Differential requirements for cell-specific elastase I enhancer domains in transfected cells and transgenic mice

Galvin H. Swift, Fred Kruse, Raymond J. MacDonald, and Robert E. Hammer

The Department of Biochemistry, 1Howard Hughes Medical Institute, and the Department of Cell Biology, The University of Texas Southwestern Medical Center, Dallas, Texas 75235 USA

The 134-bp enhancer region of the pancreatic elastase I gene is sufficient to direct pancreatic acinar cell-specific transcription in transgenic mice and in transfected cells in culture. Ten-base-pair scanner mutations in three separate enhancer domains that inactivate enhancer function in transfected pancreatic cells in culture have no significant effect in transgenic mice. Because any pair of the three domains is sufficient to direct pancreas-specific expression in mice, no one domain is required for pancreas-specific transcription. Disruption of any two domains does inactivate the enhancer function in transgenic mice. Therefore, the elastase I enhancer domains essential for function in transfected cells in culture are not essential in animals but have a redundant function not apparent in transfected cells. This redundant function is not because of the particular acinar cell line used for transfections, the nature of the reporter gene, or the state of integration of the foreign test gene. We conclude that a trans-acting transcription factor or a modification of a factor[s] present in pancreatic cells of an animal is absent in pancreatic acinar cell lines.

[Key Words: Enhancer redundancy; transgenic mice; pancreatic acinar cells]

Received December 19, 1988; revised version accepted March 13, 1989.

Differentiation occurs through the selective expression of genes contained within all cells. Regulation at the level of transcription is a common control mechanism of the genes that specify the phenotype of differentiated cells [Derman et al. 1981; Darnell 1982]. An understanding of the genetic and molecular mechanisms that activate cell-specific gene transcription requires an understanding of the nature of DNA control sequences, the transcription factors that bind those sequences, and the productive consequence of the binding that activates transcription. The extent of expression of gene products whose high levels normally characterize a terminally differentiated tissue is often limited in cells in culture, which frequently are trapped in a less than maximally differentiated state. It is not clear whether regulatory sequences [and the factors that bind them] identified by transfection of cultured cells are an accurate reflection of those used in the animal. To investigate this question, we have compared the cell-specific expression of the rat pancreatic elastase I (EI) gene in both transgenic animals and pancreatic acinar cells in culture.

EI is a representative member of the subfamily of serine proteases expressed selectively and to high levels in the acinar cells of the exocrine pancreas [MacDonald 1987]. These serine proteases, including three isozymes of trypsin, three isozymes of chymotrypsin, elastases I and II, and glandular kallikrein, are part of the complement of hydrolytic enzymes that are synthesized, stored, and secreted in massive amounts for intestinal digestion and dominate the differentiated phenotype of the exocrine pancreas [Rutter et al. 1968]. EI gene expression is rigorous and simple, it is on at a high level in pancreatic acinar cells and off in other cell types [Swift et al. 1984a] and is not modulated by hormones or by diet (Shick et al. 1984a,b).

The EI regulatory sequences necessary and sufficient for rigorous, acinar cell-specific transcription have been defined in transgenic animals. The 5'-flanking region of the rat EI gene confers pancreatic transcriptional specificity to a human growth hormone (hGH) reporter gene in animals [Omitz et al. 1985a]. Moreover, a 134-bp element within the proximal upstream sequences (nucleotides −72 to −205) is sufficient to direct pancreas-specific expression of the hGH gene using either the hGH or EI promoter [Hammer et al. 1987]. When tested in transgenic animals, this regulatory element behaves as a cell-specific enhancer that directs high levels of hGH reporter gene expression in pancreatic acinar cells regardless of its orientation or position relative to the hGH gene.

Through the analysis of the effects of 10-bp scanning mutations on expression after transfection into a mouse pancreatic acinar tumor cell line (266-6), we demonstrated that the EI enhancer contains three distinct domains: All are required for acinar cell-specific transcription [Kruse et al. 1988]. The domains include binding sites for sequence-specific DNA-binding proteins, indicating that the functional sequence elements of the EI
enhancer bind factors necessary for pancreatic expression (Kruse et al. 1988).

To determine whether the same domains of the EI enhancer are essential for pancreas-specific transcription in animals and in transiently transfected pancreatic acinar cells in culture, we have introduced key enhancer mutants into transgenic mice. We have determined that the enhancer remains active as long as any two of the three domains are unaltered, in contrast to results from cell transfections. The discrepancy between animals and cultured cells is found for different pancreatic acinar cell lines. It is not due to the particular reporter gene used or to the integration state of the reporter gene. We believe that the redundant function of the enhancer domains in mice is due to a trans-acting factor or modification of a factor(s) present in pancreatic cells of an animal but absent in pancreatic acinar cell lines.

Results

Expression of EI enhancer mutants in mice

Transgenic mouse experiments defined a pancreatic acinar cell-specific enhancer residing between 205 and 72 nucleotides 5' to the start of transcription of the rat EI gene (Hammer et al. 1987). Further dissection of the enhancer utilized transient transfections of a cell line, 266-6, derived from a pancreatic acinar cell carcinoma excised from a transgenic mouse expressing T antigen selectively in the pancreas (Ornitz et al. 1985b). A series of 10-bp substitution [scanner] mutants spanning the elastase enhancer, when tested for their ability to direct chloramphenicol acetyl transferase [CAT] reporter gene expression selectively in the 266-6 cells, revealed three essential domains [A, -90 to -120; B, -140 to -160; and C, -170 to -190] of the enhancer [Kruse et al. 1988; Fig. 1]. As assayed in the 266-6 cells, the enhancer domains are nonredundant, because mutations in any domain eliminated expression (100-fold reduction).

