The mouse κ-opioid receptor (KOR) gene is expressed in mouse embryonal carcinoma P19 cells and induced by retinoic acid (RA) within 24 h. An RA-responsive cis-acting element is identified within promoter I of the KOR gene. This element contains a GC box, a putative binding site for transcription factor Sp1. Enhanced binding of Sp1 to this GC box correlates with RA induction of KOR gene. Phosphatase inhibitor (sodium pyrophosphate) decreases RA induction of this promoter, whereas hypophosphorylation of Sp1 results in an increase in its DNA binding affinity to this promoter as demonstrated by in vitro gel retardation and in vivo chromatin immunoprecipitation assays. Consistently, the inhibitor of MEK, PD98058, dose-dependently enhances RA induction of this promoter, suggesting that the ERK signaling pathway is negatively involved in the RA induction of mouse KOR gene activities. Collectively, enhanced binding of Sp1 to promoter I of the KOR gene as a result of inhibiting the ERK pathway contributes to RA induction of this gene in P19.

Opioid receptors are located throughout the central and peripheral nervous systems and interact with opiate drugs such as morphine to affect the perception of pain, consciousness, and autonomic function. Three types of opioid receptors, μ, δ, and κ, have been defined and cloned (1, 2). These receptors belong to the superfamily of G-protein-coupled receptors and regulate a number of signaling pathways (3–8). The regulated pathways include the inhibition of adenyl cyclase activity and N-type and t-type Ca2+ channels and the activation of inwardly rectifying K+ channels. The activation of these opioid receptors also increases phospholipase C activity and causes a transient increase in the activation of the mitogen-activated protein kinase (MAPK), ERK, and ERK2–2 (9–11). The expression of opioid receptors has been examined primarily by in situ hybridization, immunohistochemistry, and ligand binding assays (12–15).

The mouse κ-opioid receptor (KOR) gene has been isolated in several laboratories (16). The genetic basis underlying the ontogeny of KOR gene has been revealed in transgenic animal models in our laboratory (17). The mouse gene contains four exons and utilizes two promoters. A total of three KOR mRNA isoforms designated as a, b, and c can be generated (18). We have previously reported the activities of dual promoters of mouse KOR gene in the P19 cell line (19). Recently, we have demonstrated the up-regulation of the endogenous KOR mRNA by depleting vitamin A in developing animals and the down-regulation of KOR mRNA in differentiating P19 cells treated with retinoic acid (RA) for 2 days or longer. This is mediated by a negative regulatory element, which contains an Ikaros binding site within intron I (promoter II) of the KOR gene (20, 21).

Interestingly, a short term (within 24 h) treatment with RA induces KOR expression in P19. We now report the mechanism mediating the early inducing effect of RA on KOR gene expression in P19 cells. We first identified a cis-acting element containing a putative Sp1 binding site (GC box) in the promoter I of the mouse KOR gene. We then demonstrated that Sp1 could bind to this GC box, and the hypophosphorylation of Sp1 enhanced its DNA binding affinity to this element. The inhibition of ERKs pathway enhanced RA induction of this promoter activity, whereas the phosphatase inhibitor suppressed RA induction of this promoter. These results demonstrated that RA-induced KOR gene expression could be mediated by the enhanced binding of Sp1 to this promoter, a consequence of hypophosphorylation of Sp1 by blocking the ERK pathway.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—Luciferase fusion plasmids were constructed by inserting various upstream regulatory sequences into a promoterless and enhancerless luciferase vector pGL3B (Promega, Madison, WI). The K45 and K19 plasmids were constructed as described previously (21). K45 contains a KOR-genomic fragment of 1320 bp from the BamHI site to the ATG codon. K19 was a truncation of K45 that contains a 904-bp KOR-genomic fragment from BamHI site to the end of exon I. Kd36, Kd37, and Kd38 constructs were generated by restriction digestion of the KOR promoter and ligation into pGL3B. Kd40 was prepared by PCR with the upstream primer bearing a NheI site and the downstream primer bearing a HindIII site. The PCR product was subcloned into NheI and HindIII sites of the pGL3B vector, and the correct clones were confirmed by DNA sequencing. Kd39 was prepared by PCR amplification of the KOR promoter fragment (bases –1019 to –883) and subcloned into pGEM-T Easy vector. This clone was then digested with SphI and SalI and then subcloned into SphI and SalI sites of the thymidine kinase promoter. Clones were confirmed by DNA sequencing.

