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Potentiation of Glucocorticoid Receptor-mediated Gene Expression by the Immunophilin Ligands FK506 and Rapamycin*

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It has recently been discovered that the steroid receptor-associated heat shock protein, hsp56, belongs to the FK506 family of immunophilin proteins. The ability of hsp56 to bind the immunosuppressive macrolide FK506 has led to the speculation that the steroid receptor and immunophilin signal transduction pathways are functionally interrelated. We have tested this idea by assessing the effects of FK506 on glucocorticoid receptor (GR)-mediated expression of the murine mammary tumor virus-chloramphenicol acetyltransferase (MMTV-CAT) reporter plasmid. We report that combined treatment with FK506 and low concentrations of dexamethasone (10^{-8} or 10^{-7} M) results in a large enhancement of MMTV-CAT gene expression over that seen in response to dexamethasone (Dex) alone. FK506 potentiation of MMTV-CAT expression did not occur at 10^{-6} M Dex or in the complete absence of hormone. We also show that potentiation of Dex-mediated MMTV-CAT expression occurs in response to rapamycin, that glucocorticoid-regulated enhancer sequences are sufficient for the FK506-mediated potentiation effect, and that this effect can be blocked by RU486 antagonist. Finally, we provide evidence that FK506 potentiation of GR-mediated gene expression is the result of increased translocation to the nucleus of the GR.

In intact cells, untransformed glucocorticoid hormone receptors (GR)[1] are localized to the cytoplasm (1, 2), and upon hormone binding these receptors rapidly translocate to the nucleus, where they serve as enhancers of specific gene expression (3, 4). When cells grown in the absence of glucocorticoid hormone are ruptured, the GR is recovered in the cytosolic fraction as a large heteromeric complex containing hsp90 (5, 6) and hsp56 (7, 8). Although much is already known about the GR/hsp90 interaction, very little is known about the role of hsp56 in steroid receptor function.

Results from several laboratories utilizing both functional assays and sequence homology data have shown that hsp56 is an immunophilin protein that can bind the immunosuppressive drugs FK506 and rapamycin. This conclusion was initially made by Yem *et al.* (9) when it was found that human Jurkat cell cytosols contain a ~60-kDa protein that binds immobilized FK506 and that this protein contains an NH_2-terminal sequence identical to that previously published for hsp56 (10). In addition, the ~60-kDa FK506-binding protein also contains internal peptide sequences homologous to COOH-terminal sequences of two other FK506-binding proteins, FKBP-12 and FKBP-13 (9). At about the same time, the cDNA sequence of rabbit liver hsp56 was published by Lebeau *et al.* (11). This sequence shows that the region between amino acids 41 and 137 is 55% homologous to the domain of FKBP responsible for peptidyl-prolyl cis-trans isomerase (rotamase) activity. As all FK506-binding proteins (FKBPs) exhibit rotamase activity *in vitro*, this observation by Lebeau *et al.* (11), as well as the follow-up study utilizing hydrophobic cluster analysis (12), led to the conclusion that hsp56 represented a novel immunophilin of the FK506- and rapamycin-binding class. In support of these observations was a report by Tai *et al.* (13), in which the authors showed that a ~60-kDa FK506-binding protein from human thymus was identical to hsp56 when their respective NH_2-terminal sequences were compared, and that untransformed GR complexes from human IM-9 cell cytosols were specifically retained by an FK506 affinity matrix. The latter observation provided the first evidence that the steroid receptor-associated form of hsp56 is indeed capable of binding FK506.

The information presented thus far suggests the possibility that steroid receptor function may be affected by the binding of FK506 or rapamycin to the receptor-associated form of hsp56. Given that both the FK506 class of drugs and glucocorticoids are potent immunosuppressive compounds (14), it has been speculated that FK506 may, at least in part, act by inducing the steroid receptor immunosuppression pathway (9). A necessary requirement for such an effect by FK506 would be the promotion of GR transformation to the transcriptionally active state. In this work, we report that FK506 treatment of intact mouse L929 cells stably transfected with various reporter plasmids results in a potentiation of glucocorticoid hormone-induced GR-mediated gene transcription.