To test whether the same domains of the enhancer were essential for function in mice, an elastase-hGH fusion gene construct [EGH3; -205 to +8 of EI joined to a hGH reporter gene at +3 of the hGH transcription unit (Hammer et al. 1987)] was modified by introducing three separate scanner mutations, -101 to -110 [EGH3-101, -151 to -160 [EGH3-151] and -181 to -190 [EGH3-181] (Fig. 1). These three scanner mutations were chosen because they represent mutations in each domain that abolish enhancer activity in transfected cells. The scanner mutant constructs were introduced into transgenic mice and assayed for their ability to direct pancreas-specific expression. All three of the mutant constructs were able to direct high-level pancreas-specific expression (Table 1), in direct contrast to the results from transient transfection of pancreatic 266-6 cells. Therefore, one domain is not required for pancreas-specific transcription. It is likely that sequence information that directs pancreas specificity is present in at least two of the three domains.

Although a particular EI gene construct gives a wide range of expression in transgenic mice, higher expression generally occurs in animals with higher transgene copy numbers [Swift et al. 1984a; Hammer et al. 1987; Davis and MacDonald 1988]. To permit a comparison of expression levels between sets of mice with varying copy numbers, we have calculated the average of the levels of hGH mRNA per pancreatic cell per transgene for each expressing animal (Table 1). The unaltered EI enhancer construct, EGH3, has a value of 10,000 hGH mRNAs per cell per gene copy (average of three animals; Hammer et al. 1987). The values for both EGH3-151 and EGH3-181 (7000 mRNAs per cell per gene for each) are

Figure 1. Modified E1 enhancers linked to the hGH reporter gene and tested for activity in transgenic mice. [Top] The sequence of the EI enhancer (-205 to -72). Bracketed sequences indicate the three domains essential for enhancer activity in transfected cells [Kruse et al. 1988]. Overlining indicates the pancreas consensus sequence [Swift et al. 1984b]. [Bottom] Scanner mutant and truncation constructs tested for enhancer activity in transgenic mice. For single- and double-scanner mutant derivatives of EGH3, the EI-flanking region from -205 to +8 was modified by 10-bp substitutions, shown as boxed sequences directly below the original sequence. A line indicates identity with the original sequence. These modified flanking regions were fused to the hGH gene at +3. The 5' end of the truncation mutants EGH13 and EGH14 was provided by digestion of the -151 and -171 scanner mutants, respectively, with XbaI.
Enhancer redundancy in mice

Table 1. Pancreatic expression of hGH mRNA for enhancer scanner mutants in transgenic mice

| Number of expressors | EGH3-101 | EGH3-151 | EGH3-181 |
|----------------------|----------|----------|----------|
| Average mRNA per expressor | 8 of 11 | 5 of 5 | 6 of 6 |
| (mRNAs/cell/gene copy) | 1,000 | 7,000 | 7,000 |

| Number | mouse | copy | mRNAs/cell |
|--------|-------|------|------------|
| 318-1  | 1     | bd*  | 362-6      |
| 352-1  | 2     | bd   | 360-3      |
| 545-6  | 1     | bd   | 361-2      |
| 537-5  | 31    | 120  | 346-2      |
| 539-1  | 3     | 140  | 360-2      |
| 539-4  | 7     | 1,100| 408-6      |
| 537-3  | 10    | 3,200| 348-4      |
| 539-2  | 1     | 8,500| 353-7      |
| 538-7  | 39    | 13,000| 346-2    |
| 537-8  | 69    | 65,000| 360-2   |

All pancreatic RNA samples were assayed for the presence of hGH mRNA by Northern blotting. Positive samples (>40 mRNA/cell) were quantitated by solution hybridization. Liver and kidney RNAs from most mice were also assayed by Northern blotting and found to have undetectable levels of hGH mRNA (for details, see Methods).

* (bd) Below detection, generally <40 mRNAs per cell.

The transcription of hGH mRNA detected in these mice (Table 1) must have been directed by the mutant enhancers.

The inactivity of mutants is not cell line specific

To test whether the differential effects of the mutations were a result of a peculiarity of 266-6 cells, another pancreatic acinar cell line, AR4-2J (derived from an aza-serine-induced pancreatic acinar cell carcinoma of a rat; Jessop and Hay 1980), was transfected with several EImCAT scanner mutants tested previously in 266-6 cells. Relative to expression of an RSV-mCAT control plasmid, expression of EImCAT is ~1000-fold greater in AR4-2J cells than in NIH-3T3 cells (data not shown), which affirms the cell-specific nature of the EI regulatory region in this second pancreatic cell line. The results with EI enhancer mutants in AR4-2J cells were congruent with those for 266-6 cells (Table 2). The mutants EImCAT-101, EImCAT-151, and EImCAT-181 were at least 100-fold reduced in CAT expression relative to the unaltered EImGAT in each cell line. The expression pattern of two other mutants was also retained; EImCAT-71 was reduced 10-fold in each, and EImCAT-131 had roughly wild-type activity in each pancreatic cell line. Therefore, the effects of EI enhancer mutants were identical in both pancreatic acinar cell lines, despite the different origins of the cell lines.

The discrepancy between cells and animals is not due to the reporter genes

The hGH reporter gene is inactive in transgenic animals (Behringer et al. 1988), but may contain cryptic regulatory elements (not present in the CAT reporter gene) capable of complementing the inactivated EI enhancer.
mutants [Russo et al. 1988]. To test whether the difference in results in animals versus cells was due to the difference in reporter genes, 266-6 cells were transfected transiently with each of the four constructs used previously in transgenic mice, EGH3, EGH3-101, EGH3-151, and EGH3-181, as well as EGH3-131, a mutation that previously had been found not to inactivate enhancer activity (Table 2), and EGH4, which has the entire enhancer region deleted. The level of hGH expression directed by each mutant enhancer (Table 3) closely paralleled the level of CAT expression directed by the same mutant enhancer (Table 2). Thus, the behavior of the mutant enhancer in the transfected acinar cell line is independent of the reporter gene.

