Retinoic Acid-regulated Expression of Fibroblast Growth Factor 3 Requires the Interaction between a Novel Transcription Factor and GATA-4*

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fgf-3 shows a complex spatial-temporal pattern of transcription during mouse development, and the gene product appears to be an important intercellular signaling molecule. Here we show that the major enhancer, which is obligatory for transcription, is composed of three elements with different properties. Both functional analyses in undifferentiated and differentiated F9 cells and characterization of DNA-protein complexes in vitro have identified the sequence motifs TTGACT(C), ATTGT, and GATA as the key transcription factor binding sites. The GTGACT(C) motif, while not essential, is required for full enhancer activity. However, binding at ATTGT is crucial for transcriptional activity and is required for cooperative binding at the proximal GATA site. The GATA binding site mediates the retinoic acid/AGT motif, while not essential, is required for full enhancer activity. However, binding at ATTGT is crucial for transcriptional activity and is required for cooperative binding at the proximal GATA site. The GATA binding site mediates the retinoic acid/dibutyryl cyclic AMP stimulation of transcription and correlates with the binding of GATA-4 which is induced by retinoic acid in differentiating F9 cells. The ATTGT and GATA motifs are inactive when placed separately on a minimal thymidine kinase (TK) promoter, but together they act as a strong retinoic acid-regulated enhancer. In undifferentiated F9 cells, gata-4 expression stimulates the fgf-3 promoter, whereas in differentiated F9 cells already expressing gata-4, no further increase in promoter activity was observed.

Fibroblast growth factors (FGFs) are an important family of intercellular signaling molecules involved in early embryonic development and organogenesis (reviewed in Refs. 1 and 2). In vitro, the FGFs demonstrate a plethora of properties including the modulation of cell proliferation, differentiation, and cell motility (reviewed in Refs. 3 and 4). The principle route of signal transduction is through a secreted FGF ligand that interacts with a high affinity cell surface receptor causing activation of its cytoplasmic tyrosine kinase (reviewed in Refs. 5 and 6). Four high affinity FGF receptor genes (FGFR1–4) have been identified, although alternative splicing by three of these FGFRs generates a more diverse set of signaling receptors. Moreover, signal transduction requires the presentation of FGFs by a second type of receptor, identified as a heparan sulfate-containing proteoglycan.

We have been particularly interested in FGF3, a ligand first identified as a proto-oncogene in virally induced mouse mammary cancer but later shown to be expressed in a complex spatial-temporal pattern during mouse embryogenesis and fetal development (7–9). In the pre-implantation conceptus, fgf-3 expression is detected in the parietal endoderm and subsequently in the embryo at a number of sites, including the primitive streak mesoderm, early hindbrain and forebrain, cerebellum, sensory cells of the inner ear, pharyngeal pouches, retina, tooth mesenchyme, and tail bud. Although these multiple locations suggest an involvement in several developmental processes, mice deficient for fgf-3 only show skeletal and inner ear abnormalities reflecting a subset of the known expression sites (10). However, the expression pattern of fgf-3 overlaps with several other FGF family members, which suggests a potential for functional compensation and a possible masking of shared roles (reviewed in Ref. 2).

To understand how the complex pattern of fgf-3 transcription is regulated, we have been analyzing the promoter to identify the transcriptional regulators that control its activity. We have previously demonstrated that a 1.7-kilobase fragment of genomic DNA, encompassing both the multiple transcription start sites and sequences upstream of the transcribed region, can act as an inducible promoter in F9 embryonal carcinoma cells but not in a number of other common laboratory cell lines tested (11). F9 cells do not normally express fgf-3 at a significant level until they are induced to differentiate by the addition of retinoic acid and dibutyryl cyclic AMP (Bt2cAMP) (12, 13). Treatment with retinoic acid and Bt2cAMP causes F9 cultures to differentiate into cells expressing markers of parietal endoderm, the cell lineage in which fgf-3 is first detected in the conceptus (8).