EXPERIMENTAL PROCEDURES

Materials

FK506 and rapamycin were gifts from Drs. Karen L. Leach and Martin R. Deibel, Jr. of the Upjohn Company (Kalamazoo, MI). The pMMTV-CAT plasmid is a glucocorticoid inducible reporter plasmid (15) containing the complete mouse mammary tumor virus-long terminal repeat promoter upstream of the gene for chloramphenicol acetyltransferase (CAT). The pΔMTV-CAT plasmid (16) contains a modified MMTV-LTR sequence upstream of the CAT reporter gene in which bases −190 to −88 have been deleted, thus eliminating three out of the four consensus glucocorticoid-regulated elements (GREs) known to reside within the MMTV-LTR (17). The $pGRE_2E1B$-CAT plasmid is a "minimal" reporter plasmid containing two copies of a GRE oligonucleotide plus one copy of the E1B TATA sequence immediately upstream of the CAT gene.[2] The pSV2neo plasmid (18) contains the gene for aminoglycoside phosphotransferase (*neo*) under

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[1] The abbreviations and trivial names used are: GR, glucocorticoid receptor; PR, progesterone receptor; GRE, glucocorticoid-regulated element; hsp, heat shock protein; dexamethasone (Dex), 9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione; MMTV, murine mammary tumor virus; DMEM, Dulbecco's modified Eagle's medium; CAT, chloramphenicol acetyltransferase; FKBP, FK506-binding protein.

[2] V. E. Allgood and J. A. Cidlowski, submitted for publication.

the control of the constitutively active SV40 early promoter. The steroidal antagonist RU486 was obtained from Roussel-Uclaf. The BuGR2 anti-glucocorticoid receptor monoclonal antibody (19) was a gift from Drs. William Hendry and Robert Harrison (University of Arkansas).

Methods

CAT Reporter Cell Lines—Establishment of the LMCAT cell line was achieved by transfecting one flask (75 cm^2) of L929 cells at approximately 50% confluence with 10 μg of pMMTV-CAT DNA plus 5 μg of pSV2neo DNA and 100 μg of Lipofectin reagent (20). Following an overnight incubation with DNA and lipid, the transfected cells were allowed to grow for 48 h. The cells were then subcultured (1:2) in DMEM medium containing 0.4 mg/ml G418 (Geneticin) antibiotic and were put to incubate at 37 °C, with frequent replacement of the selection medium, until colony growth was noted. The pooled cell culture thus derived was designated LMCAT. Establishment of the LΔMCAT and LGEC cell lines from L929 cells employed the same procedure, except that 10 μg of pΔMTV-CAT or $pGRE_2E1B$-CAT DNAs, respectively, were co-transfected with 5 μg of pSV2neo DNA. All cell lines were grown in DMEM medium containing 10% charcoal-extracted newborn calf serum and 0.2 mg/ml G418. Cultures were maintained at 37 °C in a humid atmosphere of 5% CO_2.

CAT Assay—Measurement of CAT enzyme activity was performed essentially according to the method of Gorman *et al.* (21) using [^{14}C] chloramphenicol as substrate. Following thin layer chromatography (TLC), quantitation was achieved by separately excising the spots corresponding to substrate and product and performing liquid scintillation spectroscopy. The cpm data obtained were divided by the basal (vehicle control) activity for each experiment to yield fold induction values. All experiments, unless otherwise noted, were repeated at least 3 times.

Cellular Fractionation and Immunoadsorption—In the experiments of Fig. 4, individual 75-cm^2 flasks of L929 cells treated with FK506 and Dex were fractionated into cytosolic and nuclear portions by Dounce A homogenization in hypotonic buffer (10 mM Hepes, 1 mM EDTA, pH 7.4). After centrifugation at 1,000 × *g* for 5 min, the cytosolic fraction was saved and the nuclear pellet was washed twice by resuspension and pelleting in hypotonic buffer containing 250 mM sucrose. Hypotonic buffer was then added to both the pellet and cytosolic fractions to a final volume of 0.5 ml. Each fraction was made 0.5 M for NaCl by the addition of 0.5 ml of a 1 M stock solution and incubated on ice with occasional vortexing for 1 h. After salt extraction, the nuclear pellets were centrifuged at 8,000 × *g* and the supernatants saved. BuGR2 anti-GR monoclonal antibody (20 μl) was added to both the cytosolic and nuclear extract fractions, and each sample was then adsorbed in batch to protein A-Sepharose, washed, and eluted with 2 × SDS sample buffer.