The discrepancy is not due to the state of integration

Transgenic mice have the foreign gene integrated into their chromosomal DNA, whereas in transient transfections the foreign DNA is episomal. To test whether the integration status of the enhancer led to the different results between animal and cell experiments, the Escherichia coli xanthine–guanine phosphoribosyltransferase (gpt) reporter gene [Gorman et al. 1983] was fused to unaltered or mutant EI 5'–flanking regions (~205 to +8). After transfection of 266-6 and NIH-3T3 cells with the unaltered Elgpt and the three scanner mutants, Elgpt-101, Elgpt-151, and Elgpt-181, gpt expression was assayed by colony formation in the presence of mycophenolic acid. The number of mycophenolate-resistant colonies is a measure of transcriptional activity [Gillies et al. 1983; Berg and Anderson 1984]. Although Elgpt and RSVgpt transfected 266-6 cells with similar efficiencies (Table 4), Elgpt had a relative efficiency 70-fold less than RSVgpt for NIH-3T3 cells (transfection frequencies of $1.5 \times 10^{-6}$ versus $1 \times 10^{-4}$), confirming the cell-specific nature of the EI control sequences in this stable transfection assay. Integration of the transfected DNA was confirmed by Southern blot analysis of chromosomal and nonchromosomal DNA isolated from several individual 266-6 colonies arising from the Elgpt transfection, as well as from RSVgpt control transfection (data not shown). Expression of the chromosomally integrated mutant constructs was reduced dramatically, as measured by their ability to form mycophenolate-resistant colonies relative to the unaltered Elgpt (Table 4). Thus, the mutant enhancer constructs still are inactive when they are integrated into the chromosomes of pancreatic 266-6 cells.
Enhancer redundancy in mice

To test whether the EI enhancer domains have a function in animals, EGH fusion genes with a scanner mutation in each of two domains were tested in transgenic mice. All three double-mutation constructs, EGH3-101/151, EGH3-101/181, and EGH3-151/181 (Fig. 1), had drastically reduced or no pancreas-specific expression in transgenic mice (Table 5). Therefore, the same three enhancer domains essential in cells also function in animals, but this function is redundant in animals; two of the regions must be inactivated before a loss of activity is readily detectable.

To define the minimum enhancer region capable of directing pancreas-specific expression in mice, two enhancer mutants (EGH13, truncated at −150, and EGH14, truncated at −170) were tested (Fig. 1; Table 6). Both constructs had low but significant pancreatic expression of hGH mRNA. The EGH14 construct was expressed dramatically less than the EGH3-181 mutant, implying that a functional element is removed in addition to that disrupted by the −181 to −190 scanner mutant. The EGH13 construct gave results similar to those for the EGH3-151/181 double mutant, as might be expected if transcription-factor-binding sites disrupted by the −151 to −160 and −181 to −190 scanner mutations form the important domains of the enhancer distal to −150. Two of the EGH13 mice had high levels of hGH RNA (mice 515-4 and 523-2, Table 6). It seemed possible that these two mice, both with high transgene copy number, probably also each had at least one head-to-head joining of the transgenes and thereby created a functional enhancer by linking two A domains. However, when two transgenic EGH13 mice with high levels of hGH mRNA and two with low levels (515-4, 523-2, 445-2, and 513-4) were examined for the presence of head-to-head orientation of transgenes, such an arrangement was seen only in 445-2, one of the low-level-expressing mice (data not shown). Therefore, the high level of expression of hGH in mice was apparently not due to the formation of multimeric A domains.

**Table 2. Expression of enhancer scanner mutants in transiently transfected AR4-2J and 266-6 pancreatic acinar cells**

| Construct/mutant | Expression levels (CAT enzyme activity*) in | AR4-2J | 266-6 |
|------------------|--------------------------------------------|--------|------|
|                   | ElmCAT units (%)                         | 650 (100) | 21,000 |
| RSV-mCAT         | 96                                         | 220     |      |
| mCAT             | 0.7                                        | 0.7     | 1.1  |
| EImCAT-71        | 7                                          | 7.3     | 54   |
| EImCAT-101       | 0                                          | 4.1     | 0.6  |
| EImCAT-131       | 0.7                                        | 82      | 660  |
| EImCAT-151       | 1.1                                        | 1.1     | 2.5  |
| EImCAT-181       | 0                                          | 0       | 1.3  |

* CAT enzyme activity units are expressed as pmole/hr/mg.

**Table 3. Expression of hGH directed by enhancer scanner mutants in transiently transfected 266-6 cells**

| Construct/mutant | Relative activity* |
|------------------|-------------------|
| EGH3             | 100               |
| RSV-GH [1]b      | 6200              |
| EGH4* [2]        | 0.2               |
| EGH3-101 [3]     | 2.2               |
| EGH3-131 [1]     | 200               |
| EGH3-151 [2]     | 0.2               |
| EGH3-181 [1]     | 0.6               |

* Expressed relative to the percentage of hGH protein secreted into the medium by 266-6 cells transfected with EGH3. Levels of hGH protein were adjusted according to transfection efficiency differences assayed by cotransfection of either RSV-gpt or RSV-mCAT.

**Discussion**

The activity of modified EI enhancers is dramatically different in pancreatic tissue of animals than in either of two pancreatic acinar cell lines. In the pancreas any two of the three enhancer domains are sufficient for activity, whereas all three domains are required in both pancreatic 266-6 and AR4-2J cells. Thus, more regulatory sequence information is required for expression in the cultured cells than in animals. We have demonstrated that the difference is not due to correction of the mutations in the transgenes, a particular reporter gene, or the state of integration of the transgenes. Moreover, because both available pancreatic cell lines gave identical results despite their variant origins (a T-antigen-induced mouse tumor and a chemically induced rat tumor), the difference in enhancer function is not a result of the origin or the peculiar properties of a particular cell line. Therefore, it appears that the results are due to a more fundamental difference between animals and differentiated cells in culture.

