Arrestin Binding to the G Protein-coupled N-Formyl Peptide Receptor Is Regulated by the Conserved “DRY” Sequence*

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Following activation by ligand, the N-formyl peptide receptor (FPR) undergoes processing events initiated by phosphorylation that lead to receptor desensitization and internalization. Our previous results have shown that FPR internalization can occur in the absence of receptor desensitization, suggesting that FPR desensitization and internalization are controlled by distinct mechanisms. More recently, we have provided evidence that internalization of the FPR occurs via a mechanism that is independent of the actions of arrestin, dynamin, and clathrin. In the present report, we demonstrate that stimulation of the FPR with agonist leads to a significant translocation of arrestin-2 from the cytosol to the membrane. Fluorescence microscopy revealed that the translocated arrestin-2 is highly colocalized with the ligand-bound FPR. A D71A mutant FPR, which does not undergo activation or phosphorylation in response to ligand, did not colocalize with arrestin-2. Surprisingly, an R129G mutant FPR, which does not bind G protein but does become phosphorylated and subsequently internalized, also did not bind arrestin. These results indicate that arrestin binding is not required for FPR internalization and demonstrate for the first time that a common motif, the conserved “DRY” domain of G protein-coupled receptors, is essential for phosphorylation-dependent arrestin binding, as well as G protein activation.

G protein-coupled receptors (GPCRs)† play essential roles in most physiological responses. Of particular significance is their role in the regulation of the complex signaling pathways of the immune system. Chemokine and chemotactant receptors are largely responsible for leukocyte trafficking and activation (1). The pathways responsible for receptor activation are critical to the proper functioning of a given system, and of equal importance are the pathways involved in terminating or attenuating these responses. Termination of receptor signaling has been shown to be dependent on receptor phosphorylation, primarily by the family of G protein-coupled receptor kinases (GRKs) (2). However, receptor phosphorylation alone is insufficient to preclude G protein binding and activation. For this to occur, another protein, a member of the arrestin family, must first bind the phosphorylated receptor. The binding of arrestins to phosphorylated receptors prevents G protein binding and results in an inactive receptor (3, 4). For many G protein-coupled receptors, arrestin also acts as an adapter protein, mediating internalization through clathrin-coated pits (5). Furthermore, arrestin-mediated recruitment of Src has been shown to be essential for the activation of mitogen-activated protein kinases by certain G protein-coupled receptors (6). More recently, it has been suggested that the ability of arrestins to dissociate from an internalized or internalizing receptor regulates the rate at which the receptor is resensitized and re-expressed at the cell surface (7). It is thus clear that arrestins can play multiple roles in receptor desensitization, internalization, signal transduction, and resensitization.

Mechanisms for the binding of arrestins to receptors have been described based largely on biochemical, biophysical, structural, and mutational studies of visual arrestin (8). This protein exhibits great selectivity for binding to light-activated, phosphorylated rhodopsin, with binding resulting in signal termination (9). With the recent x-ray crystallographic determination of visual arrestin, a much more precise understanding of the functional properties of arrestins has come to light (8). The structure of visual arrestin consists of two domains with a highly polar core comprised mostly of amino-terminal domain residues. The extreme carboxyl terminus of the protein lies over the cavity containing the polar core. Removal of the carboxyl terminus or mutation of the polar core of the homologous arrestin-2 protein results in an arrestin molecule that discriminates poorly between phosphorylated, activated rhodopsin and non-phosphorylated, activated rhodopsin, suggesting the phosphorylated carboxyl terminus of rhodopsin binds to and disrupts this polar region (10). Disruption of the polar core is believed to result in a large conformational change within arrestin, leading to its activation (11). A key feature of arrestin, essential to its proper functioning, is its ability not only to recognize the phosphorylation state of a receptor but also its activation state, reflecting whether ligand is bound. This has been shown for visual and non-visual arrestins and is elegantly demonstrated by studies of a mutant form of arrestin, which disrupts the polar core (partially pre-activating the protein), preventing the protein from discriminating the phosphorylation state of the receptor (10). Despite this deficit, the protein continues to display significantly higher affinity for the light-activated form of rhodopsin compared with the inactive form.
These results indicate that arrestins recognize at least two distinct features of G protein-coupled receptors.

