The vascular endothelial growth factor receptor-2 (VEGFR-2/KDR/flk-1) functions as the primary mediator of vascular endothelial growth factor activation in endothelial cells. Regulation of VEGFR-2 expression appears critical in mitogenesis, differentiation, and angiogenesis. Transcriptional regulation of the VEGFR-2 is complex and may involve multiple putative upstream regulatory elements including E boxes. Transcript initiation is dependent on an initiator (Inr) element flanking the transcriptional start site. The transcription factor, TFII-I, enhances VEGF-2 transcription in an Inr-dependent fashion. TFII-I is unusual both structurally and functionally. The TFII-I transcription factor family members contain multiple putative DNA binding domains. Functionally, TFII-I acts at both the basal, Inr element as well as at several distinct upstream regulatory sites. It has been postulated that the structure of TFII-I might allow simultaneous interaction with both basal and regulatory sites in a given promoter. As TFII-I is known to act at regulatory sites including E boxes as well as at the basal Inr element, we evaluated the possibility of Inr-independent TFII-I activation of the VEGFR-2 promoter. We found that an Inr-mutated VEGFR-2 reporter construct retains TFII-I-stimulated activity. We demonstrated that TFII-I binds to both the Inr and to three regulatory E boxes in the human VEGFR-2 promoter. In addition, reduction in TFII-I expression by siRNA results in decreased VEGFR-2 expression. We also describe counter-regulation of the VEGFR-2 promoter by TFII-IRD1. We found that TFII-I is capable of acting at both basal and regulatory sites in one promoter and that the human VEGFR-2 promoter is functionally counter-regulated by TFII-I and TFII-IRD1.

Elaboration of the complex mammalian vasculature requires precise spatial and temporal signaling. Central to this process is the vascular endothelial growth factor receptor-2 (VEGFR-2/flk-1/KDR) (1). This transmembrane receptor tyrosine kinase transduces signals from extracellular vascular endothelial growth factor (VEGF). Other receptors for VEGF have been identified, but their roles in vasculogenesis are less well defined (2). The VEGFR-2 is an early embryonic marker of endothelial cells with expression detected in mouse embryo as early as day 7 after conception (3). The critical role of VEGFR-2 in development was dramatically demonstrated through targeted disruption of the gene in mice (3). Homozygous deficient embryos died in utero between days 8.5 and 9.5 after conception due to severe abnormalities in development of the hematopoietic and endothelial systems. In addition to its role in development, VEGFR-2 has been shown to be involved in multiple pathologic conditions including tumor-induced angiogenesis (4, 5) and increased vascular permeability following ischemic insult (6).

Common to its role in both normal development and pathologic states is the process of transcriptional regulation of the VEGFR-2. The human and mouse VEGFR-2 5′-promoters have been identified (7). A number of regulatory sites have been identified including a GATA binding site (8), Sp1 and Sp3 binding sites (7, 9), as well as a NF-kB site (10) shown to be involved in both basal transcriptional activity. Deletional analysis of the 4-kb upstream region of human VEGFR-2 demonstrated that full promoter activity was dependent on the region between −225 and the transcriptional start site (7). Further, 5′-deletion to −164 resulted in almost 50% loss of activity. This deletion resulted in the loss of an E box at −170 (Fig. 1B). The minimal promoter was further defined by 3′-deletions that identified the region up to +268 as necessary for maximal promoter activity (7). The region from −225 to +268 includes a number of putative DNA binding motifs including three E boxes at −170, +70, and +184 (Fig. 1B).

Further, Wu and Patterson (11) identified a functional initiator (Inr) element in the VEGF-2 gene that is transactivated by TFII-I. The Inr element is a poorly conserved 7-base pair sequence overlapping the transcription start site that can function in TATA-less genes, such as VEGF-2, to direct transcription from a single start site (12). In addition, the Inr can enhance transcription in TATA-containing genes (12). These studies showed loss of transcriptional activity in reporter systems with disruption of the Inr sequence. Thus, TFII-I has been implicated in regulating the expression of the VEGF-2 gene.