Gel Electrophoresis and Quantitative Western Blotting—Samples were resolved by electrophoresis in 7% SDS-polyacrylamide gels as described by Laemmli (22). The relative amounts of glucocorticoid receptor protein in the cytosolic and nuclear fractions were determined via a quantitative Western blotting technique previously described (23), which employs the BuGR2 anti-glucocorticoid receptor monoclonal antibody and both peroxidase- and ^{125}I-conjugated counter antibodies.

RESULTS AND DISCUSSION

In a manuscript under consideration,[3] we have described our initial attempts to ascertain the effects of FK506 on GR-mediated gene transcription. We showed that treatment of L929 cells containing the pMMTV-CAT reporter plasmid (LMCAT cells) with FK506 alone did not result in induction of CAT gene expression. In addition, simultaneous treatment of LMCAT cells with 1 μM Dex and 10 μM FK506 resulted in CAT enzyme levels that were qualitatively similar to that induced by 1 μM Dex alone. Since then, we have determined that 1 μM dexamethasone results in maximal induction of CAT gene expression in the LMCAT cells (data not shown). Thus, any potential effect of FK506 on GR-mediated MMTV-CAT gene expression would have to occur at sub-maximal levels of Dex. In this work, we have tested this idea by treating LMCAT cells with FK506 and low concentrations of Dex. In the experiment of Fig. 1*A*, LMCAT cells were treated with 10^{-8} M Dex after a 2-h pretreatment with increasing concentrations of FK506. The results showed that 1 μM FK506 could cause a 6.7-fold increase in CAT gene expression over that seen with 10^{-8} M Dex alone, while 10 μM FK506 resulted in an even greater increase (20.9-fold induction of CAT). Although the concentrations of FK506 (1 μM or higher) required to see the potentiation effect of Fig. 1*A* appear to be high, a wide range of effective concentrations (0.1 nM to 25 μM) have been reported for FK506 used *in vitro* (24, 25). The need for micromolar amounts of FK506 in our experimental system may simply be due to the presence of a variety of FK506-binding proteins in these cells. For example, FKBP-12 is an abundant and ubiquitous cytosolic protein with an intracellular concentration of ~20 μM in human Jurkat cells (26), while FKBP-13 has been shown to be a fairly abundant protein in calf and human T cells (27). Moreover, we and others have shown that the amount of GR-associated hsp56 is a small fraction of the total cellular hsp56 (7, 10).

In order to determine if FK506-mediated potentiation occurred at other concentrations of Dex, we subjected LMCAT cells to treatment with either Me_2SO vehicle or 10 μM FK506, followed by treatment with increasing concentrations of Dex (Fig. 1*B*). Several conclusions can be made from the data of Fig. 1*B*. First, FK506 treatment alone clearly did not result in activation of CAT gene expression in these cells. Second, FK506 potentiation of Dex-mediated MMTV-CAT gene expression requires a minimum of 10^{-8} M Dex and results in a dramatic increase in CAT expression (30.9-fold) in comparison to that seen in response to 10^{-8} M Dex alone. As the level of CAT induction by 10^{-8} M Dex alone was almost negligible (1.4-fold) as compared to the basal control (1-fold), the 30.9-fold induction by the combined FK506/10^{-8} M Dex treatment may represent the most significant stimulatory effect of the FK506 drug on glucocorticoid hormone-mediated responses. Third, a marked potentiation by FK506 also occurred at 10^{-7} M hormone (59.0-fold) over that seen by hormone alone (33.2-fold), but no such potentiation was observed at 10^{-6} M Dex. In fact, FK506 treatment at 10^{-6} M Dex resulted a lower level of CAT induction (36.6-fold) than hormone alone (49.6-fold).

