INTRODUCTION

The difficulty of isolating and characterizing pluripotent precursor cells has limited the understanding of regulatory mechanisms that might potentiate gene activity prior to cell type differentiation. Gene inactivation studies can reveal whether a regulatory factor is necessary for a tissue to differentiate, but they are unable to address whether regulatory factors have functions in precursor cells in which target genes are transcriptionally silent, but the genes have the potential to initiate tissue development but they have not yet been committed to express albumin or other tissue-specific genes. The GATA-4 isoform accounts for about half of the nuclear GATA-factor-binding activity in the endoderm. GATA site occupancy persists during hepatic development and is necessary for the activity of albumin gene enhancer. Thus, GATA factors in the endoderm are among the first to bind essential regulatory sites in chromatin. Binding occurs prior to activation of gene expression, changes in cell morphology or functional commitment that would indicate differentiation. We suggest that GATA factors at target sites in chromatin may generally help potentiate gene expression and tissue specification in metazoan endoderm development.

SUMMARY

Gene inactivation studies have shown that members of the GATA family of transcription factors are critical for endoderm differentiation in mice, flies and worms, yet how these proteins function in such a conserved developmental context has not been understood. We use in vivo footprinting of mouse embryonic endoderm cells to show that a DNA-binding site for GATA factors is occupied on a liver-specific, transcriptional enhancer of the serum albumin gene. GATA site occupancy occurs in gut endoderm cells at their pluripotent stage: the cells have the potential to initiate tissue development but they have not yet been committed to express albumin or other tissue-specific genes. The GATA-4 isoform accounts for about half

GATA transcription factors as potentiators of gut endoderm differentiation

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of the albumin gene in these three states revealed an unexpected discovery (Gualdi et al., 1996). In neural tissues, no factors are bound to the albumin enhancer and, in newly specified hepatocytes, where the albumin gene is active, factors occupy sites for HNF3, a winged helix transcription factor (Lai et al., 1990), for NF-1, a ubiquitous factor, and for two uncharacterized proteins (see Fig. 1A below). However, in pluripotent gut endoderm, where the albumin gene is silent but can be activated during hepatogenesis, one of the HNF3 sites, designated eG, is occupied. HNF3β and HNF3β, which are genetically unlinked isoforms (Lai et al., 1990, 1991), are expressed in the embryonic endoderm, notochord and neural tube floor plate, and, in adult mammals, they are expressed in all tissues derived from the gut endoderm (Sasaki and Hogan, 1993; Ang et al., 1993; Monaghan et al., 1993; Ruiz i Altaba et al., 1993). Homozygous inactivation of HNF3β in mice is embryonic lethal due to a lack of both the endoderm and the notochord (Ang and Rossant, 1994; Weinstein et al., 1994), and fork head, a related protein, is essential for gut development in Drosophila (Weigel et al., 1989). Therefore, like the GATA factors, the HNF3/fork head subset of winged helix proteins appears to possess an evolutionarily conserved function in metazoan endoderm differentiation.

During our previous in vivo footprinting analysis of the albumin gene enhancer in pluripotent gut endoderm, we discovered that an uncharacterized factor occupies a binding site called eF, immediately upstream of the occupied HNF3 site (Fig. 1A; Gualdi et al., 1996). The eF site remains occupied in the liver bud, after hepatic specification, as well as in adult liver cells (McPherson et al., 1993; Gualdi et al., 1996). In the present paper, we investigate the nature of the albumin enhancer eF site-binding activity. The results allow us to identify a function for GATA transcription factors in pluripotent, undifferentiated gut endoderm and lead us to propose a general model for the function of GATA factors in cooperation with winged helix proteins in potentiating metazoan tissue differentiation from the endoderm.

MATERIALS AND METHODS

Isolation of mouse embryo tissues

Mice were the C3H strain from Taconic. Noon of the day of the vaginal plug appearance was considered as 0.5 day of gestation. Embryonic tissues were excised under a dissecting microscope at 60x with electrolytically etched tungsten needles in a few drops of phosphate-buffered saline (PBS) (Gualdi et al., 1996). To obtain the endodermal epithelial cells from the 10.5 to 11.5 day embryo gut tubes, isolated gut segments were incubated in 10 mM EDTA for 3 to 4 minutes at room temperature and then thoroughly rinsed in PBS. A tungsten needle was then inserted into the gut tube, resulting in the gut epithelium being pushed out the other end (see Fig. 5A). Tissues were then processed for RNA isolation, explant culture, nuclear extract preparation or in vivo footprinting analysis.