TFII-I, a ubiquitously expressed, multifunctional transcription factor, was originally described based on its in vitro binding to the core promoter element, Inr, as well as to the upstream E box element (13). TFII-I was independently identified

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The abbreviations used are: VEGF, vascular endothelial growth factor; VEGFR-2, VEGF receptor-2; Inr, initiator; HLH, helix-loop-helix; HDAC, histone deacetylase; TSA, trichostatin A; TF, transcription factor; siRNA, small interfering RNA; BPAE, bovine pulmonary artery endothelial cells; FACS, fluorescence-activated cell sorter; GFP, green fluorescent protein; h, human.
VEGFR-2 Transcription Counter-regulation

Overview of TFII-I and TFII-IRD1 protein structure and VEGFR2 promoter DNA sequence. A, schematic of TFII-I and TFII-IRD1 proteins. Putative DNA binding domains include a leucine zipper (LZ) and multiple HLH-like repeat domains (shown in red). Amino acid numbering is indicated. B, schematic of minimal human VEGFR-2 promoter. Portion of human VEGFR-2 promoter from −225 to +268 relative to transcriptional start site (arrow) is sufficient to confer maximal activity in reporter assays. This region includes three E boxes (blue) and an Inr (green) flanking the transcriptional start site. C, consensus sequences for Inr (green) and E box (blue). Sequences from hVEGFR-2 are indicated in black. Mutations of each site are shown below in red.

Materials—Antibodies to TFII-I were generated as described (33). Anti-TFII-I antibody 3588 generated to an epitope common to both human (amino acids 150–163) and mouse TFII-I also cross-reacts with bovine TFII-I. Antibodies were generated to TFII-IRD1 using a peptide derived from the human sequence corresponding to amino acids 94–106 Anti-His (H-3) and anti-Flk-1 (N-931) antibodies were obtained from Santa Cruz Biototechnology, Inc. Human TFII-I siRNAs constructs hTFII-I 2020 (DNA target sequence, 5'-AAGTACTTCGACCAAGACAGA-3') or hTFII-I 3390 (DNA target sequence, 5'-AAGGTATCGTTGTTGCAAG-3') and control siRNA (no known homology, 5'-AAAGATTTGTCGAAAAAGGC-3') were obtained from Qiagen.

Cell Culture—Bovine pulmonary artery endothelial (BPAE) cells were purchased from Cell Systems Corp. Cells were maintained in tissue culture medium containing 80% Dulbecco's modified Eagle's medium, low glucose (Invitrogen), 20% heat-inactivated fetal bovine serum (HyClone), 1% minimal Eagle's medium non-essential amino acid solution (100×Sigma), and 0.1% of initial mix endothelial cell growth factor (reconstituted with Dulbecco's modified Eagle's medium) (Upstate Biotechnology). Cells were maintained at 37 °C in 5% CO2. All cells were used between passage numbers 5 and 8.

Human pulmonary artery endothelial (HPAE) cells were from Clonetics (Cambrex). Cells were maintained in endothelial cell medium (Cambrex) with growth supplements (hydrocortisone, human fibroblast growth factor-B, VEGF, B3-insulin-like growth factor-1, ascorbic acid, heparin, fetal bovine serum, human epidermal growth factor, GA-1000) (EGM-2 bullet kit, Cambrex). Cells were used between passages 4 and 6.

Transfection—For luciferase assays, BPAE were seeded in 24-well dishes such that the cells were 50–80% confluent at the time of transfection. Transfection was performed using FuGENE 6 transfection reagent (Roche Diagnostics) as recommended by the manufacturer. For 24-well plates, 0.6 μl of FuGENE 6 reagent/well and 200 μg of DNA/well were optimal. For most experiments, transfection efficiency was determined by FACS of cells transfected in parallel with GFP. Cells were assayed for luciferase activity at 24–48 h after transfection. Lysates were retained for Western analysis.