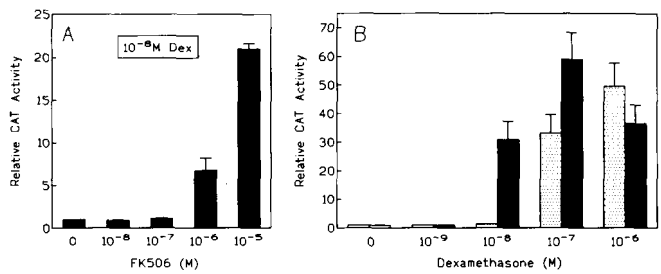

FIG. 1. **FK506 potentiates dexamethasone-induced expression of MMTV-CAT.** *Panel A*, concentration dependence of FK506 in LMCAT cells exposed to 10^{-8} M Dex. Replicate flasks (25 cm^2) of LMCAT cells were treated for 2 h with Me_2SO vehicle (0) or with the indicated concentrations of FK506. Immediately following pretreatment, Dex (10^{-8} M final concentration) was added to all flasks and the cells were cultured for an additional 20 h. CAT assays were performed and fold induction values were calculated relative to the vehicle (0) control. Values obtained represent the means ± S.E. of 3 independent experiments. *Panel B*, concentration dependence of Dex in LMCAT cells exposed to 10 μM FK506. LMCAT cells were treated for 2 h with Me_2SO vehicle (*stippled bars*) or with 10 μM FK506 (*black bars*). Following pretreatment, Dex was added to the indicated final concentrations and all flasks were cultured for an additional 20 h. CAT assays were performed, and fold induction values were calculated relative to the Me_2SO/0 Dex control. Values obtained represent the means ± S.E. of 4 independent experiments.

[3] K. A. Hutchison, L. C. Scherrer, M. J. Czar, Y.-M. Ning, E. R. Sánchez, K. L. Leach, M. R. Deibel, Jr., and W. B. Pratt, submitted for publication.

Rapamycin is an immunosuppressive macrolide structurally related to FK506 (28). Like FK506, rapamycin has recently been shown to bind hsp56 (13), as well as the low molecular weight FK506-binding protein FKBP-12 (29). With this in mind, we tested if rapamycin could also potentiate Dex-mediated induction of MMTV-CAT gene expression. In the experiment of Fig. 2, LMCAT cells were pretreated with increasing concentrations of rapamycin followed by treatment with 10^{-8} M Dex. Although the results showed only small increases (~2.2-fold) in CAT levels at 10^{-7} and 10^{-6} M rapamycin, a pronounced and consistent potentiation of Dex-mediated induction was measured (8.5-fold) in response to 10 μM rapamycin.

The experiments presented so far suggest that FK506 and rapamycin can enhance Dex-mediated expression of the MMTV-CAT reporter plasmid. Other than the requirement for Dex hormone, no evidence has yet been provided to show that the glucocorticoid receptor is required in order to see this effect. In order to demonstrate that the FK506 potentiation effect is the result of enhancement of transcription activity by the GR, we performed the experiments seen in Fig. 3. In Fig. 3*A*, LMCAT cells were pretreated with FK506 and RU486 antagonist prior to addition of 10^{-8} M Dex. The results show that RU486 can completely block the FK506-mediated potentiation effect. As the RU486 antagonist has been shown to be a specific inhibitor of GR transcription activity (30), it seems reasonable to conclude that the combined effect of FK506 and Dex on MMTV-CAT expression is mediated by GR.

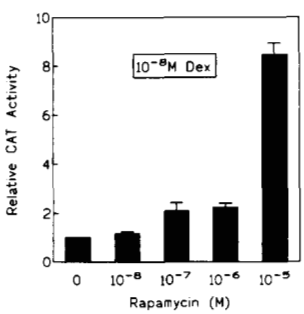

FIG. 2. **Potentiation of dexamethasone-induced MMTV-CAT expression by rapamycin.** LMCAT cells were treated for 2 h with vehicle (0) or with the indicated concentrations of rapamycin. Immediately following pretreatment, Dex (10^{-8} M final concentration) was added to all flasks and the cells were cultured for an additional 20 h. CAT assays were performed, and fold induction values were calculated relative to the vehicle (0) control. Values obtained represent the means ± S.E. of 2 independent experiments.