One common property of the two acinar cell lines that distinguishes them from acinar cells in situ is reduced expression of the endogenous cell-specific genes, although the levels of a given gene product are quite variable between the two lines. For instance, 266-6 has 2% the normal pancreatic level of EI mRNA, 8% of trypsin mRNA, and <1% of amylase mRNA; AR4-2J has 4% the normal level of EI mRNA, 1% of trypsin mRNA, and 40% of amylase mRNA (Ornitz et al. 1985b; Boulet et al. 1986; G.H. Swift, B. Davis, and R.J. MacDonald, un-
Swift et al.

Table 4. Expression of enhancer scanner mutants in stably transfected pancreatic 266-6 cells

| Construct/mutant | Average number of colonies/plate | Transformation frequency | Relative efficiency |
|------------------|----------------------------------|--------------------------|---------------------|
| Elgpt            | 87                               | $1.2 \times 10^{-4}$    | 100                 |
| RSV-gpt          | 34                               | $4.9 \times 10^{-5}$    | 41                  |
| gpt<sup>b</sup>  | 0                                | $<1.4 \times 10^{-7}$  | <0.1                |
| Elgpt-101        | 0.1                              | $1.4 \times 10^{-7}$    | 0.1                 |
| Elgpt-151        | 0                                | $<1.4 \times 10^{-7}$  | <0.1                |
| Elgpt-181        | 3                                | $4.3 \times 10^{-6}$    | 2.8                 |

<sup>a</sup> Average of two transformations.

<sup>b</sup> An enhancerless and promoterless gpt gene.

A lowered level of tissue-specific mRNAs relative to that of their counterparts in intact tissue is a common, if not universal, attribute of cultured cell lines (Clayton and Darnell 1983, Reid and Jefferson 1984). The most extensive comparisons between cells in intact tissue and in culture have used hepatocytes; in culture, these cells have greatly reduced rates of transcription of liver-specific genes (Clayton et al. 1985, Fujita et al. 1987; Isom et al. 1987). Decreased transcription of cell-specific genes in cultured cells is potentially a serious problem when these cultured cells and extracts derived from them are used to dissect the mechanisms of cell-specific transcription. Partial dedifferentiation may cause (or be caused by) the absence of some factor whose function is to ensure maximal transcription of a tissue-specific gene. In hepatoma cell lines, dedifferentiation is associated with the loss of a factor, APF, required for transcription of the albumin gene (Cereghini et al. 1988).

As for the El enhancer, the activity of a given enhancer in less-differentiated cultured cells may fail to mirror the action of that enhancer in the corresponding cell type of an animal because of the absence or decreased level of a crucial transcription factor. In animals, the trans-acting factors in pancreatic acinar cells needed for the expression of the El enhancer are clearly in excess. This is demonstrated dramatically by the absence of competition by large numbers of gene copies in transgenic animals. For example, when mice bearing an hGH reporter gene directed by the El enhancer are crossed with mice bearing 250 copies of an identical El enhancer (as part of a rat El transgene), no diminution of hGH expression occurs in progeny also bearing the 250 additional El enhancers (Davis and MacDonald 1988). Therefore, if binding of trans-acting factors to only two of three enhancer domains A, B, and C (Fig. 1) is sufficient for enhancer activity, wild-type expression of a mutant missing one of the domains would be expected, because the remaining two domains would be fully occupied by the trans-acting factors. This wild-type expression is indeed seen in mice for the EGH3-151 and EGH3-181 mutants, which inactivate one of the three enhancer domains. The somewhat lower expression of the EGH3-101 mutant may be due to a relatively greater importance of domain A, which includes the pancreas consensus region (Fig. 1), and its binding factor for activating the transcription mechanism.

Table 5. Pancreatic expression of hGH mRNA for enhancer double-scanner mutants in transgenic mice

| Number of expressors | EGH3-101/-151 | EGH3-101/-181 | EGH3-151/-181 |
|----------------------|---------------|---------------|---------------|
| Gene copy number: range (avg) | 0 of 14       | 0 of 5        | 4 of 10       |
| Average mRNA per expressor (mRNAs/cell/gene copy) | 1–170 (22)   | 1–74 (20)    | 14–71 (36)   |
| mouse gene copy number | 20           | mRNAs/cell   |
| 493-4                | 71            | bd            |
| 490-6                | 50            | bd            |
| 486-5                | 36            | bd            |
| 494-7                | 25            | bd            |
| 498-4                | 22            | bd            |
| 482-7                | 14            | bd            |
| 494-1                | 35            | 40            |
| 497-2                | 37            | 60            |
| 493-3                | 20            | 700           |
| 490-5                | 55            | 1,000         |

<sup>a</sup> [bd] Below detection, generally <40 mRNAs per cell.
present for the enhancer to function, however, cannot be explained by a lower concentration of the factors binding to the domains. If, as in animals, binding of transcription factors present in limiting amounts to a minimum of two sites is sufficient for enhancer activity and three sites are present, then the enhancer activity seen is the sum of that expressed as a result of binding to each pair of sites. (This is true regardless of whether the binding of transcription factors to each site in the pair is independent or cooperative.) Inactivation of one site (as in each of the single mutants EGH3-101, EGH3-151 and EGH3-181) would eliminate two of the three possible functional pairs; the remaining pair would still be able to bind factors, and the level of expression would drop an average of 3-fold, not the drop of ≥100-fold observed. If the actual array of sites and factors binding to them is more complex, the expected drop would be greater, but still much <100-fold. For instance, if five sites are present, and binding to four is required for activity, then removal of one site would eliminate four of five possible functional combinations and expression would drop an average of fivefold. Only if each scanner mutant eliminates two or more independently acting binding sites of a complex array [e.g., binding to six sites is required and eight sites are normally present] would the drop in functional combinations approach the magnitude of the drop observed in expression levels of reporter genes directed by any of the three domain scanner mutants in transfected cells.