Cell Culture—P19 cells were grown in α-minimal essential medium supplemented with 2.5% heat-inactivated fetal bovine serum and 7.5% heat-inactivated calf serum in an atmosphere of 5% CO2 at 37° C. RA was added at the concentration of 1 μM in ethanol.

Analyses of RNA—RNA was isolated from P19 cells using a TRIzol solution (Invitrogen) as described previously (18), and endogenous KOR mRNA isoforms were detected with an established RT-PCR protocol (18). Actin-specific primers were included for internal control in each RT-PCR.

Transient Transfection and Assay for Reporter Genes—P19 cells were transfected using the calcium phosphate precipitation method as de-
II. K19 is driven by the same KOR-genomic fragment containing only promoter I. A negative control plasmid, the pGL3B vector (basic)
luciferase (luc) reporter activities determined in P19 cells. K45 is driven by a contiguous KOR-genomic fragment containing promoters I and II. K19 is driven by the same KOR-genomic fragment containing only promoter I. A negative control plasmid, the pGL3B vector (basic), was included in transient transfection assay. RLU, relative luciferase units.

Nuclear Extract Preparation—P19 cells were harvested by centrifugation (2000 × g for 5 min) and washed in low salt buffer (10 mM Hepes, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A). Cell pellet was resuspended in the same low salt buffer supplemented with 0.1% Nonidet P-40 and incubated on ice for 15 min. The nuclei were extracted with high salt buffer (20 mM Hepes, pH 7.9, 0.4 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 10% glycerol), incubated on ice for 30 min. Nuclear extracts were then collected by centrifugation (12,000 × g for 30 min) and stored at −80 °C.

Electrophoretic Mobility Shift Assay (EMSA)—EMSA was carried out by using the double-stranded oligonucleotide designated as L204 (5′-GCATCAGACCGCCCGCCAGG-3′) end-labeled with [α-32P]dCTP. This probe was incubated with nuclear extract in EMSA buffer (10 mM Hepes, pH 7.9, 50 mM NaCl, 1 mM MgCl2, 0.1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.5% bovine serum albumin, 1 μg poly(dIdc) for 30 min at 4 °C. For competition analysis, a 100-fold molar excess of cold probe was also added. After incubation at 4 °C for 30 min, the reaction mixture was resolved on 5% polyacrylamide gels followed by PhosphorImager analysis. For the antibody supershift assay, 1 μl of Sp1 polyclonal antibody (Santa Cruz Biotechnology) and nuclear extract were allowed to react at 4 °C for 30 min followed by the addition of L204-labeled probe for 30 min at 4 °C.

Western Blot Analysis—Whole cell extracts were prepared by lysing confluent P19 cells in CytoBuser protein extraction reagent (Novagen). Protein concentration was determined with a Bradford assay. 60 μg of whole cell extract or nuclear extract was resolved on SDS-PAGE and transferred to polyvinylidene difluoride membrane (Bio-Rad). Blots were then probed with Sp1 polyclonal antibody (sc-59-G, Santa Cruz Biotechnology) (1:1000), and bound antibody was detected using ECL (Amerham Biosciences).

In Vitro Phosphatase Treatment—P19 nuclear extracts were prepared as described above with the addition of 10 mM sodium fluoride and 1 mM sodium orthovanadate to the final resuspension buffer. 10 μg of nuclear extract (20 μl of total reaction volume) was incubated at 30 °C for 20 min in 1× phosphate buffer (20 mM Tris-HCl, pH 7.4, 2 mM MgCl2, 0.1 mM EGTA, 1 mM dithiothreitol, 1× protease inhibitor mixture solution) with 40 units of calf intestinal alkaline phosphatase (Promega). After phosphatase treatment, 10 μl of 5× EMSA buffer was added, and the sample was analyzed for L204 probe binding.