Embryo tissue explant cultures, RNA isolation and RT-PCR

The embryo tissue explants were cultured in microwells coated with collagen type I on a glass slide (CoStar) in DMEM containing 10% calf serum (Hyclone) at 37°C in presence of 5% CO2 (Gualdi et al., 1990). After 48 hours of culture, the explants were released from the slides with tungsten needles, aspirated and transferred to a tube containing 100 µl of a guanidinium thiocyanate solution. The wells were washed with another 100 µl of this solution and the two fractions were pooled and processed for RNA extraction as described previously (Cascio and Zaret, 1991).

RNA was isolated from freshly dissected tissues and from individual colonies after 48 hours of culture. The RNA from each colony was subjected to primer extension (Gualdi et al., 1996), then half of the primer extension products were subjected to PCR with 10 pmoles of each 5’ oligonucleotide, where 0.3 pmoles of the 3’ oligonucleotide was phosphorylated with [γ-32P] ATP. RT-PCR methods and oligonucleotides were as described by Gualdi et al. (1996), except that the 5’ β-actin primer was AAAGACCTGTACGCAACAACAGTG. The cDNAs coding for actin and serum albumin were amplified in the same reaction in a Twin Block System (Eriomp Inc., CA) for 30 seconds at 94°C, 1 minute at 60°C and 30 seconds at 74°C. 30 µl aliquots were removed every 4 cycles, starting at 28 cycles, analysed on 6% polyacrylamide gels and subjected to autoradiography. We ran aliquots from different cycles to confirm that the reactions were not saturated.

Nuclear extracts, proteins and electromobility shift assays

Epithelial layers of the 11.5 day gestation embryo gut tubes were pooled and rinsed with ice-cold phosphate-buffered saline containing 5 µg/ml trypsin inhibitor, 5 µg/ml antipain and 5 µg/ml leupeptin (all from Sigma Chemical Co., St. Louis, MO), and transferred to a 2 ml Wheaton glass homogenizer. The tissues were disrupted with a Teflon pestle to generate a cell suspension. The cells were collected by centrifugation and nuclear extracts were prepared by the method of Schreiber et al. (1989) and Ang et al. (1993). Up to 40 µg of nuclear proteins were recovered from the pooled gut epithelia of 20 embryos at the 11.5 day stage. Embryonic livers were partially depleted of hematopoietic cells by mincing the livers and collecting the tissue matrix; the residual material was used for nuclear isolation.

The GAT-4 cDNA (Arcetri et al., 1993) was transcribed in vitro using T7 RNA polymerase and translated in a rabbit reticulocyte lysate (Promega). Mouse HNF3β was expressed in Escherichia coli and purified to homogeneity (Zaret and Stevens, 1995). Electromobility shift assays were performed with 2 µg nuclear extract according to Jackson et al. (1993). For in vitro translated proteins, the nonspecific competitor poly(dI-dC) was replaced by 100 ng of sonicated salmon sperm DNA. For probes and specific competitors shown in Fig. 1B, oligonucleotides were annealed and 5’ extensions were filled in with the Klenow polynucleotide fragment in the presence of dNTPs (including α-32P dATP for probes). The NF-Y-binding site was described by DiPersio et al. (1991). The GATA polyclonal antibody was specific for mouse GATA-4 with no cross-reactivity for the other GATA factors (Santa Cruz Biotechnology).