Therefore, it appears likely that the difference between the normal pancreas and the partially dedifferentiated pancreatic acinar cell lines 266-6 and AR4-2J is the absence of a transcriptional component of the normal pancreas in both cell lines rather than a reduction in amount of one or more of the trans-acting factors that bind domains A, B, and C. This missing component may be: [1] another DNA-binding transcription factor, [2] a transcription factor that interacts with other proteins without binding DNA, or [3] a modification of transcription factors. If model 1 is correct, then the EI enhancer would require binding by a minimum of three transcriptional activators, bringing them together in a manner that activates transcription. In animals, in addition to the transcriptional activators that bind EI enhancer domains A, B, and C, a fourth transcriptional activator that binds to a fourth domain (D) is present also. Therefore, the loss of any one of the A, B, or C domains would be compensated by the action of the fourth activator at its binding domain. This fourth activator is absent in 266-6 and AR4-2J cells; therefore, enhancer domains A, B, and C are all required for activity. Binding site D, of course, would not have been delineated by the scanner mutant analysis of the EI enhancer in 266-6 cells. If model 2 is correct, then the fourth activator may be a protein similar to the HeLa cell S300-II factor [Tsai et al. 1987] and the herpes simplex virus protein Vmw65 [O’Hare and Goding 1988; Preston et al. 1988], which do not interact directly with DNA but, rather, with protein bound to DNA. In this case, the interaction of this factor with the factors bound to any two EI enhancer domains would provide the third activator and be sufficient for transcription.

The presence of a modification system in animals but absent in the two pancreatic cell lines [model 3] is compatible with our results as well. The modification could be necessary either to activate the hypothetical fourth activator protein or to enhance the activity of the A, B, and C domain binding proteins. If the modification were directly to the A, B, and C domain binding proteins, it would have to alter their activity so that a pair of modified proteins would be sufficient to activate transcription, whereas all three would be needed if unmodified. A wide variety of noncovalent and covalent modifications to transcription factors have been observed. Binding of estrogen to the estrogen receptor is required for binding to an estrogen-responsive element and transcriptional activation [Kumar and Chambon 1988]. NF-κB, required for the activity of the κ light-chain gene enhancer in B

Table 6. Pancreatic expression of hGH mRNA for 5'-truncated enhancer mutants in transgenic mice

| Number of expressors | Average mRNA per expressor |
|----------------------|-----------------------------|
|                      | [mRNAs/cell/gene copy]      |
|                      |                             |
|                      | mouse | gene copy number | mRNAs/cell | mouse | gene copy number | mRNAs/cell |
| EGH13 (−150 truncation) | 100  |
|                       | 7 of 11 |                             |
|                      | 441-1  | 2 | bd* | 423-6  | 1 | bd |
|                      | 517-4  | 4 | bd | 426-4  | 1 | bd |
|                      | 520-5  | 5 | bd | 419-2  | 3 | bd |
|                      | 444-2  | 7 | bd | 417-5  | 3 | bd |
|                      | 522-8  | 71 | 90 | 426-7  | 2 | 140 |
|                      | 445-2  | 30 | 90 | 425-2  | 5 | 880 |
|                      | 513-4  | 65 | 130 | 414-5  | 2 | 1,000 |
|                      | 523-6  | 8 | 170 |                  |     |          |
|                      | 523-2  | 74 | 28,000 |                  |     |          |
| EGH14 (−170 truncation) | 300  |
|                       | 3 of 7 |                             |
|                      | 441-1  | 2 | bd* |
|                      | 517-4  | 4 | bd |
|                      | 520-5  | 5 | bd |
|                      | 444-2  | 7 | bd |
|                      | 522-8  | 71 | 90 |
|                      | 445-2  | 30 | 90 |
|                      | 513-4  | 65 | 130 |
|                      | 514-6  | 5 | 840 |
|                      | 514-6  | 5 | 840 |
|                      | 515-4  | 38 | 4,100 |
|                      | 523-2  | 74 | 28,000 |

* [bd] Below detection, generally <40 mRNAs per cell.
Swift et al.

cells [Atchison and Perry 1987], is present in an inactive form, apparently in complex with an inhibitor, in non-
lymphoid as well as pre-B cell lines [Sen and Baltimore 1986; Bauerle and Baltimore 1988]. One plasmacytoma
cell line has been found in which the active form of 
NF-κB is absent even after treatment that can induce the 
activation of NF-κB in pre-B cell lines [Atchison and 
Perry 1987]. Covalent modifications of transcription 
factors by both glycosylation [Jackson and Tjian 1988] 
and phosphorylation [Tienrungroj et al. 1987, Hoffeler et 
al. 1988; Sorger and Pelham 1988] also have been 
observed. Phosphorylation may increase the strength of ac-
tivation domains by increasing the net negative charge 
of those domains; indeed, phosphorylation of the yeast 
heat shock factor has been correlated with an increase in 
its transcriptional activity [Sorger and Pelham 1988].