Chromatin Immunoprecipitation Assay—P19 cells were treated with RA, PD98059, or vehicle for 20 h. Whole cell lysate was prepared after formaldehyde cross-linking as described previously (21). The cross-linking reaction was stopped by the addition of 0.125 × glycine. One-tenth diluted lysate was used for input, and the residual lysate was subjected to immunoprecipitation overnight at 4 °C using 2 μg of antibody against Sp1 (Upstate Biotechnology) or preimmune rabbit serum (Pierce). Following the reverse cross-linking step, DNA was precipitated following phenol/chloroform extraction and dissolved in 30 μl of TE buffer (10 mM Tris HCl, pH 8.0, and 1 mM EDTA, pH 8.0). PCR reactions contained 2 μl of immunoprecipitated chromatin sample with primer spanning the KOR promoter region containing the GC box, i.e. 5′-GATGCACAGTGGCTCCC-3′ and 5′-GTTCCCTGGCGGGCTGTCG-3′ in 25 μl of total volume. After 27–30 cycles of amplification, 8 μl of PCR product was analyzed on a 1.5% agarose gel.

RESULTS

RA Induces KOR Gene Activity in P19 Cells—Previously, we showed that RA was a potent suppressor of KOR gene expression in P19 cells after a long term treatment (3–5 days), and the negative regulatory sequence of the KOR was located in an element of intron I of this gene (20, 21). In an attempt to examine KOR gene expression after a short term treatment with RA, we used an established RT-PCR procedure (18) to detect the expression of specific KOR mRNA. We found that all three of the KOR mRNA isoforms, A, B, and C, were induced by RA within 24 h in P19 cells (Fig. 1, A and B). Actin expression remained constant during the time of examination as also shown in our previous study of KOR expression in P19 cells (20). In contrast, the induction of KOR messages by RA was statistically significant (*, p < 0.001; **, p = 0.006). C

![Fig. 1. RA induces KOR gene expression and promoter activity in P19 cells. A, expression of KOR mRNA isoforms A, B, and C in P19 cells was detected by RT-PCR as described under “Experimental Procedures.” Lane 1, control; lane 2, RA treatment for 24 h; lane 3, water control; lane 4, plasmid control. B, statistical analyses of isoforms A, B, and C expression in control (−RA) and RA treatment for 24 h. Data were quantified by PhosphorImager analyses. The levels of KOR isoforms A, B, and C expression were determined by normalizing KOR isoforms A, B, and C levels to actin message levels, respectively. A total of three experiments were conducted for statistic analyses. *, p < 0.001; **, p = 0.006. C, KOR-luciferase (lac) reporter activities determined in P19 cells. K45 is driven by a contiguous KOR-genomic fragment containing promoters I and II. K19 is driven by the same KOR-genomic fragment containing only promoter I. A negative control plasmid, the pGL3B vector (basic), was included in transient transfection assay. RLU, relative luciferase units.

![Lane 1](http://example.com/lane1.png) ![Lane 2](http://example.com/lane2.png) ![Lane 3](http://example.com/lane3.png) ![Lane 4](http://example.com/lane4.png)
I. It appeared that RA was able to induce the reporter expression from both reporters within 24 h (Fig. 1C), suggesting that promoter I contained the required sequence for RA induction of this gene expression in P19 cells.

A cis-Acting Element from Promoter I of KOR Gene Mediates RA Induction in P19 Cells—To further dissect the regulatory elements responsible for RA induction of KOR gene in P19 cells, serial deletion analyses were performed for various portions of promoter I. Reporter constructs of promoter I and its deletions were prepared as described under “Experimental Procedures” and designated as K19, Kd36, Kd37, Kd38, Kd39, and Kd40, respectively (Fig. 2A). The promoter activity of each construct was tested in P19 cells. Because the fragment between Kd39 and Kd40, respectively (Fig. 2A), was apparent in RA induction. A detailed examination of the 160-bp sequence revealed a GC box located between positions −1019 and −883 is devoid of promoter sequence, a thymidine kinase promoter was used as the basal promoter in the construct Kd39. As shown in Fig. 2B, four reporters (K19, Kd36, Kd38, and Kd40) that retained the sequence between −903 and −743 were induced within 24 h of RA treatment, whereas the reporters deleted in this sequence (Kd37 and Kd39) were apparently defective in RA induction. A detailed examination of the 160-bp sequence revealed a GC box (CCCCGC) located between positions −761 and −755, which is a putative binding site for transcription factor Sp1 family (23, 24). Therefore, the RA induction of the KOR gene was mediated by a DNA sequence containing a putative Sp1 binding site of promoter I. It was interesting that Kd37 appeared to be moderately induced by RA despite the lack of Sp1 binding site. However, it was noted that a putative nuclear factor-interleukin 6 element is present in this sequence, which might contribute to the moderate effect of RA on this reporter.

