Mechanisms Identified in the Transcriptional Control of Epithelial Gene Expression*

(Received for publication, August 14, 1995, and in revised form, October 24, 1995)

Guido Hennig, Oliver Löwrick, Walter Birchmeier, and Jürgen Behrens§
From the Max-Delbrück-Center for Molecular Medicine, Robert-Roelsle-Strasse 10, 13125 Berlin, Germany and the Institute for Cell Biology (Cancer Research), University of Essen Medical School, Essen 45147, Germany

Epithelial-specific gene expression is fundamental in both embryogenesis and the maintenance of adult tissues, and impairment of epithelial characteristics contributes to diseases such as cancer. We have here analyzed the 5′-region of the epithelial (E-) cadherin gene in order to understand mechanisms of epithelial-specific transcription and loss of expression during epithelial-mesenchymal transitions. The regulatory region of the mouse epithelial cadherin gene is composed of a promoter (from position −94 to the transcription start site) and a 150-base pair enhancer located in the first intron. The 5′-promoter consists of positive regulatory elements (a CCAAT-box and two AP-2 binding sites in a GC-rich region) and the palindromic element E-Pal that activates and represses transcription in epithelial and mesenchymal cells, respectively. The enhancer of the first intron stimulates the activity of heterologous promoters exclusively in epithelial cells. This epithelium-specific enhancer consists of three elements (E I to E III; E II and E III bind AP-2) that are necessary and sufficient for activity. We thus propose two regulatory mechanisms by which epithelial specificity of epithelial cadherin expression is determined: suppression of promoter activity in mesenchymal cells by E-Pal and enhancement of activity in epithelial cells by both E-Pal and the epithelium-specific enhancer.

Epithelium-specific gene expression is fundamental in both embryogenesis and the maintenance of adult tissues, and impairment of epithelial characteristics contributes to diseases such as cancer. We have here analyzed the 5′-region of the epithelial (E-) cadherin gene in order to understand mechanisms of epithelial-specific transcription and loss of expression during epithelial-mesenchymal transitions. The regulatory region of the mouse epithelial cadherin gene is composed of a promoter (from position −94 to the transcription start site) and a 150-base pair enhancer located in the first intron. The 5′-promoter consists of positive regulatory elements (a CCAAT-box and two AP-2 binding sites in a GC-rich region) and the palindromic element E-Pal that activates and represses transcription in epithelial and mesenchymal cells, respectively. The enhancer of the first intron stimulates the activity of heterologous promoters exclusively in epithelial cells. This epithelium-specific enhancer consists of three elements (E I to E III; E II and E III bind AP-2) that are necessary and sufficient for activity. We thus propose two regulatory mechanisms by which epithelial specificity of epithelial cadherin expression is determined: suppression of promoter activity in mesenchymal cells by E-Pal and enhancement of activity in epithelial cells by both E-Pal and the epithelium-specific enhancer.

Epithelia are essential and abundant tissues in most eukaryotic organs. Epithelial cells are the first identifiable embryonic cell type, which appears during compaction of the morula early in development (Fleming et al., 1993). During gastrulation, epithelial-mesenchymal transitions take place, and in this process epithelial-specific genes are repressed, and genes of the mesenchymal (and neuronal) lineages are activated (Cunningham and Edelman, 1990; Jessell and Melton, 1992). New epithelia usually derive from existing ones, i.e. from the ectoderm or endoderm, but can also be formed from the mesoderm by mesenchymal-epithelial transitions (e.g. during development of the kidney) (Saxen, 1987). In development, epithelial-mesenchymal and mesenchymal-epithelial transitions take place in a temporally and spatially controlled manner (Valles et al., 1991; Boyer and Thiery, 1993; Birchmeier and Birchmeier, 1993), whereas in tumors these changes are highly uncontrolled; loss of epithelial character is typically observed late in progression of carcinomas and correlates there with the acquisition of invasive and metastatic potential (Birchmeier and Behrens, 1994; Reichmann, 1994).