In vivo footprinting of mouse embryo tissues

Endodermal gut and head tissues were dissected from 8 to 25 embryos at a time and pooled into single tubes containing PBS at 4°C. Tissues were minced and triturated to release cells, then treated for 5 minutes at room temperature with 0.2% DMS in PBS (Bossard et al., 1997). The cells were immediately transferred to ice and diluted in ice-cold PBS, then rinsed 4 times in 1.5 ml of ice-cold PBS. The cells were suspended in 500 µl of homogenization buffer (2 M sucrose, 10 mM Hepes, pH 7.6, 25 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 10% glycerol, 0.5 mM PMSF, 0.5 mM DTT), transferred to a 2 ml Wheaton homogenizer and disrupted with a Teflon pestle. The cell lysates were layered on top of 300 µl of homogenization buffer and spun at 45,000 revs/minute for 35 minutes at 4°C with a TLS 55 rotor in a Beckman TL-100 ultracentrifuge. The nuclear pellets were resuspended in a solution of 10 mM NaCl, 10 mM Tris (pH 7.5) and 3 mM MgCl2. The nuclei were lysed with SDS and incubated overnight with pronase K at 25°C. DNA was purified by phenol-chloroform extractions, precipitated and resuspended into a solution of 10 mM Tris (pH 8.0), 1 mM EDTA. The final yields of DMS-treated DNA...
Plasmid constructs and transfection of H2.35 cells

The plasmids pAT2 and pRT1, with either the mouse albumin promoter or the RSV promoter driving thymidine kinase, respectively, contain the albumin enhancer from the mouse serum albumin gene. When in vitro translated GA TA-4 protein, using as a template a linearized plasmid bearing the albumin enhancer, when in vitro translated GATA-4 was used, 1 μg of salmon sperm DNA was added to the reaction as a nonspecific competitor. Mock-translated lysate served as a control.

RESULTS

GATA-4 as an albumin enhancer eF site-binding factor

In our previous studies, we observed in vivo footprints over the albumin enhancer eF site in embryonic gut endoderm, in nascent hepatocytes of 9.5 day mouse embryos and in adult liver, but not in non-hepatogenic tissues (see Fig. 1A for binding sites; McPherson et al., 1993; Gualdi et al., 1996). We also detected a DNase I footprint over the enhancer eF site with nuclear extracts from adult hepatic cells, but not in extracts from other tissues (Liu et al., 1991). The sequence ‘GATA’ is within the footprinted region of the eF site (Fig. 1B; Liu et al., 1991) and the transcription factors GATA-4, GATA-5 and GATA-6 are expressed in embryonic endoderm and its derivatives, such as the liver, stomach and intestine (Arceci et al., 1993; Laverriere et al., 1994; Matsuda et al., 1994). We therefore assessed whether or not the eF site-binding factor is a GATA protein.

In electromobility shift assays with adult mouse liver nuclear extracts, an eF site probe exhibited a prominent binding species that migrated identically to factors that bound to a consensus GATA sequence (Fig. 2A, lanes 1, 8), and the eF and GATA probes exhibited cross-competition (Fig. 2A; lanes 2-5 and 9-12). An antibody specific to GATA-4 completely supershifted the factor bound to the eF site in nuclear extracts from adult liver and from the liver cell line H2.35 (Fig. 2B, lanes 1-4). A GATA-4 cDNA (Arceci et al., 1993) translated in vitro gave rise to an eF site-binding protein, which migrated identically to the eF site factor in liver nuclear extracts (Fig. 2C, lanes 4, 7). An e-M oligonucleotide, with mutations of the GATA sequence (Fig. 1B), failed to compete the factor from binding to the eF site probe (Fig. 2C, lanes 6, 9). We conclude that virtually all of the eF site-binding activity on the albumin enhancer in the adult mouse liver is due to GATA-4.