Transcription Factor Sp1 Binds Specifically to the GC Box on Promoter I of KOR Gene—To determine whether RA induction mediated by the GC box containing segment (−903/−743) correlated with Sp1 binding to this GC box, EMSAs were performed using nuclear extracts prepared from P19 cells. The oligonucleotide L204 containing the putative binding site for Sp1 was used as the probe. As shown in Fig. 3A, nuclear extracts from untreated and RA-treated cells produced two major DNA-protein complexes. The formation of the larger complex, the Sp1-DNA complex, was greatly enhanced in the reactions using nuclear extracts from RA-treated P19 cells (lanes 2, 6, and 10). Complex formation was specifically competed out by unlabeled wild-type L204 probe (lanes 3 and 4) but was not affected by the same amount of L204 mutant oligonucleotide (lanes 7 and 8). Three sets of experiments were conducted, and one representative set was presented here.

A supershift experiment was performed to establish the band representing Sp1-DNA complex. As shown in Fig. 3A, lanes 9–12, the slower migrating protein-DNA complex was retarded by anti-Sp1 antibody and yielded a new complex with a slower electrophoretic mobility (lanes 11 and 12). The signal of the supershifted Sp1-DNA complex was also stronger in reactions using RA-treated P19 extract as compared with that using the
untreated P19 extract, further supporting that either Sp1 expression or its DNA binding affinity was increased in RA-treated cells. Interestingly, the faster migrating complex was also competed out but failed to be supershifted by anti-Sp1, supporting the nonspecific nature of this complex.

To examine the in vivo Sp1 binding, P19 cells were treated with 1 μM RA or 0.01% ethanol and cross-linked with formaldehyde for chromatin immunoprecipitation assay. Chromatin co-precipitated with anti-Sp1 antibody was amplified with PCR using primers spanning 300 bp, which included this Sp1 binding site. As shown in Fig. 3B, Sp1 bound to the KOR promoter I in control P19 (lane 5), and the binding was greatly increased in RA-treated cells (lane 6), which was further enhanced by a MEK inhibitor PD98059, consistent with the results of gel shift assays shown in Fig. 3A and in Fig. 5B.

**Protein Phosphatase Treatment Increases Sp1 Binding Activity**—Because DNA protein interactions were found in both RA-treated and untreated cells, we were interested in determining the expression level of Sp1 in RA-treated and control P19 cells. Western blot analysis using Sp1 antibody revealed that P19 cells expressed nearly the same amount of Sp1 in both control and RA-treated cultures (Fig. 4). This result suggested that an increase in the binding affinity, rather than the level of Sp1 expression, probably accounted for the increased Sp1-DNA complexes in RA-treated P19 cells.

A role of phosphorylation in post-translational modification of Sp1 and its DNA binding activity has been described previously (25, 26). To determine whether the phosphorylation status of Sp1 was related to its binding ability to this GC box of KOR gene in P19 cells, P19 cell extract was incubated in the absence or presence of calf intestinal alkaline phosphatase and then subjected to EMSAs. As shown in Fig. 5A, Sp1 binding activity was increased by calf intestinal alkaline phosphatase treatment (lanes 3 and 4), which was blocked by incubating the reaction with 50 mM sodium pyrophosphate (NaPi) (lanes 5 and 6), a general phosphatase inhibitor. The complete blockage of DNA-Sp1 complex formation by NaPi (lanes 5 and 6) suggested that phosphorylation probably still occurred in prepared nuclear extract. In the presence of NaPi, the equilibrium of Sp1 phosphorylation could be shifted toward hyperphosphorylation; thus DNA binding was completely inhibited. We have also observed an inhibition of Sp1 binding to DNA in the presence of NaPi alone (data not shown), which was inconsistent with results shown here. To extend this finding to an in vivo situation, a MAPK inhibitor, PD98059, was used to treat cells prior to the preparation of nuclear extracts for gel shift as shown in