Methods

Construction of plasmids

RSV-CAT [the Rous sarcoma virus [RSV] enhancer and pro-
moter linked to the bacterial CAT] and RSV-gpt (the RSV enh-
ancer and promoter linked to the E. coli gpt) were gifts of Cor-
nelia Gorman [Gorman et al. 1983]. RSV-GH (the RSV enhancer 
and promoter linked to the hGH gene) was constructed by 
fusing the enhancer and promoter on an Ndel–BamHI fragment 
of RSV-mCAT [Kruse et al. 1988] to the hGH gene at +3. To 
create EI enhancer–promoter fusions to reporter genes, a 213-
bp SalI–BamHI fragment (from nucleotides −205 to +8) con-
taining the enhancer, promoter, and mRNA start site of the 
EI gene was fused either to the hGH gene at +3 [EGH3, Hammer 
et al. 1987], to a modified CAT gene at +10 [ElmCAT; Kruse et 
al. 1988], or to a gpt gene contained within a BglII–BamHI frag-
ment of RSV-gpt [Elgpt]. Scanner mutants were prepared by oli-
gonucleotide-directed mutagenesis of the 213-bp SalI–BamHI 
fragment. The base sequence of each substitution was identical 
and consisted of overlapping recognition sites for EcoRV and 
XhoI [Kruse et al. 1988]. To create truncation mutants, substi-
tution mutants were cut with XhoI and BamHI and fused to the 
BamHI site at +3 of the hGH gene. The various scanner mu-
tant constructs are identified by appending the nucleotide 
number of the proximal base substituted onto the parent 
plasmid designation, i.e., EGH3-101, ElmCAT-101, Elgpt-101 
all contain a scanner mutant substitution at −101 to −110. 
Double-scanner mutants EGH3-101/151, EGH3-101/181, 
and EGH3-151-181 have two substitutions each at −101 to −110 
and −151 to 160, −101 to 110 and −181 to 190, and −151 to 
−160 and −181 to 190, respectively. Promoter and enhancer 
minus plasmids mCAT [Kruse et al. 1988] and gpt were used 
also. The gpt plasmid was created by replacing the BamHI– 
BamHI fragment of mCAT with the BglII–BamHI fragments of 
RSV-gpt. Cloning vectors were pUC13 for the hGH fusion con-
structs and pUC19 for the mCAT and gpt constructs.

Production and identification of transgenic mice

A few hundred molecules of plasmid-free fusion gene DNA (a 
HindIII–EcoRI fragment) were microinjected into the male 
pronucleus of F2 hybrid mouse eggs (obtained by mating 
C57Bl/6 × SJL hybrid adults), as described by Brinster et al. 
(1985). The eggs were reimplanted into pseudopregnant mice 
and allowed to develop to term. Quantitative dot-blot assays of 
transgene copy number were performed as described by Brinster 
et al. [1985], using 5 μg of tail DNA and hybridizing with a 
32P-labeled hGH probe comprising 2 kb of the hGH gene. Copy 
number is the average copy number per cell. As mice were not 
bred, mosaic mice were not identified. Thus, some mice actu-
ally have a greater copy number than reported in only a fraction 
of their cells. All activities involving mice were reviewed and 
approved by our institutional committee in accordance with 
current National Institutes of Health policy.

Cell culture and DNA transfection

The measurement of CAT activity was performed as described 
previously [Kruse et al. 1988] for 266-6, NIH-3T3, and AR4-2J 
cells. The expression of hGH fusion genes was measured by 
hGH accumulation in the culture medium [Selden et al. 1986], 
using a radioimmunoassay [Nichols Institute, San Juan Capis-
trano, California].

Mycoplasma-resistant colonies resulting from stable 
transfections of 266-6 cells and NIH-3T3 cells with gpt gene 
constructs were isolated in the following manner: Cell monol-
ayers of 266-6 cells or NIH-3T3 cells were transfected with ei-
ther RSV-gpt, Elgpt, Elgpt scanner mutants, or the promoterless 
gpt construct. After 48 hr, the transfected cells were treated 
with trypsin-EDTA, and 7 × 10⁵ 266-6 cells or 2 × 10⁶ 
NIH-3T3 cells were dispersed on 100-mm dishes in Dulbecco’s 
modified Eagle’s medium containing 10% fetal calf serum, 250 
μg/ml xanthine, 13.6 μg/ml hypoxanthine, and 2.5 μg/ml my-
cophenolic acid. Every 4 days, the culture medium was replaced 
with fresh medium containing the same supplements. After 11 
days for 266-6 cells or 13 days for NIH-3T3 cells, drug-resistant 
colonies were fixed in methanol, stained in 0.5% crystal violet, 
and counted. Transformation frequency was expressed as the 
fraction of cells plated that grew under the selective conditions.

Hybridization analysis of hGH RNA

Tissue RNA was isolated by the guanidine thiocyanate proce-
dure [Chirgwin et al. 1979; MacDonald et al. 1987]. Northern 
blot analysis of RNAs was performed essentially as described 
previously [Swift et al. 1984a]. Each Northern blot contained a 
standard panel of hGH mRNA from mouse 169-5 [Hammer et 
al. 1987] with amounts of hGH RNA per lane ranging from 
equivalent to 40-40,000 mRNA molecules per pancreatic cell 
in a 5-μg sample of total RNA. Pancreatic RNA samples from 
transgenic mice that gave a detectable hGH RNA band at 950 
nucleotides [indicating at least ~40 mRNAs per cell] were ana-
lyzed more quantitatively by solution hybridization. In addi-
tion to analyzing pancreatic RNA for all animals in this study, 
Northern blot analysis also was performed on liver and kidney 
for all [constructs EGH3, EGH3-151, EGH3-181, EGH3-101/
181] or a majority of [construct EGH3-101, EGH3-151/181, 
EGH3, EGH14] of animals of each transgenic construct [ex-
cpt for EGH3-101/151, where kidney and liver RNA of 6 of 14 
mice were analyzed]. No hGH mRNA band was visible for any 
liver or kidney sample with a limit of detection of ~20 hGH 
mRNAs per cell for liver and ~5 hGH mRNA per cell for kidney 
[varying limits of detection for pancreas, liver, and 
kidney are due to the varying amounts of RNA per cell in these 
three tissues [Swift et al. 1984a]]. Solution hybridization was 
performed as described previously [Hammer et al. 1987]. The 
probe was a 24-nucleotide oligonucleotide complementary to 
exon 2, end-labeled to a specific activity of ~3000 Ci/mmole, 
and purified by cetylpyridinium bromide precipitation [Geck 
and Nasz 1983].
Enhancer redundancy in mice