The albumin enhancer can be co-occupied by HNF3 and GATA-4

Our previous in vivo footprinting studies showed that the albumin enhancer eF site is co-occupied with the adjacent ‘eG’-binding site for HNF3 in the embryonic gut endoderm, embryonic hepatocytes and adult liver (McPherson et al., 1993; Gualdi et al., 1996). We therefore expected that the authentic eF site-binding protein would co-occupy DNA with purified HNF3 in vitro. We added increasing amounts of HNF3 protein to GATA-4 translated in a reticulocyte lysate and assayed binding to an eF-eG double site oligonucleotide. As seen in Fig. 3 (lanes 6-9), increasing amounts of HNF3 led to increasing amounts of a complex with both factors bound and a depletion of the GATA-4/DNA complex. Addition of HNF3 to the mock translated lysate had no such effect (Fig. 3, lanes 2-5). The appearance of GATA-4 and HNF3 bound to the same DNA in the presence of excess enhancer probe indicates that the factors bind cooperatively. In sum, GATA-4 and HNF3 can rise to an eF site-binding protein, which migrated identically to the eF site factor in liver nuclear extracts (Fig. 2C, lanes 4, 7). An e-M oligonucleotide, with mutations of the GATA sequence (Fig. 1B), failed to compete the factor from binding to the eF site probe (Fig. 2C, lanes 6, 9). We conclude that virtually all of the eF site-binding activity on the albumin enhancer in the adult mouse liver is due to GATA-4.
co-occupy their adjacent sites at the albumin enhancer in vitro, as seen for factors at the eF and eG sites in vivo.

**GATA factor and HNF3 are critical for albumin enhancer activity**

To assess the functional role of GATA factor binding to the albumin enhancer, we employed a transient transfection assay. Previously we showed that an albumin enhancer fragment, which confers liver-specific activity in transgenic mice (Pinkert et al., 1987) is active only in transfected hepatic cells that are cultured under differentiating conditions for hepatocytes, and that multiple liver-enriched factors that bind outside of the region of the eF and eG sites are required for enhancer activity (Liu et al., 1991; DiPersio et al., 1991; Hu et al., 1992; Jackson et al., 1993). The wild-type albumin enhancer and one containing point mutations that inhibit GA TA-4 binding to the eF site were fused to the albumin promoter upstream of the thymidine kinase gene (Fig. 4A,B). We also tested the effects of clustered point mutations that inhibit binding of HNF3 to its enhancer sites eG and eH (Fig. 4A); eH is a second, lower affinity HNF3-binding site that becomes occupied when the albumin gene becomes active in liver bud cells (Gualdi et al., 1996). The various constructs were co-transfected with an internal control into differentiating H2.35 cells (Zaret et al., 1988); we showed above that H2.35 cells contain GA TA-4 as the primary eF site-binding activity, like adult liver cells (Fig. 2B). After culturing the cells for 2 days under conditions that promote hepatic differentiation, RNA was isolated and subjected to a primer extension assay. As seen in Fig. 4C and quantitated in Fig. 4D, the eF site mutant enhancer possessed about 25% of the wild-type enhancer activity, similar to the eG site mutant. The eH site mutant enhancer possessed about 40% of the wild-type activity. Taking together the data from Figs 2 and 4, we conclude that both GATA factor and HNF3 binding are essential for the albumin enhancer in differentiating hepatic cells.

**Pluripotency of the gut endoderm in mouse extends to 11.5 days gestation**

Although endoderm outside the ventral foregut normally does not express serum albumin, we previously showed that the dorsal-posterior endoderm, which normally becomes the intestine, could give rise to albumin-expressing cells when...
isolated from inhibitory mesoderm and ectoderm and cultured for 2 days in vitro (Gualdi et al., 1996). These studies were of 9.5 day gestation embryos and proved that the endoderm remains developmentally pluripotent at that stage and that signals from the dorsal mesenchyme and ectoderm actively prevent hepatogenesis outside the ventral foregut. To enhance our ability to perform a molecular analysis of undifferentiated endoderm tissue, we investigated whether the potential to activate liver gene expression extends to 11.5 days gestation; intestinal morphogenesis begins at about 14.5 days gestation. We therefore developed a protocol that allowed us to isolate the gut endoderm epithelium from 10.5 to 11.5 day embryos, after gut tube closure. Fig. 5A shows a segment of the epithelium being manually pushed out of the gut tube. Gut endoderm segments were then individually cultured in vitro for 2 days and their RNAs assayed for albumin gene expression by RT-PCR, to assess their developmental potential. All RT-PCR reactions included primers for β-actin as an internal control for the integrity and amount of RNA assayed. In subsequent experiments, endoderm segments from 15 to 20 embryos were pooled into a single tube and used for preparing nuclear extracts or for in vivo footprinting.