Epithelial cells form continuous cell layers, and they are generally polar. In single-layered epithelia (e.g. the mature intestine), apical and basolateral cell surface are separated by tight junctions (Citi, 1993). An example of a multilayered epithelium is the skin, where basal cells (stem cells) are covered by layers of gradually differentiating cells (Fusenig et al., 1994). Thus, epithelia are extremely complex tissues, and they are highly variable in type and degree of differentiation. Typical structures in epithelia are adherens junctions and desmosomes (Buxton and Magee, 1992; Tsukita et al., 1993; Hülsken et al., 1994a), which are organelles responsible for strong intercellular adhesion; epithelial cells also form hemidesmosomes to the basement membranes at their basal side (Timpl, 1989; Sonnenberg et al., 1991). Polar epithelial cells develop special mechanisms that allow the transport of membrane proteins to either the apical or basolateral surface (Eaton and Simons, 1995). Epithelial cells express characteristic genes that are responsible for the maintenance of the epithelial phenotype; for example, components of junctions or keratins (Birchmeier and Behrens, 1994; Kouklis et al., 1994; Buxton et al., 1993), specific epithelial products (e.g. albumin in the liver) (Cereghini et al., 1987), and specific transcription factors (e.g. LFB-3 in the liver or kidney) (De Simone et al., 1991).

Recently, much progress has been made in the elucidation of the molecular basis of epithelial junction formation (Tsukita et al., 1993; Garrod, 1993; Citi, 1993; Hülsken et al., 1994b). Adherens junctions are specialized structures containing the transmembrane cell adhesion molecule epithelial cadherin,1 that recognizes and binds E-cadherin present on the neighboring cells in a Ca2+-dependent manner. The cDNA of E-cadherin codes for a signal peptide and a presequence at the amino terminus, a large extracellular domain with four repeated domains important in Ca2+-binding, a single transmembrane sequence, and a short cytoplasmic domain (Takeichi, 1991; Kemler, 1993). E-cadherin is the prototype of a family of Ca2+-dependent cell adhesion molecules and is expressed in all embryonal and adult epithelial tissues. In development, E-cadherin expression is down-regulated during epithelial-mesenchymal transitions and reappears during reversion to the epithelial phenotype. For example, E-cadherin disappears during differentiation of the dorsal ectoderm into the neural tube (Thiery et al., 1982; Nose and Takeichi, 1986), and it is induced in epithelial cells that develop from mesenchyme during morphogenesis of kidney tubules (Vestweber et al., 1985). Overall, E-cadherin is thus a faithful component in all epithelia and plays a functional role for the

1 The abbreviations used are: E-cadherin, epithelial cadherin; bp, base pair(s); CAT, chloramphenicol acetyltransferase; HLH, helix-loop-helix; PCR, polymerase chain reaction.
E-cadherin Gene Expression

maintenance of the epithelial phenotype (Imhof et al., 1983; Behrens et al., 1989). Accordingly, homozygous mutations of E-cadherin introduced into mice by homologous recombination disturbed early embryogenesis: The individual cells of the morulae lose their morphologic polarization and do not form a blastocoele. The mutant embryos cannot leave the zona pellucida and, therefore, do not implant into the uterus (Larue et al., 1994; Riethmacher et al., 1995).