The MMTV-CAT reporter construct contained in the LMCAT cells relies on the presence of four consensus glucocorticoid-regulated elements (GREs) within the MMTV promoter to effect GR-mediated enhancement of gene expression (15, 17). However, the MMTV promoter is a long (~1,200 bp) regulatory sequence known to contain binding sites for a variety of transcription factors. This fact poses the possibility that the mechanism by which FK506 enhances GR-mediated gene transcription is via activation of a transcription factor(s) distinct from the GR for which there exists a cognate recognition sequence within the MMTV promoter. Indeed, there are several examples of transcription factors (*e.g.* CACCC box binding factor) that are known to cooperatively enhance GR-mediated transcription (31, 32). We have addressed this question by asking if GRE sequences alone are sufficient for FK506-mediated potentiation. Toward this end, we have tested the effects of FK506 on an L929 cell line containing the pΔMTV-CAT reporter in which three of the four GRE sequences have been deleted (16), and on another L929 cell line containing the pGRE$_2$E1B-CAT "minimal" reporter composed of two tandemly linked synthetic GREs and a TATA box immediately upstream of the CAT reporter gene. In the experiment of Fig. 3*B*, treatment of the pΔMTV-CAT cells with FK506 and Dex (10^{-8} and 10^{-7} M) did not result in a measurable induction of CAT activity. In contrast, FK506 treatment of the pGRE$_2$E1B-CAT cells resulted in a marked potentiation of Dex-mediated CAT gene expression (Fig. 3*C*). These results suggest that GRE sequences are indeed sufficient for FK506-mediated enhancement of GR-mediated gene expression and that FK506-mediated activation of another as yet unknown transcription factor is probably not occurring. Although the levels of FK506 potentiation were lower in the pGRE$_2$E1B-CAT cells (Fig. 3*C*) than in the pMMTV-CAT cells (Fig. 1*B*), this probably reflects the fact that the pGRE$_2$E1B-CAT construct is a weaker GR-inducible promoter than pMMTV-CAT.

Taken together, the results of Fig. 3 suggest that FK506 serves to enhance Dex-mediated gene expression by a direct or indirect enhancement of transcription activity by the GR protein itself. One way in which FK506 may achieve this is by increasing the amount of GR that is transformed and subsequently translocates to the nucleus. We have investigated this possibility by assaying for the subcellular localization of GR in L929 cells subjected to various FK506 and Dex treatments (Fig. 4). From the experiment of Fig. 4*A*, it can be concluded that combined treatments with FK506 and Dex

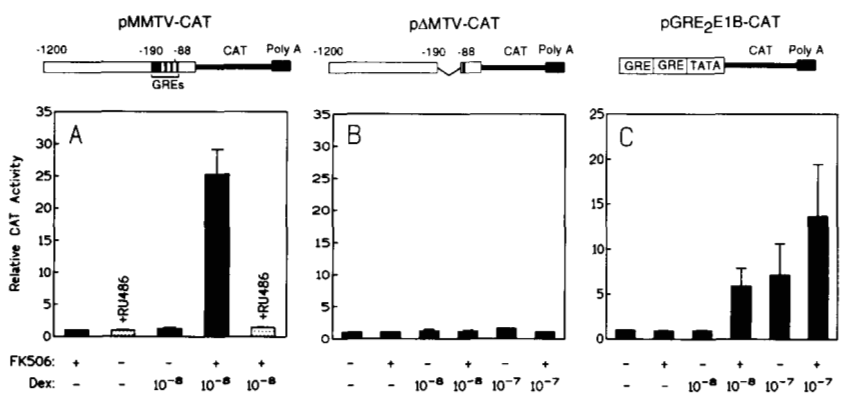

FIG. 3. **FK506 potentiation of dexamethasone-induced CAT expression is inhibited by RU486 and requires GRE enhancer sequences.** L929 cells stably transfected with the pMMTV-CAT (*panel A*), pΔMTV-CAT (*panel B*), or pGRE$_2$E1B-CAT (*panel C*) reporter plasmids were treated first with 10 μM FK506 for 2 h, followed by the indicated concentrations of Dex for an additional 20 h. In *panel A*, cells were also pretreated with RU486 antagonist (*stippled bars*). CAT activities were assayed and fold induction values were calculated relative to the vehicle (no treatment) controls (*panels B* and *C*) or relative to the FK506 treatment alone (*panel A*). Values obtained represent the means ± S.E. of 3 (*panels A* and *B*) or 2 (*panel C*) independent experiments.