Acknowledgments

We acknowledge the excellent technical assistance of Shanna Maika and Walter Ehrman. We thank Dr. Veronica Blasquez for advice on stable transfection experiments and Helen Aronovich for synthesizing oligonucleotides. The research was supported by American Cancer grant CD-354 and National Institutes of Health grants AM-27430 and GM-31689.

References

Atchison, M.L. and R.P. Perry. 1987. The role of the \( \kappa \) enhancer and its binding factor NF-\( \kappa \)b in the developmental regulation of \( \kappa \) gene transcription. *Cell* 48: 121–128.

Baerue, P.A. and D. Baltimore. 1988. Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-\( \kappa \)b transcription factor. *Cell* 53: 211–217.

Behringer, R.R., L.S. Mathews, R.D. Palmiter, and R.L. Brinster. 1986. Dwarf mice produced by genetic ablation of growth hormone-expressing cells. *Genes Dev.* 2: 453–461.

Berg, P.E. and W.F. Anderson. 1984. Correlation of gene expression and transformation frequency with the presence of an enhancing sequence in the transforming DNA. *Mol. Cell. Biol.* 4: 368–370.

Boulet, A.M., C.R. Erwin, and W.J. Rutter. 1986. Cell-specific enhancers in the rat exocrine pancreas. *Proc. Natl. Acad. Sci.* 83: 3599–3603.

Brinster, R.L., H.Y. Chen, M. Trumbauer, A.W. Senear, R. Warren, and R.D. Palmiter. 1981. Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. *Cell* 27: 223–231.

Brinster, R.L., H.Y. Chen, M.E. Trumbauer, M.K. Yagle, and R.D. Palmiter. 1985. Factors affecting the efficiency of introducing foreign DNA into mice by microinjection of eggs. *Proc. Natl. Acad. Sci.* 82: 4438–4442.

Cereghini, S., M. Blumenfeld, and M. Yaniv. 1988. A liver-specific factor essential for albumin transcription differs between differentiated and dedifferentiated rat hepatoma cells. *Genes Dev.* 2: 957–974.

Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonuclease acid from sources enriched in ribonuclease. *Biochemistry* 24: 5294–5299.

Clayton, D.F. and J.E. Darnell. 1983. Changes in liver-specific compared to common gene transcription during primary culture of mouse hepatocytes. *Mol. Cell. Biol.* 3: 1552–1561.

Clayton, D.F., M. Weiss, and J.E. Darnell. 1985. Liver-specific RNA metabolism in hepatoma cells: Variations in transcription rates and mRNA levels. *Mol. Cell. Biol.* 5: 2633–2641.

Darnell, J.E. 1982. Variety in the level of gene control in eukaryotic cells. *Nature* 297: 365–371.

Davis, B.P. and R.J. MacDonald. 1988. Limited transcription of rat elastase I transgene repeats in transgenic mice. *Genes Dev.* 2: 13–22.

Derman, E., K. Krauter, L. Walling, C. Weinberger, M. Ray, and J.E. Darnell. 1981. Transcriptional control in the production of liver-specific mRNAs. *Cell* 23: 731–739.

Fujita, M., D.C. Spray, H. Choi, J.C. Saez, T. Watanabe, L.C. Rosenberg, E.L. Hertzberg, and L.M. Reid. 1987. Glycosaminoglycans and proteoglycans induce gap junction expression and restore transcription of tissue-specific mRNAs in primary liver cultures. *Hepatology* 7: 15–95.

Gack, P. and I. Nasz. 1983. Concentrated, digestible DNA after hydroxylapatite chromatography with cetylpyridinium bromide precipitation. *Anal. Biochem.* 135: 264–268.

Gillies, S.D., S.L. Morrison, V.T. Oi, and S. Tonegawa. 1983. A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* 33: 717–728.

Gorman, C.M., L. Moffat, and B. Howard. 1983. High efficiency DNA-mediated transformation of primate cells. *Science* 221: 551–553.

Hammer, R.E., G.H. Swift, D.M. Ornitz, C.J. Quaife, R.D. Palmiter, R.L. Brinster, and R.J. MacDonald. 1987. The rat elastase I regulatory element is an enhancer that directs correct cell-specificity and developmental onset of expression in transgenic mice. *Mol. Cell. Biol.* 7: 2956–2967.

Hoeffler, W.K., R. Kovelman, and R.C. Roeder. 1988. Activation of transcription factor IIIC by the adenovirus E1A protein. *Cell* 53: 907–920.

Isom, H. I. Georgoff, M. Sallidt-Goergieff and J.E. Darnell. 1987. Persistence of liver-specific messenger RNA in cultured hepatocytes: Different regulatory events for different genes. *J. Cell Biol.* 105: 2877–2885.

Jackson, S.P. and R. Tjian. 1988. O-glycosylation of eukaryotic transcription factors: Implications for mechanisms of transcriptional regulation. *Cell* 55: 125–133.

Jessop, N.W. and R.J. Hay. 1980. Characteristics of two rat pancreatic exocrine cell lines derived from transplantable tumors. *In Vitro* 16: 212.