The N-formyl peptide receptor (FPR) is a chemoattractant receptor found predominantly on leukocytes (1). We have demonstrated that following ligand stimulation, this receptor is rapidly phosphorylated on its carboxyl terminus (12). This phosphorylation, likely mediated by GRK2, is essential for the subsequent desensitization and internalization of the receptor (13, 14). However, studies of U937 cells expressing receptor mutants partially defective in phosphorylation have revealed that, unlike the paradigm outlined above, receptor internalization can occur in the absence of desensitization (15). This suggested that FPR internalization and desensitization are mediated by distinct mechanisms. Using a dominant negative arrestin mutant (arrestin-2 319–418), which binds to clathrin but not activated receptors, we have subsequently confirmed in HEK cells that internalization of the FPR occurs in an arrestin-independent manner. Furthermore, co-expression of a dominant negative dynamin mutant or a dominant negative clathrin mutant also had no effect on FPR internalization, substantiating that neither clathrin nor caveolae are involved in this process. Lastly, fluorescence microscopy revealed that the β2-adrenergic receptor, which does internalize via clathrin-coated pits, does not colocalize with the FPR during simultaneous internalization of both receptors. Together, these results provide clear evidence that the FPR need not bind arrestins to be processed for internalization.

In this study, we demonstrate that stimulation of the FPR does indeed result in a translocation of arrestin-2 from the cytosol to the membrane and furthermore that the translocated arrestin-2 colocalizes with the FPR. Despite not being required for internalization, arrestin-2 can clearly be seen to be associated with endosomes containing internalized FPR. Activation of the FPR is required for this association as demonstrated by the lack of arrestin colocalization with an inactive D71A mutant form of the FPR. However, another mutant form of the FPR, R123G, which does not bind G protein but does become phosphorylated and undergoes internalization, was also shown not to bind arrestin. These results suggest that the binding of arrestin to G protein-coupled receptors may be regulated in part by the same receptor activation signal utilized by G proteins, namely the highly conserved “DRY” sequence, located at the interface between the third transmembrane helix and the cytoplasm.

**EXPERIMENTAL PROCEDURES**

**Materials**—The cDNA encoding the FPR was originally obtained from a human HL-60 granulocyte library (17). The generation of FPR mutants has been described previously (18). Anti-arrestin-2 rabbit polyclonal antiserum was generously supplied by Dr. Jeffrey Benovic, Thomas Jefferson University. Texas Red-conjugated goat anti-rabbit antiserum was generously supplied by Dr. Jeffrey Benovic, Thomas Jefferson University. Texas Red-conjugated goat anti-rabbit antibodies was from Vector Laboratories. FMLF, GTP-γ-S, and HRP-conjugated goat anti-rabbit antibodies were purchased from Sigma. N-Formyl-Nle-Leu-Phe-Nle-Tyr-Lys fluorescein was from Molecular Probes.

**Transfection Assay**—U937 cells (105) were collected, washed once in PBS, and resuspended into 10 ml of PBS. One-half of the sample was stimulated with 50 μM FMLF for 8 min at 37 °C. The reaction was stopped with the addition of 10 ml of cold PBS. Stimulated and unstimulated cells were then pelleted, resuspended in 750 μl of PBS plus protease inhibitor mixture (Calbiochem), and sonicated. The membrane and cytosolic fractions were separated by centrifugation at 30,000 × g for 30 min. The membrane fraction was resuspended once and recentrifuged. Western blot analysis was carried out to determine the relative arrestin concentration in each fraction.

**Western Blot Analysis**—Proteins were separated by SDS polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Gelman) with a semi-dry transfer apparatus (Owl Scientific). Membranes were blotted with antibody against the indicated arrestin protein followed by an HRP secondary antibody. The blots were developed using ECL Plus (Amersham Pharmacia Biotech) and imaged using a phosphorimager (Molecular Dynamics).

**RESULTS AND DISCUSSION**

The role of arrestins in the desensitization, and more recently internalization, of numerous G protein-coupled receptors has been thoroughly documented (21, 22). However, our recent work in the characterization of phosphorylation-deficient FPR mutants has suggested that desensitization and internalization of this receptor occur through distinct mechanisms (15). Subsequently, we have demonstrated that FPR internalization proceeds via a pathway that is not only independent of arrestin but also independent of dynamin and clathrin. The mediators of this mode of internalization have yet to be described. Despite this, our results have led to questions regarding the existence of an interaction between arrestins and the FPR and the role, if any, of arrestins in FPR function.