A number of potential regulatory elements within the 5′ proximal region of the fgf-3 promoter were identified by DNase-1 protection assays using nuclear extracts from F9 cells (11). Functional studies using site-directed mutagenesis showed that three of the DNase-1 protected regions were necessary for full transcriptional activity, whereas two others were suppressors of transcription. Of the three regions showing enhancer activity, one designated PS4A was found to be absolutely required for promoter activity. In this paper, we characterize the PS4A region, demonstrating that it can be divided into three subregions. The centrally located subregion is essential for enhancer activity and binds a protein that aids the binding of adjacent 5′ and 3′ transcription factors. We also demonstrate that within PS4A the 3′ binding protein is GATA-4 and that this transcription factor confers retinoic acid inducibility of transcription upon the fgf-3 promoter.
GATA-4 Regulation of Fgf-3 Transcription

EXPERIMENTAL PROCEDURES

Plasmid DNA and Transfections—The construction of pgf-3/CAT was described previously (11). Mutations in the PS4A region of pgf-3/CAT were introduced using a two-step PCR procedure with primers containing a selected deletion or base substitutions as described (14). pTKLuc was constructed by inserting 136 nucleotides (−85 to +51) of the HSV TK promoter from pBLCAT2 (15) upstream of the luciferase gene in pG-2B (Nippon Gene, Tokyo). For derivatives of pTKLuc containing three copies in tandem of individual or combined elements identified in PS4A, complimentary synthetic oligonucleotides were designed with 5′ blunt and 3′ EcoRI cohesive ends. After annealing the complimentary oligonucleotides, the resulting duplex DNA was ligated into pTKLuc at the Snr1 and EcoRI sites located upstream of the TK promoter. Sequences of a single copy of each plasmid insert are presented in Fig. 8. pgf-3-2Luc was constructed by inserting the 1.7-kilobase {\(g\text{-f}}-3\text{-CIT}\) into pGV-B2 at the KpnI/XhoI site. A gata-4 expression plasmid, pG4S1, was constructed by cloning a PCR fragment from a gata-4 mouse cDNA (16) into pCR3 (Invitrogen) at the EcoRI site. DNA transfection experiments were carried out in 6-cm dishes using either pG4S1 (6 μg) or pCR3 (6 μg) with the reporter pgf-3/Luc (3 μg) and with pRL-CMV (1 μg) (Nippon Gene, Tokyo) as internal control. Procedures for F9 cell culture, transfections of F9 cells with plasmid DNA, isolation of stably transfected cells, preparation of cell extracts, and CAT and luciferase assays have been described previously (11, 12).

Preparation of Nuclear Extracts and Recombinant Gata-4 Proteins—Nuclear extracts were prepared from undifferentiated F9 cells or differentiated F9 cells after 3 days of treatment with retinoic acid (10 \(\text{−6} \text{ M}\)) and dibutyryl cyclic-AMP (10\(^{-4} \text{ M}\)) by the method described previously (17). Recombinant Gata-4 protein carrying a histidine tag at the C terminus (HT-GATA-4) was obtained using Bac-to-Bac Baculovirus Expression Systems (Life Technologies, Inc.). In brief, a gata-4 mouse cDNA (16) was inserted in the genome of AcNPV, and the resulting recombinant virus was used to infect SF9 cells. HT-GATA-4 was purified from infected cell extracts using a Ni-nitrilotriacetic acid resin.

Electrophoretic Mobility Shift Assays—Oligonucleotide probes were labeled with [γ-{\(\text{32P}\)}]ATP (5000 Ci/mM, Amersham Pharmacia Biotech) using T4 polynucleotide kinase (New England BioLabs) and annealed by heating at 80 °C for 10 min followed by slow cooling. Nuclear extract (7 μg) and/or recombinant HT-GATA-4 protein (about 10 pg) were preincubated with 1 μg of nonselective competitor poly(dI-poly(dC)) (Amersham Pharmacia Biotech) in 20-μl reaction mixtures for 15 min at 4 °C. The reaction buffer consisted of 20 mM HEPES/KOH (pH 7.9), 50 mM KCl, 1 mM dithiothreitol, 2 mM MgCl\(_2\), 1 mM EDTA, 0.1% Triton X-100, and 20% glycerol. When indicated, double-stranded competitor oligonucleotides were included. After addition of 25 fmol of {\(\text{32P}\)}-labeled probe, the reaction was incubated for 15 min at room temperature. Finally, for supershift experiments, 2 μl of antiserum was added and the reaction incubated for a further 30 min at room temperature. Antiserum to GATA-4 and GATA-6 were purchased from Santa Cruz Biotechnology. In some initial experiments (not shown), we used an antiserum to GATA-4, kindly provided by Dr. David B. Wilson (Washington School of Medicine, St. Louis, MO) (18). Samples were loaded on 6% polyacrylamide, 0.5× Tris borate-EDTA gels and run at 200 V for 2 h at 4 °C. Gels were dried and exposed to x-ray films.