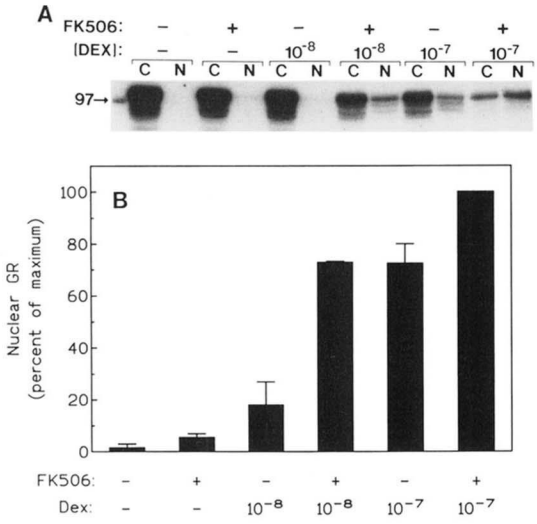

FIG. 4. **FK506 potentiates dexamethasone-induced nuclear translocation of GR.** L929 cells were treated with vehicle or 10 μM FK506 for 2 h. After pretreatment, Dex was added to the indicated final concentrations and the cells were cultured for an additional 20 h. The cells were then fractionated into cytosolic (*C*) and nuclear pellet (*N*) compartments. Each fraction was salt-extracted, immunoadsorbed with BuGR2 anti-GR monoclonal antibody and subjected to quantitative Western-blotting. *Panel A*, autoradiogram of typical Western blot. The panel represents an autoradiogram made from the Western blot in which ^{125}I-conjugated counter antibody was employed. The 97-kDa molecular mass standard is indicated. *Panel B*, relative amounts of GR protein in the nuclear fractions. After visualization of GR protein bands by peroxidase staining, GR bands corresponding to the nuclear fractions only were excised and subjected to liquid scintillation spectroscopy. All cpm values obtained were normalized as percent of maximum value (10 μM FK506 + 10^{-7} M Dex). The *bars* indicate the means ± S.E. for 2 independent experiments.

result in clear increases in the amounts of GR in the nuclear pellet fractions over that seen in response to Dex hormone alone. Similarly, the amounts of GR in the cytosolic fractions were decreased in response to the FK506/Dex treatments. This observation, namely that FK506 can enhance GR translocation to the nucleus, makes it unlikely that the FK506 potentiation effect is the result of CAT mRNA stabilization or some other post-transcriptional effect. When the relative amounts of GR in the nuclear fractions are quantitated (Fig. 4*B*), the amount of GR in the nuclear fraction of cells treated with 10^{-8} M Dex plus FK506 is roughly the same as that seen in the nuclear pellet of cells treated with 10^{-7} M Dex alone (72.9 *versus* 72.5%, respectively). It should be noted that the relative CAT activities resulting from the 10^{-8} M Dex/FK506 *versus* 10^{-7} M Dex alone treatments (Fig. 1*B*) were also of roughly equal values (30.9- *versus* 33.2-fold, respectively). This suggests that under these conditions there may be a 1:1 correlation between the amount of GR in the nucleus and the extent of GR-mediated gene transcription.

The results so far presented are consistent with a model in which FK506 potentiates GR-mediated gene expression by enhancing transformation of the GR and its subsequent translocation to the nucleus. Although it is tempting to speculate that FK506 achieves this by directly binding the hsp56 subunit of the untransformed, cytosolic GR complex, it is also possible that FK506 acts indirectly on the GR complex through the other known FK506-binding proteins, namely, FKBP-12 and FKBP-13 or a diverse group of proteins described by the laboratories of Schreiber (33) and Harding (34). Yet another alternative is that the GR-independent hsp56 complex (10, 35), which is known to contain hsp90 and hsp70 as well as several as yet unidentified proteins, may be involved in mediating the effects of FK506 on GR action. However, the recent report by Tai *et al.* (13), demonstrating that GR complexes can be specifically purified via an FK506 affinity matrix, suggests that FK506 can indeed bind GR-associated hsp56 and a direct effect of FK506 on GR transformation *in vivo* remains the most logical explanation for the results described herein.