Since down-regulation of E-cadherin expression is a frequent event late in progression of human carcinomas and since modulation of E-cadherin expression plays a major role during development, we and others have begun to analyze the E-cadherin promoter and have found epithelial specificity in a fragment 178 bp upstream of the transcription start site (Behrens et al., 1991; Ringwald et al., 1991; Bussemakers et al., 1994). This promoter fragment contains a GC-rich region, a CCAAT-box, and a 12-bp palindromic element, which we named E-Pal. We have furthermore found that epithelium-specific transcription correlates with factor binding to these elements in vivo and to a loosening of chromatin structure in the promoter region (Hennig et al., 1995). Other epithelium-specific promoters have recently also been examined: The upstream regulatory regions of the epithelium-specific human papilloma viruses (HPV) 16 and 18 contain several binding sequences for ubiquitous cellular transcription factors (cf. Cripe et al., 1990; Hoppe-Seyler and Butz, 1994; Bernard and Apt, 1994). Epithelial specificity thus appears to be achieved by different combinations of these cellular factors. Specific factors have also been characterized that contribute to epithelial specificity; for instance, a mesenchyme-specific member of the NF-1 family represses transcription in fibroblasts but not in epithelial cells (Apt et al., 1993). Other epithelium-specific activators and repressors such as KRF-1, a coactivator of transcriptional enhancer factor-1 and YY1 have recently been described (Mack and Laimins, 1991; Ishii et al., 1992, Bauknecht et al., 1992).

Here we report that epithelium-specific expression of the E-cadherin gene is achieved by two different mechanisms; E-Pal in the upstream promoter activates or suppresses transcription in epithelial or mesenchymal cells, respectively. A new epithelium-specific enhancer (ESE) was discovered in the first intron of the gene, that enhances transcription in a tissuespecific manner and binds nuclear factors specifically in epithelial cells.

MATERIALS AND METHODS

Plasmid Constructs—The E-cadherin promoter-CAT constructs are derivatives of the deletion –178/+92 cloned in the pCAT basic vector (Behrens et al., 1991). To generate the –94 or –78 deletion constructs, a HindIII/PstI fragment (–178 to –79) was replaced by a double-stranded oligonucleotide, or the blunt-ended vector was directly religated, respectively. To obtain mutants of E-Pal, the pCAT-box, or the GC-rich region, restriction fragments of the –178/+92 construct were replaced by corresponding mutant oligonucleotides. The restriction sites PstI (–99), NarI (–54), EcoD (–22), or KspI (+18) were used for the exchange. The CCAAT sequence was mutated to TCCG, and the AP-2 binding sites in subregions GCI and GCII were mutated to GCCGAGGCGAGGGAAGGGTTACCCTTG and GGTCCTCCCCACTAG, respectively.

For analysis of the intronic enhancer, a 1.9-kilobase genomic BamHI fragment containing intron 1 and parts of intron 2 was ligated into the TK-CAT construct (pBLCAT2) (Lucodow and Schütz, 1987). Deletions of the enhancer were made with exonuclease III (Pharmacia Biotech Inc.), and all fragments were ligated into TK-CAT. The AP-2 expression construct SPRSV AP-2 was kindly provided by Dr. T. Williams (New Haven, CT). In the construct AP-2TA (Williams and Tjian, 1991), nucleotides 153–413 were removed. All sequences were confirmed by dinuclease sequencing.

Cells, Transfections, and CAT Assays—Cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Cell lines are described in Friksen et al. (1991) and Behrens et al. (1991). For transfection experiments, cells were seeded at a density of 10⁶ per 10-cm tissue culture dish and transfected with usually 5 µg of the mutant promoters using calcium phosphate coprecipitation and cotransfected plasmids Rous sarcoma virus lacZ (Behrens et al., 1991) or pCH110 (Pharmacia).

In Vitro DNase I Footprint Assays—Nuclear extracts were prepared as described (Behrens et al., 1991). For DNase I footprint assays, an XbaI/KspI fragment of the E-cadherin promoter (positions –178 to +17) or the intronic enhancer (positions 672–311 from the BamHI site in intron 1) was labeled with Klenow enzyme. For the AP-2 footprint assays, 30 ng of purified recombinant transcription factor AP-2 (Promega) were employed. In the competition footprint assays of the enhancer region, 50 ng of the double-stranded oligonucleotides were added to the reaction: E II, 5′-GATCCCCCTGTGTGCCCTGAGGG-GGTTTTC and 5′-GATCCAAA-TTCTGCGGG, respectively.