Northern Blot Analysis—Total RNA was isolated from undifferentiated or differentiated F9 cells using an RNA Extraction Kit (Amersham Pharmacia Biotech), and poly(A){\(^+\)} RNA was selected using mRNA Purification Kit (Amersham Pharmacia Biotech). Procedures for Northern hybridization were described previously (18). 1 μg of poly(A){\(^+\)} RNA for each sample was electrophoresed in 1.2% agarose gels and transferred to GeneScreen membrane (NEN Life Science Products). gata-4 or gata-6 transcripts were detected using appropriately labeled PCR fragments. These were prepared by RT-PCR using differentiated F9 cell RNA as template, and the following primers: gata-4, AACGGAAAGCCACAAAGCCGT and CAGGGCAGGGGAGATAAGG; gata-6, TTGTCCAGCATGGGACACATGGTTG and AACACATGATTGTGCACAGG.

RESULTS

PS4A Is a Critical Enhancer Element That Can Bind Multiple Factors—A schematic depiction of the proximal {\(g\text{-f}}-3\text{-CIT}\) promoter with the previously identified DNase-1 protected regions (PS) is shown in Fig. 1A. Targeted deletion of these DNase-1 protected sites showed that the region designated PS4A (located approximately 60 nucleotides upstream of P1) was essential for {\(g\text{-f}}-3\text{-CIT}\) expression in F9 cells (11). To analyze the PS4A enhancer in more detail, a series of six smaller nonoverlapping deletions across this element were introduced into the expression plasmid pgf-3/CAT, and their effect on CAT expression tested (Fig. 1, B and C). As transient transfection assays do not satisfactorily reproduce the retinoic acid induction of the {\(g\text{-f}}-3\text{-CIT}\) promoter, the effects of the deletions within PS4A were assessed in stably transfected F9 cells. For these assays, cultures containing more than 100 transfected clones were pooled to minimize positional effects caused by integration at random sites within the genome. Two of the deletions designated 4A{\(_{\alpha}\}) and 4A{\(_{\delta}\}) abolished detectable CAT activity, whereas two others 4A{\(_{\beta}\}) and 4A{\(_{\lambda}\}) showed substantial reductions. Deletion mutants 4A{\(_{a}\}) and 4A{\(_{e}\}) gave values similar to the parental {\(g\text{-f}}-3\text{-CIT}\) expression vector, indicating that these sequences are not a crucial part of the enhancer element.

To delineate the sequences within these subdomains of PS4A that are necessary for enhancer activity, base substitutions of one, two, or three closely located bases were introduced into the expression plasmid pgf-3/CAT (Fig. 2A). The CAT activity estimated in pools of F9 cells stably transfected with these base substitution mutants confirmed that regions encompassed by 4A{\(_{\alpha}\}), c, d, and f were necessary to maintain enhancer activity.
bands 5 and 6 (Fig. 3). To localize the binding sites of the different complexes, three overlapping probes designated 4Ax, 4Ay, and 4Az were used in place of PS4A (Fig. 3). This approach showed that the complexes common to uF9NE and dF9NE (bands 1–3 and two minor undesignated bands below 3) were clearly associated with the 4Ax probe, whereas the major complexes formed using dF9NE on the full-length probe bound to 4Ay only at a significantly reduced level. Using probe 4Az, no bands were observed with uF9NE and only a weak complex (band 4) migrating at a novel position was observed with dF9NE. These latter findings suggest that neither 4Ay or 4Az probes could support efficient formation of the main complex(es) (bands 5 and 6) identified with the full-length 4A probe and dF9NE.

Identification of Core Binding Sites in PS4A—To correlate PS4A enhancer activity with transcription factor binding, an extensive set of base substituted probes was generated for EMSA that also included those mutations used for the functional analysis (Figs. 2, 4, and 5B). Base substitutions in the 4Ax region of PS4A resulted in a complete or partial loss of bands 1, 2, and 3, which correlated with reduced promoter activity observed in both undifferentiated and differentiated F9 cells (compare Figs. 2 and 4). Thus, from the combined point mutational analyses, the sequence GTGACT(C) was deduced as the binding domain for the three complexes of the 4Ax region (Figs. 4 and 5B). The crucial nucleotides of the motif are GTGA because their substitution (probes 4AxM12, M12.1, M12.2, and M0) resulted in loss of the three complexes. Changing the 3′ T to a G (4AxM13) resulted in a site with an apparent increased binding domain for the three complexes of the 4Ax region (compare Figs. 2 and 4). However, 4AxM13 did not display the same results as the original probe. A similar result was observed with 4AxM14, which had no effect on the three bands observed with 4AxM13. This suggests that the 3′ T is not essential for binding of the three complexes.