To investigate the potential interaction between the FPR and arrestins, we utilized a model promonocytic cell line, U937, stably transfected with the wild type FPR (23). Analysis of whole cell lysates by Western blot revealed that this cell line predominantly expresses arrestin-2 (data not shown). To deter-
mine the cellular location of the arrestin-2, cells were incubated for 10 min either in the presence or absence of agonist, disrupted by sonication, and separated into cytosolic and membrane fractions by centrifugation. In the nonstimulated cells, approximately 15% of the total arrestin-2 was associated with the membrane fraction (Fig. 1). Following a 10-min treatment with agonist, a significant translocation occurred with approximately 50% of the arrestin-2 now detected in the membrane fraction. Because arrestin is known to reside predominantly in the cytosol in unstimulated cells (24), this result is consistent with the activated FPR being able to recruit arrestin to the membrane.

Given that activation of the FPR induced arrestin-2 translocation to the plasma membrane, we next examined cells utilizing fluorescence microscopy techniques to investigate whether the translocating arrestin was being directed to sites containing the FPR. Cells were stimulated with a fluorescent agonist to identify and track the FPR as it was processed. The cells were then immediately fixed and permeabilized, arrestin-2 staining with anti-arrestin antibodies followed by a secondary antibody conjugated to Texas Red. Shown are representative microscopic images displaying the cellular localization of FPR and arrestin-2. Three independent experiments were performed with identical results.

FIG. 1. Translocation of arrestin-2 from the cytosol to the membrane following fMLF stimulation of FPR-transfected cells. A, FPR-transfected U937 cells were treated with 50 μm fMLF for 8 min at 37 °C. Cells were then sonicated to generate cytosolic and membrane fractions. Subsequent Western analysis of arrestin-2 in either the cytosolic (Cyt) or membrane (Mem) fractions in the absence or presence of agonist was performed. B, quantitation of arrestin-2 associated with the membrane fraction as a percentage of total arrestin-2 in either untreated (unstim) or treated (+fMLF) cells. Shown are the means ± S.E. of three independent experiments.

To characterize this interaction further, we employed two mutant forms of the FPR, R123G and D71A, that we have described previously (26). The R123G mutation is at the cytoplasmic boundary of the third transmembrane domain, and the D71A is located in the second transmembrane segment. The Arg-123 site is part of the highly conserved DRY consensus sequence that is conserved in all identified G protein-coupled receptors (27). Although the Asp and Tyr can permit a small number of substitutions, the Arg is unalterable. The Asp-71 residue is also highly conserved in the great majority of G protein-coupled receptors, with only a small number exhibiting substitutions. In our previous work we have demonstrated that neither the R123G nor the D71A mutant receptor was able to mediate a ligand-induced calcium response as seen with the wild type FPR. This suggested a defect in the ability of these mutant receptors to either bind or activate G protein (26). Although we initially hypothesized that both mutants were unable to attain the active receptor conformation, differences in receptor phosphorylation and internalization suggested otherwise (15).

To demonstrate the functional capabilities of the R123G and D71A mutants, we used membrane preparations to investigate receptor activity with respect to G protein coupling. Utilizing spectrofluorometric techniques that detect ligand dissociation from receptors, it can be determined whether a given receptor is capable of interacting with G proteins. Two distinct rates of ligand dissociation can be observed dependent on whether a G protein is bound to the receptor. The receptor-G protein complex has a higher affinity for ligand than receptor alone. This can also be viewed in spectrofluorometric experiments as the ligand dissociation rate being sensitive to guanine nucleotide (GTPγS). An initial slow dissociation rate is present that is followed by a fast dissociation rate after the addition of GTPγS with the wild type receptor (Fig. 3A). Neither the Arg-123 nor the Asp-71 mutants display any nucleotide sensitivity, and there is no initial slow dissociation rate (Fig. 3A). Ligand dissociates at a single rate rather than two distinct rates as observed with the wild type receptors, indicating that 70% of the wild type FPR is initially coupled to G protein (Fig. 3B). Interestingly, although both mutant receptors exhibit single rate dissociation kinetics, the R123G rate is slower than the D71A, suggesting that the Arg-123 receptor may be able to
undergo a conformational change in the presence of ligand, possibly to a higher affinity, activated state.