For siRNA and FACS, HPAE were seeded in 6-cm dishes such that...
they were 50–80% confluent at the time of transfection. Transfection was performed using FuGENE 6 transfection reagent (Roche Applied Science) as recommended by the manufacturer. siRNAs were co-transfected with 1 μg of pCMV2 as carrier DNA. Cells were assayed by FACS 24–48 h after transfection.

For experiments with trichostatin A (TSA), BPAEs were transfected as above in 24-well plates. Six hours after transfection, TSA diluted in Me2SO or an equivalent amount of Me2SO was added to wells. Luciferase activity was assayed 24 h after the addition of TSA.

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Electrophoretic Mobility Shift Assay—Oligonucleotides representing the Inr and E box domains of the human VEGFR-2 promoter (Table I) as well as the reverse complement of each oligonucleotide were synthesized that the decreased activity was related to disruption of the conserved A(5-70) sequence overlaps the transcriptional start site, we hypothesized that TFII-I might be acting at a second putative TFII-I binding site in the VEGFR-2 promoter. This enhancer activity suggested that TFII-I might be acting at a second putative binding site or E box. The resulting promoter constructs showed varying absolute activities, but all displayed a significant increase in luciferase activity (data not shown).

Luciferase Assays—All data are expressed as relative luciferase activity (firefly/Renilla or firefly/β-galactosidase, as indicated) and represent the mean of three individual transfections in a given experiment. Each experiment was performed at least three times. Data are shown as mean and standard deviation.

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indicated. The transfected TFII-I (isoform 4) is apparent in the upper band of immunoreactivity. Other blots.

Promoter with point mutations of putative TFII-I binding sites.

Right basal (luciferase activity with TFII-I/luciferase activity with pCIS) for each promoter construct. All experiments were performed in triplicate.

TFII-I or empty expression vector (pCIS). Total DNA/transfection was constant. Data are expressed as -fold increase in luciferase activity over mean of the relative luciferase activity (firefly/Renilla)

were also transfected with TFII-I or empty vector (pCIS) as indicated. Total DNA/transfection was constant. Data are expressed as 

fold increase in luciferase activity over basal luciferase activity (pCIS) as indicated. Total DNA/transfection was constant. Data are expressed as 

fold increase in luciferase activity over basal luciferase activity (pCIS) as indicated. Total DNA/transfection was constant. Data are expressed as 

TABLE II

| Reporter construct | TFII-I-stimulated luciferase activity/basal luciferase activity ± S.D. |
|--------------------|---------------------------------------------------------------|
| pGL2 Inr           | 4.55 ± 0.74                                                   |
| pGL2 Inr mutant    | 2.53 ± 0.56                                                   |
| pGL2 Basic         | 0.83 ± 0.36                                                   |

E boxes (−170), (+70), and (+184). This binding was decreased by the addition of an anti-His antibody to the binding reactions (Fig. 3D). The binding to E box (+70) could only be detected on longer exposures and appears to be lower affinity. The binding to the Inr could be competed by cold E (+184) but not by cold Inr mutant probe (Fig. 3A and data not shown). Similarly, binding to E box (−170) and (+184) was competitively by unlabeled Inr probe but not by cold E box (−170) mutant or cold E box (+184) mutant (Fig. 3B and data not shown). Binding to E box (+184) was more efficiently competed by cold E box (+184) than by unlabeled Inr probe (Fig. 3D). Binding to E box (+70) was competed similarly by unlabeled E box (+70), E box (+184), and Inr (see Fig. 7C).

Counter-regulatory Effect of TFII-IRD1—TFII-IRD1 acts as an antagonist of TFII-I at the c-fos promoter (32). We sought to characterize this effect at the VEGFR-2 promoter. Co-transfection with reporter and TFII-IRD1 resulted in the dramatic reduction of TFII-I-induced luciferase activity (Fig. 4A). In addition, TFII-IRD1 decreased basal VEGFR-2 reporter activity, suggesting that the effect also altered native bovine TFII-I activity. Western blots of cell lysates do not indicate a significant effect of TFII-IRD1 on TFII-I protein expression (Fig. 4A). TFII-IRD1 appears to block TFII-I enhancement of luciferase activity by a mechanism other than a one-to-one interaction based on the near maximal inhibition of TFII-I activation by significantly lower molar quantities of transfected TFII-IRD1.

