52 to the motor protein dynein, delayed nuclear translocation of GR, and differential affect of the PP1ase domain fragments on nuclear translocation. Only the PP1ase domain of FKBP52 that binds to dynein (15) delays nuclear translocation and GR activity. Disruption of the transport machinery by overexpression of the p50 subunit dynamitin also reduced GR activity.

Although overexpression of FKBP52 had no effect on GR activity in mammalian cells, it enhanced GR-dependent transcription in yeast (27). Conversely, FKBP51 produced no effect in yeast, but inhibited GR in mammals. This difference could be explained by the significantly different cellular background provided for steroid signaling by the two systems. In contrast to mammalian cells, where steroid signaling is optimized, yeast is devoid of steroid receptors, the large FKBP5s, and potentially other GR regulators, resulting in only low GR activity. Therefore, factors such as FKBP52, which promote GR signaling, produce an effect in yeast but not in the mammalian environment already optimized for GR. Conversely, TPR factors such as FKBP51, which do not support GR, produce an effect in mammalian cells but not in yeast. Nevertheless, the importance of hsp90 interaction and of the PP1ase enzymatic activity for FKBP52 that we report here was also found in yeast (27). Thus, our results do not contradict the findings in yeast (27) but, rather, reveal another level of regulation available in mammals.

No difference in GR-dependent transcription was reported for yeast containing or lacking dynein (27). It is not clear whether this represents another difference in the mechanisms available in yeast for GR signaling or whether the influence of possible dynein binding of FKBP52 could not be observed under the experimental setting chosen. Nuclear translocation was not investigated in the yeast report (27). It is also not known whether FKBP52 binds to yeast dynein. Apparently, in mammals, small differences in the PP1ase domain between FKBP51 and FKBP52 lead to differential binding to dynein.

What could be the significance of the differential dynein interaction of FKBP51 and FKBP52 that presumably evolved in higher organisms? The major effect of the disruption of the binding of the GR heterocomplex to dynein appears to be a delayed nuclear translocation. Under physiological conditions,
GR is occupied mainly after stress (46). It has been reported that the level of corticosterone peaks already at 30 min after acute stress in rats and returns to nearly basal levels shortly after 1 h (47). Therefore, after acute stress, when hormone levels are saturating, the inhibitory function of FKBP51 on nuclear translocation should become more important than the inhibitory effect on hormone binding affinity. Expedited nuclear translocation may also have become important in mammals in some specialized cells such as neurons, where the traveling distances can be quite long. The physiological relevance of GR signaling in neurons has been amply demonstrated (46).

Apparently, increasing the levels of FKBP51 represents a cellular mechanism to decrease the potency and efficacy of corticosteroids with manifold physiological implications. For example, it is interesting to note that FKBP51 is induced by DEX (48, 49). This represents a direct autoregulatory loop to control glucocorticoid action.

We propose that three characteristics of the large immunophilins are essential for efficient GR signaling in mammals: first, interaction with hsp90; second, association with dynein; and third, PPiase enzyme activity. Although FKBP52 fulfills all three requirements, FKBP51 lacks interaction with dynein. Moreover, the conformation of FKBP51 has been postulated to prevent the PPiase domain from acting on the relevant prolines of GR (27). It will be interesting to understand for which prolines the PPiase activity of FKBP52 is required.

The first characteristic, interaction with hsp90 via the TPR domain, is necessary to compete with other TPR proteins to gain access to the GR heterocomplex. The second characteristic, binding to dynein via the PPiase domain, is required to associate with the transport machinery. Because the PPiase domain of FKBP51 is unable to bind to dynein, FKBP51 disrupts GR-dynein association and thereby impairs nuclear translocation. The third characteristic, PPiase enzyme activity, seems necessary for efficient hormone binding. It appears that, under our experimental conditions, alterations affecting hormone binding have a stronger effect than alterations affecting nuclear translocation. The PPiase-deficient FKBP52 still interacts with dynein, but it strongly inhibits GR-dependent transactivation. We cannot exclude, however, that the PPiase enzymatic activity may, in addition, be required for efficient nuclear translocation. On the other hand, the PPiase enzyme activity of FKBP51 makes no active contribution to the inhibitory action of this protein. Conditions that disrupt nuclear translocation but most likely do not change hormone affinity lead to a less severe impairment of GR, e.g. overexpression of the PPiase fragment of FKBP52, of dynamitin, or of the FKBP52 chimera with the PPiase domain of FKBP51.

Recently, a switch from FKBP51 to FKBP52 in the cytosolic GR heterocomplex following hormone binding has been reported (26). We propose that this switch is also important in promoting efficient nuclear translocation. At elevated amounts of FKBP51 (as observed in New World primates that have a 26-fold increased ratio of FKBP51/FKBP52 as compared with human (28) or induced in cell culture (this study)), no detectable exchange takes place.

Although our data in mammals and those in yeast (27) indicate that the most important domain to characterize the difference between FKBP51 and FKBP52 with regard to GR is the N-terminal PPiase domain, we cannot rule out that other domains also contribute to this difference. Interestingly, regions other than the TPR domain influence interaction with hsp90 in a manner that is different for FKBP51 and FKBP52 (44). Future research will elucidate the fine tuning that the other domains may exert to modulate the GR-related functions of the two immunophilins.
45. Burkhardt, J. K., Echeverri, C. J., Nilsson, T., and Vallee, R. B. (1997) J. Cell Biol 139, 469–484
46. De Kloet, E. R. (2000) Eur. J. Pharmacol. 405, 187–198
47. Paskitti, M. E., McCreary, B. J., and Herman, J. P. (2000) Brain Res. Mol. Brain Res. 80, 142–152
48. Baughman, G., Harrigan, M. T., Campbell, N. F., Nurrish, S. J., and Bourgeois, S. (1991) Mol. Endocrinol. 5, 637–644
49. Baughman, G.; Wiederrecht, G. J., Campbell, N. F., Martin, M. M., and Bourgeois, S. (1995) Mol. Cell. Biol. 15, 4395–4402
50. Chambraud, B., Rouviere-Fourmy, N., Radanyi, C., Hsiao, K., Peattie, D. A., Livingston, D. J., and Baulieu, E. E. (1993) Biochem. Biophys. Res. Commun. 196, 180–186
51. Callebaut, I., Renoir, J. M., Lebeau, M. C., Massol, N., Burny, A., Baulieu, E. E., and Mornon J. P. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6270–6274
52. Radanyi, C., Chambraud, B., and Baulieu, E. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11197–11201
53. Massol, N., Lebeau, M. C., Renoir, J. M., Faber, L. E., and Baulieu, E. E. (1992) Biochem. Biophys. Res. Commun. 187, 1330–1335
FK506-binding Proteins 51 and 52 Differentially Regulate Dynein Interaction and Nuclear Translocation of the Glucocorticoid Receptor in Mammalian Cells
Gabriela M. Wochnik, Joëlle Rüegg, G. Alexander Abel, Ulrike Schmidt, Florian Holsboer and Theo Rein

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