To determine the extent to which the receptors internalize under the conditions used in microscopy experiments, cells expressing either wild type, R123G, or D71A FPR were treated with fluorescent agonist for 8 min and washed extensively to remove fluorescent ligand from the exterior of the cell. Samples were then assayed by flow cytometry where detected fluorescence represents fluorescein that has been internalized with receptors into the cell (Fig. 3C). Approximately 70% of the total wild type cell surface receptors have been internalized under these conditions, and approximately 50% of the cell surface R123G mutant FPR was internalized. Under the same conditions, there is essentially no internalization of the D71A receptor. This is consistent with the rates and extents of internalization demonstrated previously for other agonists (28).

We next examined the ability of both of the mutant receptors to redistribute arrestins following stimulation. Fluorescence microscopy experiments were performed to determine whether either the R123G or D71A mutant receptor was able to colocalize with arrestin-2. Both receptors, like the wild type receptor, initially appeared diffuse, spread evenly over the cell surface (data not shown). After incubation with fluorescent agonist, the R123G receptor appears punctate, similar to wild type receptor, with a significant fraction of the receptor appearing in endosomes (Fig. 4). The D71A receptor, however, remains diffuse over the cell surface reacting very differently to agonist treatment. Stimulation of the cells has no effect on the appearance of arrestin-2, and there is no apparent colocalization with either mutant receptor. The result for the R123G mutant FPR was particularly surprising, because it has been shown to undergo ligand-dependent phosphorylation (28). To support these findings, arrestin-2 translocation experiments were performed using the R123G and D71A cells lines. For both cell lines, there is no change in the fraction of arrestin that is membrane-associated upon ligand stimulation, with <10% of the total arrestin being associated with the membrane fraction in either the agonist-treated or unstimulated cells (data not shown). These results indicate that receptor activation is required for receptor processing and arrestin binding as demonstrated by the D71A FPR mutant but that receptor phosphorylation alone, in the presence of ligand, is insufficient for arrestin binding as demonstrated by the R123G FPR mutant, which undergoes phosphorylation and internalization but does not activate G proteins.

**Fig. 3. G protein coupling and internalization of the D71A and R123G mutants of the FPR.** A, the rate of ligand dissociation can be used as an indicator of receptor-G protein coupling. Membrane preparations from wild type (WT), R123G, and D71A FPR-expressing cells were used in a fluorometric assay as described under "Experimental Procedures." The dissociation of the fluorescent ligand can be followed over time. A change from a slow dissociation rate to a fast dissociation rate after the addition of GTP-γ-S (100 μM) indicates that the receptor was coupled to a G protein. Plots are representative of three experiments, each done in duplicate. B, the rate of ligand dissociation was determined for the wild type and mutant receptors. The bar graph represents the percentage of receptors displaying an initial slow dissociation rate (G protein-coupled state) for each receptor type. *, kinetic data for both the D71A and R123G mutant receptors could only be fit to a single exponential rate suggesting an absence of the initial slow dissociation rate for these receptors (mean ± S.E.). C, receptor internalization of the wild type and mutant FPR. In these experiments, cells expressing each receptor type were treated with N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys fluorescein for 8 min at 37 °C. Subsequent wash steps removed surface-associated fluorescent ligand. Receptor internalization was assessed by flow cytometry with the residual fluorescent intensity representing internalized ligand (mean ± S.E. of two experiments).

**Fig. 4. Colocalization of arrestin-2 with mutant forms of the FPR.** U937 cells stably transfected to express either wild type (WT) FPR, or the mutant receptor R123G or D71A, were incubated for 8 min at 37 °C with N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys fluorescein. The cells were then fixed with 2% paraformaldehyde and permeabilized with saponin. Arrestin-2 was visualized by staining with anti-arrestin antibodies followed by a secondary antibody conjugated to Texas Red. Images shown are representative micrographs from three separate experiments.