RESULTS

Characteristics of the E-cadherin Promoter—We and others have previously analyzed the E-cadherin promoter and found epithelial specificity in a –178 bp fragment upstream of the transcription start site (Behrens et al., 1991, Ringwald et al., 1991, Bussemakers et al., 1994). This fragment contains a GC-rich region with subregions GCI and GCII, a CCAAT-box, and a 12-bp palindromic element that we named E-Pal (Fig. 1A). We demonstrate here that deletion or mutation of E-Pal resulted in a 5-fold increase of promoter activity in mesenchymal cells, e.g., fibroblasts or smooth muscle cells (Fig. 1B). Similarly, mutation of E-Pal leads to an increase (2–4-fold) of promoter activity in E-cadherin-negative carcinoma cells (Table I). A slight decrease of promoter activity was observed when E-Pal was mutated or deleted in E-cadherin-expressing carcinoma cell lines. In the mouse mammary epithelial cell line EpFosER, which looses E-cadherin expression following activation by estrogen, the E-Pal mutation also leads to an increase of promoter activity (Table I). These data demonstrate that E-Pal is a negative regulatory element in mesenchymal E-


**TABLE I**

Activity of the E-cadherin promoter in E-cadherin-expressing and nonexpressing human carcinoma cell lines and in an inducible mouse mammary epithelial cell line.

| Cell lines | E-cadherin expression | Promoter activity | Relative activity of the promoter with mutated E-Pal² |
|------------|-----------------------|-------------------|---------------------------------------------------|
| Breast     | +                     | 32                | 0.9                                               |
| MCF-7      | +                     | 8                 | 3.7                                               |
| MDA-MB-435 | −                     | 9                 | 2.5                                               |
| MDA-MB-231 | −                     | 47                | 0.6                                               |
| Lung       | −                     | 9                 | 2.4                                               |
| Pancreas   | +                     | 25                | 0.8                                               |
| Capan-2    | −                     | 8                 | 2.1                                               |
| MIA-PaCa-2 | −                     | 30                | 0.8                                               |
| Bladder    | −                     | 8                 | 2.0                                               |
| Mammary    | Ep-FosER³             | 35                | 1.0                                               |
| Ep-FosER   | (not activated)       | −                 | 2.2                                               |

¹ The cell lines and the endogenous E-cadherin expression are as described in Fri xen et al. (1991).
² E-cadherin promoter activity was determined relative to the activity of the SV40 promoter/enhancer as described (Behrens et al., 1991).
³ The E-Pal mutation (mut) is described in Fig. 1. Activity is expressed relative to the −178 bp construct.

The mouse mammary epithelial cell lines were kindly provided by Dr. H. Beug (Institute for Molecular Pathology, Vienna, Austria). The cell lines express FosER fusion protein that either was not activated or was activated by estrogen.

E-cadherin-negative) cells but has a weak positive activity in epithelial cells.

We attempted to identify regulatory factors that control the E-cadherin promoter through the E-Pal element, by comparing the functional effects of specific mutations of E-Pal with the capacity of nuclear factor binding in gel retardation assays. Mutations in the center or in the 3'-half of E-Pal (mut 1 to mut 4) increased promoter activity in fibroblasts (Fig. 2A), in contrast to a mutation of the 5'-side (mut 5). A specific nuclear factor from fibroblasts was found to bind to the E-Pal element in gel retardation assays (Fig. 2B), which could be competed only by the wild-type oligonucleotide and the one mutated in the 5'-half of E-Pal. This suggests that binding of a specific factor (repressor) of fibroblasts to E-Pal correlates with suppression of promoter activity. However, we are aware of the fact that a similar band shift is seen when nuclear extracts from epithelial cells are examined (not shown, but see Behrens et al. (1991)). We next examined the contribution of the CCAAT-box and the GC-rich region to transcriptional activity of the E-cadherin promoter. Mutation of either the core sequence of the CCAAT-box or one of the two consensus binding sites for the transcription factor AP-2 (cf. Williams and Tjian, 1991) strongly reduced promoter activity in epithelial cells (Fig. 3A). These mutations also reduced the activity of the promoter with a mutated E-Pal in fibroblasts (Fig. 3B). Mutation of all three elements completely abolished promoter activity. These data show that both the CCAAT-box and the GC-rich region represent positive regulatory elements in both epithelial and mesenchymal cells. The GC-rich region of the E-cadherin promoter binds the transcription factor AP-2, as revealed by footprint analysis (Fig. 4). Mutation of each of the two AP-2 binding sites in the subregion GCI or GCII narrowed the footprint with both recombiant AP-2 and nuclear extracts on the respective sides. Footprint formation at both sites was inhibited by an oligonucleotide containing the AP-2 binding site of the SV40 enhancer (data not shown; cf. Imagawa et al. (1987)). Furthermore, a cotransfected dominant-negative mutant of AP-2 that lacks the transactivation domain inhibited activity of both the −178 and −58 bp promoters in a concentration-dependent fashion (Table II). These data indicate that AP-2 or a closely related factor regulate the E-cadherin promoter by binding in a tandem arrangement to the GC-rich region.