Probes with base substitutions in the subregions 4Ay and 4Az, resulted in a loss or severe reduction of the major complexes (bands 5 and 6) unique to dF9NE (with the exception of 4AyM3, Fig. 4). It was surprising that mutations across two subregions caused the loss of the same complex(es) because the deletion analysis suggested the presence of two distinct sites: one located in 4Ay (4AΔε and 4AΔΔ deletions) and the other associated with retinoic acid/Bt2cAMP inducibility in 4Az (4AΔφ deletion; Fig. 1). To identify the core nucleotides in 4Ay necessary for formation of this major complex, a set of probes containing single base substitutions at alternate nucleotides across the region was used in a further EMSA (Fig. 5C). From
binding proteins associated with the subregions 4Ax and 4Ay.

EMSA with 32P-labeled probes containing base substitutions identical to those introduced into the expression plasmid pfgf-3 used for EMSA with dF9NE (bands 5 and 6), whereas substitution of the T residue 5' and M10) did not affect formation of the major complexes (4AyM7) virtually abolished complexes 5 and 6. Moreover, for example, single base changes at the first T (4AyM6) or at G motif necessary for efficient binding of protein to this region.

this experiment, the sequence ATTGT was deduced as the motif necessary for efficient binding of protein to this region. For example, single base changes at the first T (4AyM6) or at G (4AyM7) virtually abolished complexes 5 and 6. Moreover, these base substitutions also reduced formation of complexes associated with the 4Ay region, suggesting cooperativity between proteins binding at 4Ax and 4Ay. Probes containing point mutations immediately 3' of this sequence (4AyM8, M9 and M10) did not affect formation of the major complexes (bands 5 and 6), whereas substitution of the T residue 5' of the motif (4AyM5) diminished their formation. Mutations affecting the ATTGT motif also abolish activity of the fgf-3 promoter (compare Figs. 2 and 4).

Complexes associated with the ATTGT motif were also found to be partially dependent on the integrity of the 3' GATA motif in the 4Az subregion (Fig. 4). This suggested a cooperativity of binding between the proteins associated with 4Ay and 4Az, in addition to that found between 4Ax and 4Ay associated proteins. To establish the GATA motif as the protein binding site in 4Az, a probe encompassing the 4Ay and 4Az subregions was used as a target in an EMSA with unlabeled competitor probes containing single point mutations in and proximal to the GATA site (Fig. 6A). Competitor probes in 10-fold excess, containing base substitutions in the G, A, or T of the GATA motif (4AyM5) diminished their formation. Mutations affecting the ATTGT motif also abolish activity of the fgf-3 promoter (compare Figs. 2 and 4).

Identification of Gata-4 as a Binding Protein for 4Az—To determine whether F9 cells express known GATA proteins that could be candidates for binding to PS4A, RNA from undifferentiated and differentiated F9 cells was analyzed for expression of the GATA family of transcription factors. An initial screen by RT/PCR using primers unique to six different GATA genes (data not shown), revealed a low level of gata-1 expression in undifferentiated F9 cells that was diminished upon retinoic acid/Bt2cAMP differentiation. gata-2 and gata-3 RNA were both present at very low levels in undifferentiated F9 cells and were slightly induced upon differentiation. gata-3 was not detected, but gata-4 and gata-6 were strongly induced in F9 cells following addition of retinoic acid/Bt2cAMP. The induction of gata-4 and gata-6 mRNA by retinoic acid/Bt2cAMP was confirmed by Northern analysis (Fig. 6B). To determine whether Gata-4 and/or Gata-6 contribute to complex formation on PS4A, EMSA analysis was performed in the presence of an antiserum directed to either GATA-4 or GATA-6 (Fig. 7A). The antiserum to GATA-4 caused a substantial loss of the major complex and the appearance of a new band with a more severely retarded position. In contrast, the antiserum to GATA-6 was unable to cause a similar supershift of the major complex although this antiserum was less efficient at super-shifting Gata-6 on a probe containing two consensus GATA sites (Fig. 7B).
These results provide strong evidence that the major complexes formed on PS4A in the presence of dF9NE contain Gata-4.