**TABLE I**

| FPR   | Ligand affinity | G protein binding | Internalization | Arrestin colocalization |
|-------|-----------------|-------------------|-----------------|------------------------|
| WT    | high/low        | +                 | +               | +                      |
| D71A  | low             | -                 | -               | -                      |
| R123G | low             | -                 | -               | +                      |

The results of our experiments are summarized in Table I. After stimulation with agonist, the wild type FPR assumes a fully active conformation, activates G protein, localizes to discrete membrane sites, recruits arrestin, and internalizes. The D71A mutant FPR appears totally inactive, exhibiting none of
the features of the wild type receptor with the exception that it is able to bind ligand, be it in the low affinity state. The R123G mutant FPR represents an interesting intermediate. It binds ligand only with low affinity, indicating it does not couple to G protein. This has been confirmed previously in calcium mobilization experiments (26). Although we had at first believed this mutant was inactive like the D71A mutant, we were surprised to discover that it became phosphorylated and internalized almost as well as the wild type receptor. If receptor internalization required arrestin binding, then this mutant would also have to bind arrestin. However, our recent studies in HEK cells suggested that the FPR could internalize in an arrestin-independent manner. In the current study, when we examined the ability of the R123G mutant FPR to colocalize with arrestin in U937 cells, we found that no such interaction occurred, demonstrating that in a native myeloid cell line, FPR internalization occurs in the absence of arrestin binding.

Our data further indicate the importance of the Arg-123 site not only in the interaction with G protein, but with arrestin as well. It is interesting to note that the R123G mutant is recognized by the kinase, as we have demonstrated previously, resulting in substantial ligand-dependent phosphorylation. This suggests that the R123G mutant may be able to form an overall active-like conformation state that can be recognized by the kinase but not by G protein or arrestin. This is supported by Fig. 3B, in which the ligand dissociation rate from the R123G mutant differs from that of the D71A mutant. If the D71A mutant is incapable of forming an active conformation and wild type can form a fully active conformation, the R123G mutant may represent a new intermediate state of the receptor. An alternative interpretation could propose that in addition to being an important point of contact for G protein, the Arg-123 site governs the phosphorylation pattern produced by the kinase, altering it in such a way that arrestin can no longer bind, even though phosphorylation-dependent internalization can take place. Even in this situation, a crucial role for Arg-123 is indicated in the regulation of receptor processing.

The role of the DRY sequence in two other GPCRs, the gonadotropin-releasing hormone receptor (29) and the α1b-adrenergic receptor (16), has been extensively modelled. Work with both receptors suggests that the arginine in the DRY sequence plays a key structural role in receptor activation. Scheer et al. (16) speculate that for the α1b-adrenergic receptor, mutations of the arginine residue can induce different states of the receptor. Ballesteros et al. (29) suggest that a highly conserved aspartate, which is on helix 2 in most GPCRs and on helix 7 in the gonadotropin-releasing hormone receptor, forms a salt bridge with the arginine of the DRY sequence in the active state of the GPCR. This conflicts with the view by the Scheer et al. (16), which argues that the arginine is not engaged in a salt bridge interaction. Molecular modelling by the latter group suggests that the Arg of the DRY sequence is embedded within the receptor in the inactive state, and upon ligand-mediated receptor activation, the ensuing conformational change results in the movement of the arginine side chain to the cytoplasmic surface, where it becomes solvent-exposed. This suggests that the exposure of the arginine side chain is a crucial triggering event in G protein binding and/or activation. This model supports our conclusion that this same site is equally important in triggering the binding of arrestin, consistent with the ability of arrestin to distinguish between active, phosphorylated and inactive, phosphorylated receptors.

In conclusion, we describe a novel function of a recognized G protein-coupled receptor domain. The DRY motif of G protein-coupled receptors, known to be involved in G protein activation, is shown here to be critical to arrestin recognition and binding, demonstrating the use of a conserved receptor signal to signify the activation state of the receptor to multiple binding partners. This represents the first report, to our knowledge, of the mapping of a site used by arrestins to assess the activation state of a receptor, independent of the phosphorylation state of the receptor. It remains to be determined whether this paradigm will extend to other G protein-coupled receptors. In addition, we directly demonstrate that internalization of the FPR occurs in the absence of arrestin binding, suggesting the existence of alternative adapter proteins that recognize phosphorylated receptors and mediate their internalization.