We sought to further define the mechanism by which TFII-IRD1 alters TFII-I effect on the VEGFR-2. In COS 7 cells, overexpressed TFII-IRD1 was reported to decrease the translocation of overexpressed TFII-I to the nucleus. We addressed this possible mechanism by transfection of BPAE with either TFII-IRD1 or an equivalent molar quantity of the empty pCIS2 expression vector. Cells were co-transfected with GFP as a marker of transfection. Cells were visualized after staining for TFII-I with a rhodamine labeled anti-rabbit secondary antibody following incubation with a rabbit polyclonal anti-TFII-I antibody. We compared the intensity of rhodamine labeling in TFII-IRD1-transfected and pCIS2-transfected cells (Fig. 4B).
We noted decreased nuclear TFII-I staining but did not detect increased cytoplasmic staining in these cells expressing only native TFII-I. This observation was confirmed by confocal imaging of these cells (Fig. 4C). This finding could be consistent with TFII-IRD1 decreasing nuclear TFII-I through a mechanism other than blocking nuclear translocation. Alternatively, the lack of apparent increase in cytoplasmic TFII-I may be a function of the distribution of the TFII-I over a large cytoplasmic volume. Regardless of mechanism, these data supported the interpretation that the inhibitory effect of TFII-IRD1 is exerted at least in part through decreasing TFII-I expression in the nucleus.

A second mechanism for TFII-IRD1 antagonism might be through the recruitment of HDACs to the promoter. TFII-I has been shown to co-purify with the HDAC3 complex (34, 35). In overexpression studies, TFII-IRD1 can also interact with HDAC3 (35). To investigate the possible role of HDAC recruitment in the TFII-IRD1-mediated inhibition of the VEGFR-2, we made use of the potent and general HDAC inhibitor, TSA. BPAE were transfected with VEGFR-2 luciferase reporter construct and pCIS, TFII-I, or TFII-IRD1. Six hours after transfection, cells were treated with TSA ranging from 0.1 to 20 nM. Luciferase assays were performed after 36 h with TSA (Fig. 4D). Treatment with TSA at 20 nM nearly completely reversed antagonism of VEGFR-2 minimal promoter by TFII-IRD1. These data lent support to the hypothesis that TFII-IRD1 acts, in part, via recruitment of HDACs.

**VEGFR-2 Protein Expression Is Decreased by TFII-I siRNA and TFII-IRD1**—Regulation of VEGFR-2 protein expression is critical in normal vasculogenesis as well as in pathological angiogenesis. We have demonstrated that TFII-I can up-regulate the VEGFR-2 promoter in reporter assays. To determine whether VEGFR-2 protein expression is altered in response to changes in the amount of TFII-I, we made use of two human TFII-I siRNA constructs. Human pulmonary artery endothelial cells were transfected either with an active TFII-I siRNA construct or with a control siRNA. Expression of TFII-I was determined by FACS using rabbit anti-TFII-I antibody and Cy5-labeled anti-rabbit secondary in fixed and permeabilized cells (Fig. 5A). Two independent siRNAs both resulted in a significant decrease in TFII-I staining. Cells transfected in parallel experiments were incubated with phycoerythrin-anti-VEGFR-2 antibody (Fig. 5B). Cells transfected with active TFII-I siRNA showed significantly decreased VEGFR-2 cell surface expression in comparison with cells transfected with control siRNA. The effect on VEGFR-2 expression is also noted in immunoblots of cells sorted for a marker of transfection (H2Kk) (Fig. 6A) as described under "Experimental Procedures." When normalized to actin level, siRNA 1 reduces TFII-I and VEGFR-2 immunoreactivity to about 60% of the control siRNA (Fig. 6B). SiRNA 2 is more potent at decreasing TFII-I level as well as VEGFR-2 level (Fig. 6B). The effect of the human TFII-I siRNAs on VEGFR-2 could be rescued by co-transfection with bovine TFII-I (data not shown).