**Fig. 5. Sp1 binding to the GC box on promoter I is enhanced by dephosphorylation.** A, EMSA with nuclear extracts treated with phosphatase in vitro. Dephosphorylation of P19 nuclear extract from RA-treated or control culture was conducted by treating 15 μg of nuclear extract with 40 units of calf intestinal alkaline phosphatase in the presence of 50 mM sodium pyrophosphate. B, the effect of a MAPK inhibitor PD98059. Cells were treated with RA and/or PD98059 prior to the collection of nuclear extract for gel shift. Lane 1, control; lane 2, 1 μM RA for 24 h; lane 3, 1 μM RA for 24 h, and 100 μM PD98059 for 1 h; lane 4, control; lane 5, 10 μM PD98059 for 30 min; lane 6, 10 μM PD98059 for 8 h; lane 7, control culture. C, effects of in vivo dephosphorylation. Kd38 was introduced into P19 cells, and reporter activities were determined. −RA, control culture; +RA, RA treatment for 24 h; −NaPi, untreated; +NaPi, 10 mM NaPi treated for 4 h; RLU, relative luciferase units. D, Western blot of whole cell extracts or nuclear extract from P19 treated or untreated with RA for 24 h. Cells were cultured in the presence of 10 mM sodium pyrophosphate for 4 h. Western blot analyses using the Sp1 antibody were detailed under “Experimental Procedures.” The positive signal of the endogenous Sp1 is indicated with an arrow.
Retinoic Acid Induction of $\kappa$-Opioid Receptor Gene

Fig. 6. RA induction of promoter I activity is enhanced by MEK inhibitor PD98059. A, plasmid Kd38, which contains the GC box, was introduced into P19 cells. Specific reporter activities were determined at 30 min following the addition of PD98059 at different concentrations. Open bars, control cultures; filled bars, RA-treated cultures for 24 h. B, plasmid Kd33 was introduced into P19 cells, and reporter activities were determined at different time points following the addition of 10 $\mu$M PD98059. RLU, relative luciferase units.

Fig. 5B. It is obvious that RA induced the Sp1-DNA complex formation (compare lanes 1 and 2), which was further enhanced by PD98059 (compare lanes 2 and 3). The effect of PD98059 was readily detectable within 30 min (lane 5) as compared with untreated cells (lane 4) and even more dramatic in longer treatment (lane 6 for 8 h and lane 7 for 24 h). To confirm that dephosphorylation of Sp1 affected KOR expression, the GC box containing reporter Kd38 was used to transfect P19 cells in the presence of sodium pyrophosphate for 4 h with or without RA. A dramatic decrease in RA induction was observed in NaPi-treated cells (Fig. 5C). Western blot analysis using Sp1 antibody showed no marked difference in the amount of Sp1 expressed in RA-treated and control P19, analyzed for both whole cell extracts and nuclear extracts. Furthermore, NaPi had no effects on Sp1 protein levels (Fig. 5D). These results suggested that hypophosphorylation of Sp1 enhanced Sp1 binding to the GC box of the KOR promoter, which resulted in enhanced KOR gene expression. RA induction of KOR gene in P19 was correlated with the decrease in Sp1 phosphorylation.

Inhibition of ERK1/ERK2 Mediates RA-induced Promoter I Activity of KOR Gene—Sp1 has been recognized as one target of the transcription factors phosphorylated by protein kinases such as protein kinase C (PKC), protein kinase A (PKA), and MAPKs (27–30). We have shown that hypophosphorylation of Sp1 contributed to its enhanced ability to bind to the KOR promoter. To examine which kinase pathways could be involved in the RA induction of KOR in P19, we used various inhibitors for different kinases to treat P19 cells transfected with KOR reporter Kd38 for either control or RA-treated cultures. Transfected cells were induced with RA for 24 h followed by an incubation with different concentrations of PKA and PKC inhibitors (H7, chelerythrine chloride, and calphostin C) and two inhibitors of the MAPK pathway (the MEK inhibitor PD98059 and the p38 MAPK inhibitor SB202190) for 30 min. It appeared that only PD98059 enhanced RA stimulation of this reporter as shown in Fig. 6A. The negative results of other kinase inhibitors were not shown. Furthermore, this reporter was up-regulated by PD98059 without RA treatment, and the enhancing effect of PD98059 was time-dependent (Fig. 6B). These results revealed that hypophosphorylation of Sp1 by blocking the ERK signaling pathway up-regulated the KOR gene activity and that RA induction of the KOR gene involved such a blockage of this signaling pathway in P19 cells.