Characteristics of the Intronic Enhancer of the E-cadherin Gene—In a recent in vivo analysis, we identified DNase I-hypersensitive sites in the first and second introns of the E-cadherin gene that are specific for E-cadherin-expressing cells (Hennig et al., 1995). The respective genomic regions were here examined for enhancer activity on the TK minimal promoter (Fig. 5). The used 1.9-kilobase BamH1 fragment of the first and part of the second intron enhanced promoter activity 4.5-fold in epithelial cells; deletion of intron 2 sequences to position 672 led to a further (12.5-fold) enhancement of activity, which may indicate the presence of a negative regulatory region between positions 1100 and 672. Further deletion abolished activity, thus localizing the enhancer to a region of 200 bp of intron 1 (compare the activity of constructs 672 and 475 in Fig. 5). Similar results were obtained when the deletion fragments...
were examined with the SV40 minimal promoter. Enhancer activity was also observed when the 1.9-kilobase fragment was tested in the reverse orientation (in the TK and SV40 promoter) or at a distance of 2.7 kilobases from the transcription start site of the SV40 promoter (data not shown).

Footprint analysis of the enhancer in intron 1 revealed binding of nuclear factors to three subregions, EII to EIII (Fig. 6). The sequences EII and EIII are specifically protected by nuclear extracts from E-cadherin-expressing carcinoma cell lines (MCF-7 and RT 112) but not from E-cadherin-negative carcinoma cells (MDA-MB-231 and T 24); region EI was protected by extracts from both cell types. DNA sequencing of the protected areas revealed that EI to EIII are GC-rich; EII and EIII contain sequences that match the AP-2 consensus site (Fig. 7; cf. also Williams and Tjian (1991)).

The contribution of the individual elements EI to EIII to the function of the intronic enhancer was examined by using various PCR-generated subfragments (Fig. 8): a 149-bp fragment comprising all three elements exhibited strong enhancer activ-

### Table II

| Cell lines | Amount of dominant-negative AP-2 | Promoter activity |
|------------|----------------------------------|-------------------|
|            | µg | % | 2178 | 258 |
| CSG 120/7  | 1  | 100 | 100 |
|            | 5  | 38  | 42  |
| MCF-7      | 1  | 100 | 100 |
|            | 5  | 54  | 38  |
|            | 5  | 19  | 18  |