We have demonstrated that TFII-IRD1 can antagonize TFII-I activation of the VEGFR-2 promoter. To determine whether this results in altered VEGFR-2 protein levels, we transfected BPAE with TFII-IRD1 or the empty pCIS vector. Twenty-four hours after transfection, cell lysates were separated by SDS-PAGE and immunoblotted for VEGFR-2, TFII-IRD1, TFII-I, and actin as indicated (Fig. 7). Decreased VEGFR-2 immunoreactivity is noted in the lysates transfected with TFII-IRD1. Of note, TFII-I levels do not appear to be altered.

**DISCUSSION**

The dynamic regulation of VEGFR-2 expression is critical in normal vascular development. The transcription factor, TFII-I, is known to play a role in this regulation by acting at the basal transcriptional Inr site (11). TFII-I, however, has an unusual structure with multiple potential DNA binding domains. In other promoters, it has been shown to bind at both basal and upstream regulatory sites as well as to interact with a number of other ILH transcription factors. These characteristics have led to the hypothesis that TFII-I may function in a novel fashion to bridge basal and upstream regulatory sites and to integrate signals from multiple pathways (18). This function would make TFII-I particularly capable of the type of dynamic regulation of VEGFR-2 that occurs during vascular development.

Earlier experiments (11) demonstrated a role for TFII-I in regulating the human VEGFR-2 promoter via a poorly conserved Inr motif flanking the transcriptional start site. In this study, we have examined the possibility that TFII-I is capable of regulating the VEGFR-2 promoter by interaction with other sites. Specifically, we have demonstrated that a luciferase re-
porter driven by an Inr-mutated VEGFR-2 promoter construct retains TFII-I-dependent enhancement of expression. The reduction in absolute activity seen for the Inr mutant promoter may result from the disruption of the conserved A at the transcriptional start site. The VEGFR-2 Inr mutation would be predicted based on studies of mutagenesis of the terminal deoxynucleotidyltransferase Inr to virtually abolish Inr-mediated activity (12). We also showed that TFII-I binding to an Inr probe cannot be competed by a cold Inr mutant competitor, suggesting that the mutation disrupts binding. We nevertheless observed a consistent TFII-I-dependent increase in luciferase activity with the Inr mutant. This supported the idea that TFII-I interacts functionally with other sites in the VEGFR-2 promoter.

TFII-I has been demonstrated to interact with the E box in the adenovirus major late promoter (13, 15). The VEGFR-2 promoter contains three such motifs (7). We introduced mutations in each E box motif based on published mutagenesis studies identifying a critical core sequence (15). The E box mutants also showed a wide range in absolute luciferase activity, but each showed similar enhancement with TFII-I. This was suggestive that TFII-I might interact with multiple sites in the promoter. We were further able to demonstrate TFII-I DNA binding oligonucleotide probes spanning E box (−170), (−70), and (−184). As might be expected, a cold Inr competitor diminished TFII-I binding to the E box probes. Based on competition by cold E box and Inr oligonucleotides, we would expect that