DISCUSSION

We have previously reported that the repression of the mouse KOR gene by RA treatment for a long duration in P19 cells was mediated by an Ikaros binding site located within intron I of this gene. The repression was attributed to the later induction of Ikaros protein by RA in P19 (20, 21). This study was conducted to investigate the early effect of RA treatment on KOR gene expression in P19 and to examine the possible underlying molecular mechanisms.

RA induced all three mRNA isoforms of the mouse KOR gene in P19, and promoter I was responsible for this positive regulation of RA within 24 h. Based on the deletion analyses, it was concluded that a cis-acting element containing a GC box in promoter I of the mouse KOR was crucial for its induction by RA. Sp1 was shown to bind to this GC box, and its DNA binding ability was greatly increased in RA-treated P19 cells (Fig. 3, A and B). However, Sp1 protein expression was not affected by RA in P19 cells after 24 h treatment as shown in Western blot analyses (Fig. 4). Interestingly, the enhanced binding of Sp1 to KOR promoter I after RA treatment was because of its hypophosphorylation. Several studies have indicated that the phosphorylation of Sp1 enhances its DNA binding activity (31, 32). On the other hand, Zhu et al. (33) reported that the dephosphorylation of Sp1 increased its binding to the Sp1-like cis-motif in the adipocyte amino acid transporter gene promoter in differentiating 3T3-L1 preadipocytes. Our results provided evidence that increased Sp1 binding to the KOR promoter could also involve its dephosphorylation in P19 cells as nuclear extract treated with calf intestinal alkaline phosphatase significantly increased the binding affinity of Sp1 to this GC box (Fig. 5A). Furthermore, the kinase pathways responsible for modification of Sp1 was probably related to the ERKs as the induction was enhanced by PD98059 (Figs. 3B and 5B).

Sp1 is a target of the MAPK pathway (27, 29), and MAPK is central to a signal transduction pathway that triggers cell proliferation or differentiation. The activation of ERKs through phosphorylation results in their translocation into the nucleus where they can phosphorylate distinct transcription factors (34, 35). These kinases can be inactivated by a family of dual specificity tyrosine phosphatases such as the MAPK phosphatase and the protein serine/threonine phosphatase 2A (36, 37). The fact that ERK signaling pathway could be involved in the early inducing effect of RA on KOR promoter correlates well with the biological effects of ERK pathways in cell differentiation. It is possible that RA increased either one of the protein phosphatases, thereby dephosphorylating Sp1 in P19 cells. Alternatively, RA could inhibit ERK activity and somehow shift the balance of phosphorylation versus dephosphorylation of Sp1 toward its hypophosphorylated state. This remains to be examined experimentally. An interesting construct Kd37, which lacks this Sp1 site, was slightly induced by RA. It was noted that a putative nuclear factor-interleukin 6 element was present in this sequence. Further experiments are needed to evaluate its potential interaction with the RA signaling pathway in KOR gene regulation.

This is the first report showing that the opioid receptor gene expression in P19 cells can be up-regulated by RA treatment. Thus, RA obviously can exert a biphasic effect on the expression of this opioid receptor gene. The early effect (within 24 h) of RA on the mouse KOR gene expression in P19 is an induction, which is mediated through a GC box containing the regulatory sequence of promoter I of this gene. Enhanced binding of Sp1 to this GC box is attributed to its hypophosphorylation, which involves a blockage of ERK signaling pathway in P19 cells. The late effect (2–3 days) of RA is a repression (21), which is mediated by the recruitment of histone deacetylases through
the induced Ikaros transcription factor, to promoter II (the intron I) of this gene. It is very interesting that promoter I of the KOR gene plays a role in its up-regulation, whereas promoter II (intron I) plays a role in its down-regulation.

P19 cells have been used as a model system to examine neuronal gene expression and regulation by various hormones and cytokines. It is clear that opioid receptor gene expression, at least for the KOR gene, is highly sensitive to a disturbance in hormonal homeostasis in animals (17). The study of KOR gene regulation by RA in P19 cells represents one of our goals to dissect the hormonal regulatory events and pathways for KOR gene expression. It is tempting to speculate that the unique expression pattern of this gene as seen in transgenic mice. It will also be interesting to examine how the ERK signaling pathway may play a role in the integration of hormonal signals to the regulation of this particular family of neural genes.

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