*CSG 120/7 is a mouse salivary gland carcinoma cell line which expresses E-cadherin.
+ The dominant-negative variant of AP-2 was generated by deleting the cDNA sequences coding for the transactivation domain (Williams and Tjian, 1991). Cotransfections were performed with E-cadherin promoter-CAT constructs, increasing amounts of dominant-negative AP-2, and decreasing amounts of control vector (total transfected cDNA, 5 µg).
+ Activities of the 2178 bp and 258 bp E-cadherin promoter-CAT constructs in the presence of control vector without the AP-2 sequence were set to 100%.
ity in both orientations on the TK promoter in MCF-7 epithelial cells. Two copies of the enhancer showed 40-fold enhancement of activity (not shown). Interestingly, removal of any of the individual elements abolished enhancer activity, indicating that the integrity of the whole E I to E III cluster is sufficient and necessary to confer enhancer activity. The fragment of 149 bp also confers enhancer activity to the TK promoter in several other E-cadherin-expressing cell lines (Table III). No enhancer activity was found in fibroblasts and dedifferentiated carcinoma cells. We therefore named the 149-bp region ESE, i.e. epithelium-specific enhancer. Detailed footprint analysis of the ESE element with various nuclear extracts revealed general protection of the elements E I to E III in E-cadherin-expressing carcinoma cells; the element E I is protected in E-cadherin-negative carcinoma cells, and the elements E II and E III are protected in fibroblasts (Table III). Region E I contains a consensus binding sequence for the transcription factor H4TF-1 (cf. Dailey et al. (1988); we have not examined this factor any further). Binding of nuclear factors of MCF-7 cells to both regions E II and E III was competed by an AP-2 but not by an SP-1 binding site oligonucleotide (Fig. 9; Kadonaga et al. (1987)). Oligonucleotides containing the sequences of either E II or E III interfered with factor binding to both regions. Moreover, regions E II and E III were also protected by recombinant AP-2 (Fig. 9).

**DISCUSSION**

We report here that epithelium-specific regulation of the E-cadherin gene is controlled by two different mechanisms. First, the E-Pal element in the promoter 5’ of the transcription start site acts as a positive or negative element in epithelial or mesenchymal cells, respectively. Second, a tissue-specific enhancer (ESE) in the first intron promotes transcription exclusively in epithelial cells. We also demonstrate that both these mechanisms are disturbed in carcinoma cells that have progressed to a less differentiated state and are E-cadherin-negative. We suspect that the E-cadherin gene is activated and repressed by similar mechanisms during mesenchymal-epithelial transitions in development.

Restriction of E-cadherin Promoter Activity to Epithelial Cells Is in Part Due to Cell Type-specific Repression—We describe here three cis-acting elements in the E-cadherin promoter that exhibit positive regulatory activity in epithelial cells: E-Pal, the CCAAT-box, and a GC-rich region. We have previously shown that the E-Pal element also confers positive regulatory activity to a SV40 promoter in epithelial cells (Behrens et al., 1991). In nonepithelial cells, CCAAT-box and GC-rich region exhibit positive transcriptional potential, but this activity is masked by E-Pal, i.e. becomes apparent only when E-Pal is mutated or deleted. We also provide here evidence that a specific factor binds to E-Pal in fibroblasts and that binding specificity of this factor correlates with biological activity. Transcriptional repressors that act via binding to a specific promoter element have been described by others (see Renkaw-
We demonstrate here that the CCAAT-box in gel retardation assays, and these did not appear when the CCAAT sequence was mutated. Competition by specific oligonucleotides revealed that various members of the CCAAT-box family of proteins such as CP-1 and C/EBP (Chodosh et al., 1988; Landschulz et al., 1988) are candidates for activation of the E-cadherin promoter (data not shown). We have not analyzed the CCAAT-box of the E-cadherin promoter any further. Binding and functional data suggest that the transcription factor AP-2 or closely related factors regulate the activity of the GC-rich region of the E-cadherin promoter. (i) In DNase I footprint assays, two subregions GC1 and GCII were identified that bound purified AP-2 with similar characteristics as factors present in nuclear extracts. Binding to both sites GC1 and GCII could be competed with a single AP-2 specific oligonucleotide. (ii) In transient transfection experiments, AP-2 that lacks the transactivation domain suppressed the activity of the E-cadherin promoter in a concentration-dependent manner. Cis-elements representing AP-2 binding sites have recently been implicated in the regulation of promoters of various epidermal cytokeratins (Leask et al., 1991, Snape et al., 1991, Byrne and Fuchs, 1993). However, it is unlikely that AP-2 has an exclusive role in epithelium-specific gene expression since it is found in ectodermal derivatives but not in several other epithelial tissues that express E-cadherin (Mitchel et al., 1991).