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REFERENCES

1. Prossnitz, E. R., and Ye, R. D. (1997) Pharmacol. Ther. 74, 75–102
2. Ferguson, S. S., Barak, L. S., Zhang, J., and Caron, M. G. (1996) Can. J. Physiol. Pharmacol. 74, 1095–1110
3. Ferguson, S. S., and Caron, M. G. (1998) Semin. Cell Biol. 9, 119–127
4. Krupnik, J. G., and Benovic, J. L. (1998) Annu. Rev. Pharmacol. Toxicol. 38, 299–319
5. Goodman, O. B., Jr., Krupnick, J. G., Santini, F., Gurevich, V. V., Penn, B. R., Gagnon, A. W., Keen, J. H., and Benovic, J. L. (1996) Nature 383, 447–450
6. Lutterrell, L. M., Daaka, Y., and Lefkowitz, R. J. (1999) Curr. Opin. Cell Biol. 11, 177–183
7. Oakley, R. H., Laporte, S. A., Holt, J. A., Barak, L. S., and Caron, M. G. (1999) J. Biol. Chem. 274, 32248–32257
8. Hirsch, J. A., Schubert, C., Gurevich, V. V., and Sigler, P. B. (1999) Cell 97, 257–269
9. Gurevich, V. V., Dion, S. B., Onorato, J. J., Ptasienski, J., Kim, C. M., Sternemurr, R., Housey, M. M., and Benovic, J. L. (1996) J. Biol. Chem. 271, 720–731
10. Kovoor, A., Celver, J., Abdryashitov, R. I., Chavkin, C., and Gurevich, V. V. (1999) J. Biol. Chem. 274, 6831–6834
11. VishvNatakayi, S. A., Paz, C. L., Schubert, C., Hirsch, J. A., Sigler, P. B., and Gurevich, V. V. (1999) J. Biol. Chem. 274, 11451–11454
12. Prossnitz, E. R., Kim, C. M., Benovic, J. L., and Ye, R. D. (1995) J. Biol. Chem. 270, 1130–1137
13. Prossnitz, E. R. (1997) J. Biol. Chem. 272, 15213–15219
14. Hsu, M. H., Chiang, S. C., Ye, R. D., and Prossnitz, E. R. (1997) J. Biol. Chem. 272, 29426–29429
15. Maestas, D. C., Potter, R. M., and Prossnitz, E. R. (1999) J. Biol. Chem. 274, 29791–29795
16. Scheer, A., Panelli, F., Costa, T., de Benedetti, P. G., and Cotecchia, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 808–813
17. Prossnitz, E. R., Quehenberger, O., Cochrane, C. G., and Ye, R. D. (1991) Biochem. Biophys. Res. Commun. 179, 471–476
18. Prossnitz, E. R., Quehenberger, O., Cochrane, C. G., and Ye, R. D. (1993) Biochem. J. 294, 581–587
19. Sklar, L. A., Vilven, J., Lynam, E., Nolden, D., Bennett, T. A., and Prossnitz, E. R. (2000) Biotechniques 28, 956–985
20. Gilbert, T. L., Prossnitz, E. R., and Sklar, L. A. (1999) J. Recept. Signal Transduct. Res. 19, 327–340
21. Pitcher, J. A., Freedman, N. J., and Lefkowitz, R. J. (1998) Annu. Rev. Biochem. 67, 655–692
22. Lefkowitz, R. J. (1998) J. Biol. Chem. 273, 18677–18680
23. Kew, R. R., Peng, T., DiMartino, S. J., Madhavan, D., Weinman, S. J., Cheng, D., and Prossnitz, E. R. (1997) J. Leukocyte Biol. 61, 329–337
24. Barak, L. S., Ferguson, S. S., Zhang, J., and Caron, M. G. (1997) J. Biol. Chem. 272, 27497–27500
25. Zhang, J., Barak, L. S., Anborygh, P. H., Laporte, S. A., Caron, M. G., and Ferguson, S. S. (1999) J. Biol. Chem. 274, 10999–11006
26. Prossnitz, E. R., Schreiber, R. E., Bokoch, G. M., and Ye, R. D. (1995) J. Biol. Chem. 270, 10686–10694
27. Prohent, W. C., Snyder, L. A., Schuster, D. I., Brousset, J., and Sealfon, S. C. (1992) DNA Cell Biol. 11, 1–20
28. Prossnitz, E. R., Gilbert, T. L., Chiang, S., Campbell, J. J., Qin, S., Newman, W., Sklar, L. A., and Ye, R. D. (1999) Biochemistry 38, 2240–2247
29. Ballesteros, J., Kitanovic, S., Guarnieri, F., Davies, P., Fronnen, B. J., Konvicka, K., Chi, L., Millar, R. P., Davidson, J. S., Weinstein, H., and Sealfon, S. C. (1998) J. Biol. Chem. 273, 10445–10453