Kiledjian, M., Li-Kuo Su, and T. Kadesch. 1988. Identification and characterization of two functional domains within the murine heavy-chain enhancer. *Mol. Cell. Biol.* 8: 145–152.

Kruse, F., C.T. Komro, C.J. Michnoff, and R.J. MacDonald. 1988. The cell-specific elastase I enhancer comprises two domains. *Mol. Cell. Biol.* 8: 893–902.

Kumar, V. and P. Chambon. 1988. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. *Cell* 55: 145–156.

MacDonald, R.J. 1987. Expression of the pancreatic elastase I gene in transgenic mice. *Hepatology* 7: 425–515.

MacDonald, R.J, R.E. Hammer, G.H. Swift, B.P. Davis, and R.L. Brinster. 1986. Transgenic progeny inherit tissue-specific expression of rat elastase I genes. *DNA* 5: 393–401.

MacDonald, R.J., G.H. Swift, A.E. Przybyla, and J.M. Chirgwin. 1987. Isolation of RNA using guanidinium salts. *Methods Enzymol.* 152: 219–227.

O’Hare, P. and C.R. Goding. 1988. Herpes simplex virus regulatory elements and the immunoglobulin octamer domain bind a common factor and are both targets for virion transactivation. *Cell* 52: 435–445.

Ondek, B., L. Gloss, and W. Herr. 1988. The SV40 enhancer contains two distinct levels of organization. *Nature* 333: 40–45.

Ornitz, D.M., R.D. Palmiter, R.E. Hammer, G.H. Swift, and R.J. MacDonald. 1985a. Specific expression of an elastase-human growth hormone fusion gene in pancreatic acinar cells of transgenic mice. *Nature* 313: 600–613.

Ornitz, D.M., R.D. Palmiter, A. Messing, R.E. Hammer, C.A. Pinkert, and R.L. Brinster. 1985b. Elastase I promoter directs expression of human growth hormone and SV40 T antigen genes to pancreatic acinar cells in transgenic mice. *Cold Spring Harbor Symp. Quant. Biol.* 50: 399–409.

Palmiter, R.D. and R.L. Brinster. 1986. Germ-line transformation of mice. *Annu. Rev. Genet.* 20: 465–499.

Palmiter, R.D., T.M. Wilkie, H.Y. Chen, and R.L. Brinster. 1984. Transmission distortion and mosaicism in an unusual transgenic mouse pedigree. *Cell* 36: 869–877.

Preston, C.M., M.C. Frame., and M.E.M. Campbell. 1988. A complex formed between cell components and an HSV structural polypeptide binds to a viral immediate early gene regulatory DNA sequence. *Cell* 52: 425–434.
Swift et al.

Reid, L.M. and D.M. Jefferson. 1984. Culturing hepatocytes and other differentiated cells. Hepatology 4: 548–559.

Russo, A.F., E.B. Crenshaw, S.A. Lira, D.M. Simmons, L.W. Swanson, and M.G. Rosenfeld. 1988. Neuronal expression of chimeric genes in transgenic mice. Neuron 1: 311–320.

Rutter, W.J., J.D. Kemp, W.S. Bradshaw, W.R. Clark, R.A. Ronzio, and T.G. Sanders. 1968. Regulation of specific protein synthesis in cytodifferentiation. J. Cell Physiol. 72: Suppl. 1, 1–18.

Schaffner, G., S. Schirm, B. Muller-Baden, F. Weber, and W. Schaffner. 1988. Redundancy of information in enhancers as a principle of mammalian transcription control. J. Mol. Biol. 201: 81–90.

Schick, J., H. Kern, and G. Scheele. 1984a. Hormonal stimulation in the exocrine pancreas results in coordinate and anti-coordinate regulation of protein synthesis. J. Cell Biol. 99: 1569–1574.

Schick, J., R. Verspohl, H. Kern, and G. Scheele. 1984b. Two distinct adaptive responses in the synthesis of exocrine pancreatic enzymes to inverse changes in protein and carbohydrate in the diet. Am. J. Physiol. 247: 611–616.

Selden, R.F., H.K. Burke, M.E. Rowe, H.M. Goodman, and D.D. Moore. 1986. Human growth hormone as a reporter gene in regulation studies employing transient gene expression. Mol. Cell. Biol. 6: 3173–3179.

Sen, R. and D. Baltimore. 1986. Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. Cell 47: 921–928.

Sorger, P.K. and H.R.B. Pelham. 1988. Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell 54: 855–864.

Swift, G.H., R.D. Hammer, R.J. MacDonald, and R.L. Brinster. 1984a. Tissue-specific expression of the rat pancreatic elastase I gene in transgenic mice. Cell 38: 639–646.

Swift, G.H., C.S. Craik, S.J. Stary, C. Quinto, R.G. Lahaie, W.J. Rutter, and R.J. MacDonald. 1984b. Structure of the two related elastase genes expressed in the rat pancreas. J. Biol. Chem. 259: 14271–14278.

Tienrungroj, W., E.R. Sanchez, P.R. Houseley, R.W. Harrison, and W.B. Pratt. 1987. Glucocorticoid receptor phosphorylation, transformation, and DNA binding. J. Biol. Chem. 262: 17342–17349.

Tsai, S.Y., I. Sagami, H. Wang, M.-J. Tsai, and B.W. O’Malley. 1987. Interactions between a DNA-binding transcription factor (COUP) and a non-DNA binding factor (S300-II). Cell 50: 701–709.
Differential requirements for cell-specific elastase I enhancer domains in transfected cells and transgenic mice.

G H Swift, F Kruse, R J MacDonald, et al.

*Genes Dev.* 1989, 3:
Access the most recent version at doi:10.1101/gad.3.5.687

References

This article cites 49 articles, 17 of which can be accessed free at: http://genesdev.cshlp.org/content/3/5/687.full.html#ref-list-1

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.