The albumin gene was silent in the gut tube at the time of tissue isolation and in gut tube segments that were cultured intact for 2 days, as expected (Fig. 5B, lane 2; 5C, lane 1). Note that this portion of the gut tube, at about 14.5 days gestation, normally becomes the intestine. However, when the gut epithelium was isolated and cultivated for 2 days in the absence of its surrounding mesenchymal-derived tissue, the endoderm inevitably activated albumin gene expression (Fig. 5C, lanes 2-4). When

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**Fig. 4.** Binding sites for GATA factor and HNF3 are essential for albumin enhancer activity. (A) DNA sequence of the enhancer region and point mutations used in plasmids for transfection. The eF site mutation inhibits GATA factor binding (data not shown). (B) Structure of plasmid pAT2, containing the albumin promoter driving thymidine kinase (tk) and position of insertion of the 830 bp, Nhel-AvalII albumin enhancer fragment (NA). Darker region of enhancer segment indicates the position of the sequence shown in A. (C) Primer extension analysis of RNA from transiently transfected H2.35 cells. Upper band, primer extension product from RSV-tk internal control plasmid; lower band, product from pAT2 and designated derivatives (ALB). (D) Quantitation of enhancer activity. Means and standard deviations from 3 independent transfection experiments are shown.

**Fig. 5.** Pluripotency of gut endoderm from 10.5 day gestation mouse embryos. (A) Endodermal epithelium being extruded from a segment of the gut tube from a 11.5 day gestation mouse embryo (see arrow). (B) RT-PCR analysis of RNA from isolated gut endoderm, without being cultured, using primers that detect both actin and serum albumin mRNA. The X indicates the position where an albumin product would migrate. 6X, DNA size marker lane. (C) RT-PCR analysis of RNA from the designated 10.5 day embryo tissues that had been cultured in vitro for 2 days prior to RNA isolation. The intact gut sample was from a cultured segment where the endoderm was not removed from its surrounding mesenchyme. The data show that when the mesenchyme is removed, the isolated endoderm, which normally develops into the intestine, has the potential to express serum albumin mRNA. Identical results were obtained from 11.5 day embryo tissue samples (data not shown). Data shown are representative of multiple independent experiments.
negative control tissues were isolated and subjected to the same culture regimen, they failed to activate albumin expression (e.g., see Fig. 5C, lane 5 for neural tube; data not shown for other tissues). These data demonstrate that the gut endoderm retains its pluripotency to activate liver genes at the 11.5 day stage and it is normally repressed from doing so by mesenchymal signals that promote or permit intestinal development.

**Nuclear GATA proteins in undifferentiated, pluripotent gut endoderm**

To address whether or not GATA factors in general and GATA-4 in particular, could be the albumin enhancer eF site-binding factor during development, we investigated nuclear extracts from mouse embryonic endoderm and liver. The eF site probe exhibited a broad electromobility shift band with nuclear extracts from livers of 12.5 day gestation embryos and only a small fraction of the binding activity was supershifted with the GATA-4 antibody (Fig. 6A, lanes 1, 2). The bulk of the GATA site-binding activity is due to GATA-1, GATA-2 and GATA-3 proteins, which are especially abundant in hematopoietic cells that predominate in the fetal liver (Yamamoto et al., 1990; Leonard et al., 1993).

In nuclear extracts from endoderm fragments pooled from 15-20 embryos, the major eF site-binding activity at 11.5 days gestation embryos and only a small fraction of the binding activity was supershifted with the GATA-4 antibody (Fig. 6A, lanes 1, 2). The bulk of the GATA site-binding activity is due to GATA-1, GATA-2 and GATA-3 proteins, which are especially abundant in hematopoietic cells that predominate in the fetal liver (Yamamoto et al., 1990; Leonard et al., 1993).

In vitro footprints: In vivo footprints:

In vivo footprinting of the albumin enhancer. Panels show LM-PCR analysis of guanosine residues on the enhancer top strand protected by proteins from DMS methylation. See Figs 1B and 4A for DNA sequence. Lanes 1-3, in vitro footprinting of proteins bound to plasmid DNA containing the albumin enhancer and then treated with DMS. Lane 1, no protein; 2, with purified HNF3α; 3, with GATA-4 translated in vitro. Lanes 4 and 5, in vivo footprinting of 11.5 day embryonic tissues treated with DMS. Lane 4, gut endoderm epithelium; lane 5, head neuroectodermal tissues. Relevant factor-binding sites are indicated by brackets. Black dots, positions of stronger DMS protection; open dots, weaker protection. ‘X’ marks the eH site for HNF3 which is unoccupied in gut endoderm.