Properties of the Novel ESE in the E-cadherin Gene—The ESE of the E-cadherin gene was discovered by the analysis of DNase I hypersensitive sites followed by analysis of deletion constructs and PCR fragments for enhancer activity. The enhancer region corresponds to hypersensitive site 4 in the first intron of the E-cadherin gene, as described previously (Hennig et al., 1995). Transcriptional enhancers are generally associated with loosened chromatin structure, as in the case of the SV40 and poloma virus enhancer; these enhancers are characterized as nucleosome-free regions and are hypersensitive toward DNase I (Serfling et al., 1985). In the epithelial region of the ESE of the E-cadherin gene (150 bp) activates transcription from heterologous promoters by a factor of 15 in MCF-7 cells (40-fold when used in a tandem arrangement) that is independent of orientation and distance. Detailed analysis of the ESE revealed three elements, E1 to EIII, which are all GC-rich. These elements are necessary and sufficient for enhancer activity in several E-cadherin-expressing epithelial cell lines. In contrast, the ESE is inactive in all E-cadherin-negative cell lines such as fibroblasts and dedifferentiated carcinoma cells. The ESE therefore has a modular structure, which is characteristic for several other enhancers, e.g. the CD2 or SV40 enhancers (Serfling et al., 1985; Lake et al., 1990). In the epithelium-specific enhancer of papilloma viruses, numerous elements contribute to enhancer activity, but the involved sequences are generally bound by ubiquitous transcription factors (see Hoppe-Seyler and Butz (1994) for a review). We found that the ESE of the E-cadherin gene is active in cells that
express all three factors that bind to E I to E III, but is inactive when one factor is lacking. We also demonstrated that AP-2 or related factors bind to E II and E III; again epithelium-specific enhancer cannot be explained by the action of AP-2 only. It is possible that recently identified splice variants of AP-2 (Meier et al., 1995) play a role in the specific regulation of ESE.

The expression of the E-cadherin gene is thus under the control of an epithelium-specific promoter plus an epithelium-specific enhancer. We suggest that the combination of these two regulatory mechanisms provides additional specificity and strength of expression of the E-cadherin gene. This is thus different to the regulation of the related L-CAM gene (a chicken homologue of E-cadherin). There, a low activity and nonspecific promoter gains tissue specificity when combined with an enhancer in the second intron (Sorkin et al., 1993). In the P-cadherin gene, an enhancer is also located in the second intron (Hatta et al., 1994). In addition promoter and enhancer of the E-cadherin gene act differently in mesenchymal cells. Here the enhancer is inactive, but the promoter contributes to repression through the E-Pal element. To our knowledge, a combination of an epithelium-specific enhancer with an epithelium-specific promoter that acts as a repressor in mesenchymal cells has not previously been found.

Acknowledgments—We thank Beate Voss for technical assistance. We also thank I. Wiznrowicz for excellent secretarial help.