![Fig. 4. Mechanism of TFII-IRD1 antagonism of TFII-I activation of the VEGFR-2 promoter.](image-url)
transfected with H2Kk plasmid as described under “Experimental Procedures.” Transfected cells were enriched by adhesion to MACSelect MicroBeads and magnetic separation as recommended by the manufacturer. SDS lysates from the selected cells were separated on SDS-PAGE and immunoblotted with the antibody indicated. The band of VEGFR-2 immunoreactivity is broader than in lysates from cells not selected using MACSelect MicroBeads. This is likely secondary to an effect on the entry of this large protein into the gel. In comparing cells transfected with TFII-IRD1 with those transfected with empty vector, we did observe an apparent contrast, BPAE show little cytoplasmic TFII-I staining at baseline. In comparing cells transfected with TFII-IRD1 with those transfected with empty vector, we did observe an apparent decrease of nuclear staining but with no apparent increase in cytoplasmic staining. These results could be consistent with TFII-IRD1 blockade of translocation; the lower levels of endogenous TFII-I as compared with levels in TFII-I-transfected cells might make detection of an increase in the cytoplasm difficult. On the other hand, overexpression of TFII-I in COS 7 cells might have resulted in altered distribution from the native state.

Both TFII-I and TFII-IRD1 have been described to interact with HDAC3 (34, 35). As TFII-IRD1 has been demonstrated to repress a number of promoters (30, 32), this seemed a plausible mechanism for the inhibition of TFII-I activation of the VEGFR-2 promoter. In fact, the general HDAC inhibitor, TSA, reversed the TFII-IRD1-mediated inhibition of the VEGFR-2 driven reporters. Thus, the effect of TFII-IRD1 might be indirect and might involve binding to a distinct site in the VEGFR-2 promoter. Recent studies, however, have demonstrated co-immunoprecipitation of TFII-I and TFII-IRD1 (17).

The process of vasculogenesis requires the integration of multiple signals to modulate the patterns of expression of VEGFR-2. This study did not exclude the possibility of other
factors interacting with the VEGFR-2 promoter directly. It provided evidence, however, that at the VEGFR-2 promoter, TFII-I may subserve a more complex function than simply as a basal transcription factor at the Inr. This is a paradigm for which there is emerging evidence in a number of promoter systems (18).

The interaction of TFII-I with the VEGFR-2 promoter might be modeled in several ways. In one model, a TFII-I homodimer interacts simultaneously with multiple sites in the VEGFR-2 promoter. The affinity of each of the sites for TFII-I differs. In the second model, different molecules of TFII-I (heterodimers) interact with unique sites in the VEGFR-2 promoter. The differences in interaction might result from the known splicing variations of TFII-I. A third model reflects the known ability of TFII-I to interact with other HLH factors. Interaction with different factors might alter the affinity of the TFII-I complex for specific binding sites in the VEGFR-2 promoter. All or some of these models might operate simultaneously within a cell depending on the intracellular milieu. This complexity of interaction affords TFII-I a unique capacity to modulate VEGFR-2 expression and to integrate multiple signaling pathways. The counter-regulatory effect of TFII-IRD1 provides a potent brake to the activation pathway. We speculate that disruption of TFII-I expression in endothelial cells during development will result in significant pathology and might explain the failure to identify individuals with Williams Syndrome with homozygous deletion or mutation in TFII-I.