**Occupancy of the eF GATA factor site in the chromatin of undifferentiated gut endoderm**

Previously, we used in vivo footprinting to discover that the albumin enhancer eF and eG (HNF3) sites are occupied in the prospective dorsal-posterior gut endoderm from 9.5 day mouse embryos (Gualdi et al., 1996). The questions remained as to whether or not the footprint pattern persists in 11.5 day gut endoderm, which is the earliest period with which we could
perform the protein analysis shown in Fig. 6, and whether or not the DMS protections at the eF site in vivo correspond to the protections elicited by authentic GATA protein.

We pooled gut endoderm segments from 11.5 day embryos and treated the cells with dimethyl sulfate (DMS). DMS permeates intact cells and methylates guanosines in nuclear DNA that are not protected by bound protein. As a negative control, we also treated neuroectodermal cells from embryo heads. DNA was isolated, cleaved at methylated guanosines with piperidine and the cleavages were mapped by ligation-mediated PCR (Mueller and Wold, 1989; Rigaud et al., 1991; Bossard et al., 1997). As a positive control for the guanosine protection pattern exhibited by GATA-4 and HNF3 proteins, we DMS-treated the recombinant factors bound in vitro to plasmid DNA containing the albumin enhancer. As seen in Fig. 7 (compare lanes 1 and 2), purified HNF3 protein protected guanosines at the eG and eH sites, as previously observed (Gualdi et al., 1996). In vitro translated GATA-4 protected guanosine 523 in the GATA sequence (Fig. 7, lane 3, upper black dot; see Fig. 1B for sequence) as well as guanosines at enhancer positions 530-532, with the guanosine 532 most strongly protected (lower black dot). Control reactions exhibited no such protections (Fig. 7, lane 1).

Embryo head cells at 11.5 days gestation, which lack GATA-4 and HNF3, exhibited a DMS cleavage pattern similar to free DNA, indicating an absence of bound factors (Fig. 7, lane 5). By contrast, gut endodermal cells at 11.5 days, which contain both factors, exhibited protections at the eG and eH sites like that observed by GATA-4 and HNF3 proteins in vitro (Fig. 7, lane 4). Similar GATA and HNF3 site occupancies was previously observed in 9.5 day endoderm (Gualdi et al., 1996). No significant protections were observed over the eF site on the bottom strand (data not shown and Gualdi et al., 1996). Protections at the eH site for HNF3 were not observed at either the 9.5 (Gualdi et al., 1996) or 11.5 day stages (Fig. 7, lane 4), which may be explained by the 3- to 4-fold lower affinity of the eH site for HNF3 compared to the eG site (Zaret and Stevens, 1995). Certain other bands exhibited protections and enhancements in the experiment shown in Fig. 7, but these were not consistent in different assays. In conclusion, both the GATA and HNF3 sites are occupied in the chromatin of a target gene that is silent, yet has the potential to be activated in endodermal development.

**DISCUSSION**

The goal of this study has been to obtain an mechanistic understanding of the developmental concept of determination. Gene knockout and ectopic expression studies can identify transcriptional regulatory factors that are required for tissue formation and differentiation, but they do not provide insight into how such factors endow a pluripotent cell with its potential to differentiate. In this paper, we have used in vivo footprinting, antibody analysis and in vitro tissue differentiation assays to show that the GATA transcription factors occupy a chromatin site in a pluripotent embryonic tissue. Notably, the target gene, albumin, is silent in the endoderm, but it has the potential to be activated by developmental signals. We therefore define GATA factors as potentiators of gene activity and suggest that, more generally, determination itself might be dependent upon potentiators occupying their selected target sites in pluripotent cells.