Gata-4 Binding Is Dependent on Another Factor Binding at ATTGT—The dependence of Gata-4 binding on the presence of an unknown factor at the ATTGT motif was further investigated using purified recombinant Gata-4 protein (HT-GATA-4) in an EMSA (Fig. 7B). Alone, HT-GATA-4 protein was not able to bind the 4A probe, but in the presence of uF9NE it formed a clear complex with the expected retardation characteristics. Moreover, probes containing base substitutions in either the ATTGT motif (4AyM7) or GATA site (4AzM1) failed to form a complex, even in the presence of uF9NE. These results support the idea that in the context of PS4A, Gata-4 binding is dependent both on the presence of the GATA site and an unknown second protein that binds at the ATTGT motif.

Both the ATTGT and GATA-binding Proteins Are Necessary for Enhancer Activity on a Heterologous Promoter—The function of the three component binding motifs within the PS4A enhancer were tested individually and in combination for their ability to activate a minimal TK promoter (Fig. 8A). For all constructs, three copies of the elements were appended to the TK promoter, driving expression of a luciferase marker gene. The TK promoter alone gave no significant activity in pools of stably transfected F9 cell clones. However, the complete PS4A enhancer conferred a 100-fold increase in activity to the promoter in undifferentiated cells and nearly a 2000-fold enhancement following treatment with retinoic acid/Bt2cAMP, thereby mimicking the transcription pattern of the endogenous fgf-3 gene. Individual elements containing binding sites GTGACT(C), ATTGT, or GATA were not able to activate the promoter, and the combination of the GTGACT(C) and ATTGT binding sites was also inactive. By contrast, the ATTGT and GATA motifs together conferred a substantial inducible activity, which was further increased when the two binding sites were placed in their normal context. These results are entirely consistent with the EMSA data showing the pivotal role of the ATTGT binding site for the formation of a multimeric protein complex on the PS4A enhancer. They also show that the GATA site is necessary for the induction of transcription by retinoic acid and Bt2cAMP and that the GTGACT(C) motif contributes substantially to the full activity of the complete enhancer.

To determine whether the induction of gata-4 expression in F9 cells is sufficient to account for the stimulation of fgf-3 transcription upon F9 cell differentiation, the undifferentiated cells were transfected with a gata-4 expression vector or an empty vector control, and the level of fgf-3 promoter activity measured (Fig. 8B). The results show that expression of gata-4 in undifferentiated F9 cells resulted in a dramatic increase in fgf-3 promoter activity, whereas it had little effect in differentiated cells. This would be expected because endogenous Gata-4 is already expressed at high levels in differentiated F9 cells.

**DISCUSSION**

The results presented here clearly demonstrate that the PS4A enhancer is essential for the transcription of fgf-3 in F9 cells. Functional and in vitro analyses of PS4A have delineated three distinct regions in the enhancer, each associated with a different transcription factor binding site.

Base substitutions in the most 5’ motif GTGACT(C) resulted in the loss of enhancer activity, further supporting the importance of this site for enhancer function. The presence of both the ATTGT and GATA motifs is necessary for full enhancer activity, with the ATTGT motif playing a pivotal role in the formation of a multimeric protein complex.

**Fig. 7.** Gata-4 forms a complex on PS4A but requires both an intact GATA and ATTGT site. A, EMSA with a PS4A probe and uF9NE or dF9NE in the presence of antiserum to GATA-4 or GATA-6. Arrows indicate complexes formed in the presence of dF9NE which are further retarded by antiserum to GATA-4 (left of panel). Shown are control tracks using a probe with two consensus GATA sites (right of panel). B, EMSA using either probe 4A or probes with base substitutions that affect binding at one of the three defined sites (see Figs. 2, 4, and 5) and incubated with uF9NE and recombinant HT-GATA-4. The position of the complex containing HT-GATA-4 is marked by an arrow.