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REFERENCES

1. Antakly, T. & Eisen, H. J. (1984) *Endocrinology* **115,** 1984–1989
2. Qi, M., Hamilton, B. J. & DeFranco, D. (1989) *Mol. Endocrinol.* **3,** 1279–1288
3. Evans, R. M. (1988) *Science* **240,** 889–895
4. Beato, M. (1989) *Cell* **56,** 335–344
5. Sánchez, E. R., Toft, D. O., Schlesinger, M. J. & Pratt, W. B. (1985) *J. Biol. Chem.* **260,** 12398–12401
6. Mendel, D. B., Bodwell, J. E., Gametchu, B., Harrison, R. W. & Munck, A. (1986) *J. Biol. Chem.* **261,** 3758–3763
7. Tai, P. K. K., Maeda, T., Nakao, K., Wakim, N. G., Huhring, J. L. & Faber, L. E. (1986) *Biochemistry* **25,** 5269–5275
8. Sánchez, E. R. (1990) *J. Biol. Chem.* **265,** 22067–22070
9. Yem, A. W., Tomasselli, A. G., Heinrikson, R. L., Zurcher-Neely, H., Ruff, V. A., Johnson, R. A. & Deibel, Jr., M. R. (1992) *J. Biol. Chem.* **267,** 2868–2871
10. Sánchez, E. R., Faber, L. E., Henzel, W. J. & Pratt, W. B. (1990) *Biochemistry* **29,** 5145–5152
11. Lebeau, M.-C., Massol, N., Herrick, J., Faber, L. E., Renoir, J.-M., Radanyi, C. & Baulieu, E.-E. (1992) *J. Biol. Chem.* **267,** 4281–4284
12. Callebaut, I., Renoir, J.-M., Lebeau, M.-C., Massol, N., Burny, A., Baulieu, E.-E. & Mornon, J.-P. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 6270–6274
13. Tai, P.-K., Albers, M. W., Chang, H., Faber, L. E. & Schreiber, S. L. (1992) *Science* **256,** 1315–1318
14. Jain, A. B., Todo, S., Fung, J. J., Venkataramanan, R., Day, R., Bryant, J., Abu-Elmagd, K. M., Alessiani, M., Takaya, S., Tzakis, A. & Starzl, T. E. (1991) *Transplant. Proc.* **23,** 3023–3025
15. Danielsen, M., Northrop, J. P. & Ringold, G. M. (1986) *EMBO J.* **5,** 2513–2522
16. Hollenberg, S. M. & Evans, R. M. (1988) *Cell* **55,** 899–906
17. Cato, A. C. B., Miksicek, R., Schutz, G., Arnemann, J. & Beato, M. (1986) *EMBO J.* **5,** 2237–2240
18. Southern, P. J. & Berg, P. (1982) *J. Mol. Biol. Appl. Genet.* **1,** 327–341
19. Gametchu, B. & Harrison, R. W. (1984) *Endocrinology* **114,** 274–279
20. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. & Danielsen, M. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 7413–7417
21. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051
22. Laemmli, U. K. (1970) *Nature* **227,** 680–685
23. Tienrungroj, W., Sánchez, E. R., Housley, P. R., Harrison, R. W. & Pratt, W. B. (1987) *J. Biol. Chem.* **262,** 17342–17349
24. Goto, T., Kino, T., Hatanaka, H., Okuhara, M., Kohsaka, M., Aoki, H. & Imanaka, H. (1991) *Transplant. Proc.* **23,** 2713–2717
25. Dumont, F. J., Staruch, M. J., Flattery, A., Altmeyer, A. & Sigal, N. H. (1991) *Transplant. Proc.* **23,** 2870–2872
26. Siekierka, J. J., Wiederrecht, G., Cryan, J., Hung, S. H. Y., Comisky, M. & Sigal, N. H. *Transplant. Proc.* **23,** 2720–2721
27. Jin, Y.-J., Albers, M. W., Lane, W. S., Bierer, B. E., Schreiber, S. L. & Burakoff, S. J. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 6677–6681
28. Sawada, S., Sizuki, G., Kawase, Y. & Takaku, F. (1987) *J. Immunol.* **139,** 1797–1803
29. Van Duyne, G. D., Standaert, R. F., Schreiber, S. L. & Clardy, J. (1991) *J. Am. Chem. Soc.* **113,** 7433–7434
30. Moguilewsky, M. & Philibert, D. (1984) *J. Steroid Biochem.* **20,** 271–276
31. Schule, R., Muller, M., Otsuka-Murakami, H. & Renkawitz, R. (1988) *Nature* **332,** 87–90
32. Schule, R., Muller, M. Kaltschmidt, C. & Renkawitz, R. (1988) *Science* **242,** 1418–1420
33. Fretz, H., Albers, M. W., Galat, A., Standaert, R. F., Lane, W. S., Burakoff, S. J., Bierer, B. E. & Schreiber, S. L. (1991) *J. Am. Chem. Soc.* **113,** 1409–1411
34. Rosborough, S. L., Fleming, M., Nelson, P. A., Boger, J. & Harding, M. W. (1991) *Transplant. Proc.* **23,** 2890–2893
35. Perdew, G. H. & Whitelaw, M. L. (1991) *J. Biol. Chem.* **266,** 6708–6713