Genetic studies of GATA-4 originally emphasized a role for the factor in mouse heart development (Kuo et al., 1997; Molkentin et al., 1997). But GATA-4-deficient embryonic stem cells can populate the heart in chimeric embryos (Narita et al., 1997a), suggesting that the cardiac requirement for GATA-4 is either redundant (Jiang and Evans, 1996) or not intrinsic. Although varying results have been reported for the necessity of GATA-4 for visceral endoderm differentiation in vitro (Soudais et al., 1995; Grephin et al., 1997), GATA-4 is intrinsically required in embryos for the morphogenesis of the visceral endoderm and portions of the foregut and hindgut endoderm (Narita et al., 1997b). Thus, the cardiac defect seen in the original GATA-4 knockout embryos (Kuo et al., 1997; Molkentin et al., 1997) may be due to a lack of signals from the disrupted endoderm. In sum, two ongoing functions of GATA-4 in the gut endoderm seem to be to promote the tissue’s intrinsic development as well as the endoderm’s previously established ability to influence cardiogenesis of the adjacent mesenchyme (e.g., see Schultheiss et al., 1995; Gannon and Bader, 1995; Nascone and Mercola, 1996).

Using these issues as an example, we would like to distinguish regulatory factor activities that are important for genetic potentiation (and determination) from factor activities that maintain pluripotent tissue function. Note that in vivo footprints would not be evident unless GATA site occupancy occurs in the majority of cells in the population, and most or all of the endoderm will give rise to various gut-derived tissues. Thus, we suggest that in the same endodermal cell where GATA-4 activates genes required to maintain morphogenesis and cardiac induction, the factor occupies albumin gene chromatin and potentiates expression. Considering that GATA-like factors are critical for ongoing endoderm function in mice, frogs, flies and worms (see Introduction), it seems feasible that GATA-like factors also potentiate gene activity in those diverse metazoan contexts.

There are several ways that GATA factors and other potentiators could facilitate gene activation during cell specification. Data in this paper and others (Liu et al., 1991; Hu et al., 1992; Jackson et al., 1993) demonstrate that the albumin enhancer, like many other tissue-specific regulatory elements, must be competent to bind at least five activator proteins for the enhancer to possess significant activity; binding of GATA and HNF3 alone is insufficient (see data in Fig. 4). By having two of the requisite activators bound, prior to cell type specification, it reduces the number of binding events required to shift the enhancer from an inactive to an active state when hepatocytes are specified. Thus, gene activation may occur quickly and more synchronously in a cell population than if all activators were required to bind de novo.

Once potentiators are bound to target genes in their silent state, they may engage co-activator proteins (Tsang et al., 1997; Durocher et al., 1997; Blobel et al., 1998) or directly modify chromatin structure. For example, the winged helix DNA-binding domain of HNF3 has structural similarity to linker histone protein (Clark et al., 1993; Ramakrishnan et al., 1993) and purified HNF3 protein has been shown to position a nucleosome over the albumin enhancer sequence in vitro similar to that seen over the enhancer in adult liver in vivo (McPherson et al., 1993; Shim et al., 1998; Cirillo et al., 1998).
For the β-globin promoter, preincubation with purified GATA-1 protein, prior to nucleosome assembly in vitro, is sufficient to overcome chromatin-mediated repression (Barton et al., 1993). Having potentiators with different abilities bound to the same regulatory sequence, such as GATA and HNF3 bound to the albumin enhancer in the endoderm, could allow for various chromatin modifying activities to be engaged during tissue determination.

It is interesting to note that, by using the more sensitive reverse transcriptase-PCR assay instead of in situ methodology, tissue-specific gene activation has been found to occur virtually coincident with developmental induction of both the pancreas and the liver from the endoderm, prior to morphological differentiation (Gittes and Rutter, 1993; Gualdi et al., 1996). Indeed, a subset of the newly induced genes may be essential for morphogenesis itself. This observation underscores the rapidity with which dramatic changes in gene expression take place in development. By occupying target sites in chromatin and thereby ‘priming the pump’ for genes that may be expressed in a pluripotent cell, genetic potentiators such as GATA proteins can simplify the task of tissue specification and provide a molecular basis for understanding developmental determination. Further work will be required to know if the mechanisms proposed here are general for GATA factors in endoderm differentiation throughout the metazoans.

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GATA factors as potentiators in endoderm