REFERENCES

Apt, D., Chong, T., Liu, Y., and Bernard, H. U. (1993) J. Virol. 67, 4455–4463.
Bauknecht, T., Angel, P., Royer, H. D., and zur Hausen, H. (1992) EMBO J. 11, 4607–4617.
Behrens, J., Mareel, M. M., Van Roy, F. M., and Birchmeier, W. (1989) J. Cell Biol. 108, 2435–2447.
Behrens, J., Löwrick, O., Klein-Hilpass, L., and Birchmeier, W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11495–11499.
Bernard, H.-U. and Apt, D. (1994) Arch. Dermatol. 130, 210–215.
Birchmeier, C., and Birchmeier, W. (1993) Ann. Rev. Cell Biol. 9, 511–540.
Birchmeier, W., and Behrens, J. (1994) Biochim. Biophys. Acta 1196, 11–26.
Boukamp, P., Chen, J., Gonzalez, F. J., Jones, P. A., and Fusenig, N. E. (1992) J. Cell Biol. 116, 1257–1271.
Broyer, B., and Thiery, J. P. (1993) APMIS 101, 257–268.
Bussemakers, M. J., Giroldi, L. A., van Bakkenhoek, A., and Schalken, J. A. (1994) Biochem. Biophys. Res. Commun. 203, 1294–1299.
Buxton, R. S., and Magee, A. I. (1992) Semin. Cell Biol. 3, 157–167.
Buxton, R. S., Cowin, P., Franke, W. W., Garrod, D. R., Green, K. J., King, I. A., Koch, P. J., Magee, A. I., Rees, P. D., Stanley, J. R., and Steinberg, M. (1993) J. Cell Biol. 121, 481–483.
Byrne, C., and Fuchs, E. (1993) Mol. Cell. Biol. 13, 3176–3190.
Cereghini, S., Raymondjean, M., Garcia-Carranca, A., Herboonen, P., and Yamin, M. (1987) Cell 50, 627–638.
Chodosh, L. A., Baldwin, A. S., Carthew, R. W., and Sharp, P. A. (1988) Cell 53, 11–24.
Citi, S. (1993) J. Cell Biol. 121, 485–489.
Criere, T. P., Alderborn, A., Anderson, R. D., Parkkinnien, S., Bergman, P., Haugen, T. H., Pettersson, U., and Tunek, L. P. (1990) New Biol. 2, 450–463.
Cunningham, B. A., and Edelman, G. M. (1990) in Morphoregulatory Molecules (Edelman, G. M., Cunningham, B. A., and Thiery, J.-P., eds.) pp. 9–40, J. Wiley and Sons, Inc., New York.
Daley, L., Roberts, S. B., and Heintz, N. (1988) Genes Dev. 2, 1700–1712.
DeSimone, V., De Magistris, L., Lazzaro, D., Gerstner, J., Monaci, P., Nicosia, A., and Cortese, R. (1991) EMBO J. 10, 1435–1443.
Drummond, I. A., Madden, S. L., Rohrer Nutter, P., Bell, G. I., Sukhatme, V. P., and Rauscher, F. J. (1992) Science 257, 674–678.
Eaton, S., and Simons, K. (1995) Cell 82, 5–8.
Fleming, T. P., Javed, Q., Collins, J., and Hay, M. (1993) J. Cell Sci. (Suppl.) 17, 119–125.
Fusenig, N. E., Limat, A., Stark, H. J., and Breitkreutz, D. (1994) J. Dermatol. Sci. 10, 210–215.
Garrard, D. R. (1993) Curr. Opin. Cell Biol. 5, 30–40.
Gorman, C. M., Merling, G. T., Wittingham, M. C., Pastan, I., and Howard, B. H. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6777–6781.
Hatta, M., and Takeichi, M. (1994) Dev. Growth & Differ. 36, 509–519.
Hennig, H., Behrens, J., Truss, M., Frisch, S., Reichmann, E., and Birchmeier, W. (1995) Oncogene 11, 475–484.
Hoppe-Seyler, F., and Butz, K. (1994) Mol. Carcinogen. 10, 134–141.
Hülsken, J., Birchmeier, W., and Behrens, J. (1994a) J. Cell Biol. 127, 2061–2069.

2 M. Faraldo and A. Cano, personal communication.
Mechanisms Identified in the Transcriptional Control of Epithelial Gene Expression
Guido Hennig, Oliver Löwrick, Walter Birchmeier and Jürgen Behrens

J. Biol. Chem. 1996, 271:595-602.
doi: 10.1074/jbc.271.1.595

Access the most updated version of this article at http://www.jbc.org/content/271/1/595

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 69 references, 32 of which can be accessed free at http://www.jbc.org/content/271/1/595.full.html#ref-list-1