**Fig. 8.** PS4A acts as a retinoic acid/BT2cAMP regulated enhancer in conjunction with a minimal TK promoter. A, activity of the TK promoter containing individual or pairwise combinations of elements (in triplicate) identified in the PS4A enhancer. B, activity of pfgf-3/Luc in undifferentiated and differentiated F9 cells in the presence or absence of a gata-4 expression plasmid.
in a complete or partial loss of the most highly retarded complexes formed on a PS4A probe in EMSA and a concomitant reduction of enhancer activity when assayed in both undifferentiated and differentiated F9 cells (Figs. 2, 4, and 5B). However, none of these base substitutions completely abolished enhancer activity, showing that the GTGACT(C) binding site is not essential for enhancer function but nevertheless contributes to its overall activity. The deduced binding site is only one base different from a consensus AP1 site (GTGACTCA); however, in vitro prepared AP1 composed of c-Fos and c-Jun did not bind to a 4A probe (data not shown). The complexes associated with the GTGACT(C) site were not supershifted by antisera that recognize members of the Jun or Fos families of proteins. The evidence to date suggests that the protein(s) binding at the GTGACT(C) site is/are not related to the AP1 family of transcription factors.

Functional and DNA binding analyses revealed ATTGT as the essential motif for PS4A enhancer activity (Figs. 2, 4, and 5C). Interestingly, probes used in EMSA that encompassed the ATTGT site alone did not bind complexes associated with this motif as efficiently as longer probes that included the 3' GATA motif (Fig. 3). Thus a substantial proportion of the complexes that appeared to be associated with the ATTGT site were in fact located at the GATA site because Gata-4 binding depended on an intact ATTGT site (see below). Furthermore, integrity of the ATTGT motif was also shown to be necessary for efficient binding at the GTGACT(C) site (bands 1, 2, and 3 in Figs. 4 and 5C). These findings demonstrate the interdependence of protein binding at the three sequence motifs. Enhancer reconstitution experiments using a minimal TK promoter also demonstrated a critical interdependence between the ATTGT and GATA sites for enhancer activity and confirmed the GTGACT(C) site as contributing to, but not essential for, activity (Fig. 8A). Furthermore, together the ATTGT and GATA motifs were sufficient to confer retinoic acid- and Bt,cAMP-regulated activity to the TK promoter.

A strong interaction has been described between Gata-4 and the cardiac transcription factor Nkx2-5, which are co-expressed in cardiomyocytes and show a synergy in activating the ANF promoter (19). However, there is little similarity between the Nkx2-5 core binding site (GCAAGTG) and ATTGT (19). Interestingly, in these experiments, a physical interaction was shown between Gata-4 and Nkx2-5, as well as a strong specificity for Gata-4 when it was co-expressed with Gata-6 (see below).

In vertebrates, the GATA family presently constitutes six main GATA family members (35, 36). Mutational analysis of PS4A in the early development of the extraembryonic tissues, visceral and parietal endoderm (35, 36). Mutational analysis of PS4A in the expression vector pfgf-3/CAT showed that the loss of retinoic acid inducibility was associated with the GATA site (Figs. 1 and 2). Furthermore, the differentiation of F9 cells by retinoic acid/Bt,cAMP into parietal endoderm-like cells correlates with the concomitant induction of gata-4 and gata-6 RNA as well as fgf-3 (Refs. 13, 16, and 37, and Fig. 6B). Antiserum to GATA-4, but not GATA-6, supershifted complexes associated with the GATA/ATTGT sites, providing good evidence that Gata-4 is the main GATA family member responsible for the retinoic acid inducibility. However, at present we cannot exclude a similar role for Gata-6 because the antiserum to Gata-6 was less effective at supershifting it even on a consensus binding site (Fig. 7A). Nevertheless, the ability of Gata-4 to bind to the GATA site, and its dependence on an intact ATTGT site, was confirmed using recombinant Gata-4 protein (Fig. 7B). In addition, the expression of gata-4 in undifferentiated F9 cells from an introduced cDNA greatly stimulates fgf-3 promoter activity, whereas there was no similar effect in differentiated F9 cells that already express endogenous gata-4 and gata-6 (Fig. 8B).

The increase in gata-4 transcription in differentiating F9 cells mediated by retinoic acid and Bt,cAMP has also been implicated in the transcriptional regulation of platelet-derived growth factor α receptor and the J6 serpin genes (38–40). Thus, there is now good evidence to suggest that the induction of gene expression by retinoic acid can be mediated directly through the activation of retinoic acid receptors or indirectly by its ability to induce GATA proteins.

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