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REFERENCES

1. Millauer, B., Wizigmann-Voos, S., Schnurch, H., Martinez, R., Moller, N. P., Risau, W., and Ullrich, A. (1993) Cell 72, 835–846
2. Neufeld, G., Cohen, T., Gengrinovitch, S., and Poltorak, Z. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 604–609
3. Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Risau, W., and Ullrich, A. (1993) Cell 72, 835–846
4. Millauer, B., Longhi, M. P., Plate, K. H., Shawver, L. K., Risau, W., Ulrich, A., and Strawn, L. M. (1996) Cancer Res. 56, 1615–1620
5. Brekken, R. A., Overholser, J. P., Stastny, V. A., Waltenberger, J., Minna, J. D., and Thorpe, P. E. (2000) Cancer Res. 60, 5177–5124
6. Kazi, A. A., Lee, W. S., Wagner, E., and Becker, P. M. (2000) Am. J. Physiol. 279, L460–L467
7. Patterson, C., Ferrelle, M. A., Hsieh, C. M., Yoshizumi, M., Lee, M. E., and Haber, E. (1995) J. Biol. Chem. 270, 23111–23118
8. Minami, T., Rosenberg, R. D., and Aird, W. C. (2001) J. Biol. Chem. 276, 5395–5402
9. Hata, Y., Duh, E., Zhang, K., Robinson, G. S., and Aiello, L. P. (1998) J. Biol. Chem. 273, 19284–19303
10. Ilii, B., Puri, P., Morgante, L., Capogrossi, M., and Gaetano, C. (2000) Circ. Res. 86, E110–E117
11. Wu, X., Breitman, M. L., and Schuh, A. C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 376, 60162–30167
12. Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B., and Smale, S. T. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5605–5609
13. Roy, A. L., Alpert, N., Gengrinovitch, S., and Poltorak, Z. (1997) Cancer Res. 57, 2813–2820
14. Yang, W., and Desiderio, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 604–609
15. Roy, A. L., Du, H., Gregor, P. D., Novina, C. D., Martinez, E., and Roeder, R. G. (1997) EMBO J. 16, 7091–7104
16. Cheriyath, V., and Roy, A. L. (2000) J. Biol. Chem. 275, 26300–26308
17. Tantin, D., Tussie-Luna, M. I., Roy, A. L., and Sharp, P. A. (2004) J. Biol. Chem. 279, 5440–5448
18. Roy, A. L. (2003) Gene (Amst.) 274, 1–13
19. Cheriyath, V., and Roy, A. L. (2001) J. Biol. Chem. 276, 8377–8383
20. Clark, M. P., Chow, C. W., Rinaldo, J. E., and Chalkley, R. (1998) Nucleic Acids Res. 26, 3813–3820
21. Morikawa, N., Clarke, T. R., Novina, C. D., Watanabe, K., Haag, C., Weiss, M., Roy, A. L., and Donahoe, P. K. (2000) J. Biol. Chem. 275, 1075–1083
22. Johansson, E., Skogman, E., and Thelander, L. (1995) J. Biol. Chem. 270, 30162–30167
23. Molley, C. M., and Sealy, L. (2000) J. Virol. 74, 6511–6519
24. Kim, D. W., Cheriyath, V., Roy, A. L., and Cochran, B. H. (1998) Mol. Cell. Biol. 18, 3310–3320
25. Bayarsaihan, D., and Ruddle, F. H. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7342–7347
26. Hinsley, T. A., Cunliffe, P., Tipney, H. J., Brass, A., and Tassabehji, M. (2004) Protein Sci. 13, 2588–2599
27. Osborne, L. R., Campbell, T., Daradich, A., Scherer, S. W., and Tsui, L. C. (1999) Genomics 57, 279–284
28. Tipney, H. J., Hinsley, T. A., Brass, A., Metcalfe, K., Donnai, D., and Tassabehji, M. (2004) Eur. J. Hum. Genet. 12, 551–560
29. Yan, X., Zhao, X., Quan, M., Guo, N., Geng, X., and Zhu, X. (2000) Biochem. J. 345, 749–757
30. Polly, P., Haddadi, L. M., Issa, L. L., Subramaniam, N., Palmer, S. J., Tay, E. S., and Hardeman, E. C. (2003) J. Biol. Chem. 278, 36693–36610
31. Vullhorst, D., and Buonanno, A. (2003) J. Biol. Chem. 278, 8370–8379
32. Tussie-Luna, M. I., Bayarsaihan, D., Ruddle, F. H., and Roy, A. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7789–7794
33. Danoff, S. K., Taylor, H. E., Desiderio, S. (2004) Nature 432, 931–938
34. Wen, Y. D., Cress, W. D., Roy, A. L., and Seto, E. (2003) J. Biol. Chem. 278, 1841–1847
35. Tussie-Luna, M. I., Bayarsaihan, D., Seto, E., Ruddle, F. H., and Roy, A. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 12897–12812
36. Bayarsaihan, D., Bitchevaia, N., Enkhamandah, B., Tussie-Luna, M. I., Leckman, J. F., Roy, A., and Ruddle, P. (2003) Gene Expr. Patterns 3, 579–589