Int. J. Dev. Biol. 40: 1097-1108 (1996)

Original Article

Expression of *c-ETS-1* and *uPA* genes is associated with mammary epithelial cell tubulogenesis or neoplastic scattering

ALBINE DELANNOY-COURDENT[1], WILLIAM FAUQUETTE[1], XUE FEN DONG-LE BOURHIS[1], BÉNONI BOILLY[1], BERNARD VANDENBUNDER[2] and XAVIER DESBIENS[1]*

[1]*Centre de Biologie Cellulaire, Unité Dynamique des Cellules Embryonnaires et Cancéreuses, Bâtiment SN 3, Université des Sciences et Technologies de Lille I, Villeneuve d'Ascq and* [2]*CNRS URA 1160, Institut Pasteur, Lille, France*

ABSTRACT Although the inductive interactions which trigger epithelial morphogenesis have been extensively described, little is known about the transcription factors involved in these processes. During mammary gland morphogenesis, we report the expression of the transcription factor *c-ets-1* and one of its target genes *uPA* in mesenchymal cells during early stages of epithelial invasion, and later in epithelial cells themselves. *In vitro* studies show that both *c-ets-1* and *uPA* mRNAs can be induced in cultured normal mammary epithelial cells in response to medium conditioned by MRC-5 fibroblasts. In contrast, invasive tumorigenic cell lines from the mammary epithelium express constitutively *c-ets-1* and *uPA* while non-invasive tumorigenic cells do not. In three dimensional co-cultures in collagen gels, a preferential expression of these genes is detected in epithelial cells migrating through the gel either at the tips of normal ducts or in cancerous cells which are scattering. These genes are also expressed in the neighboring fibroblasts. In MRC-5 fibroblasts, conditioned media from tumorigenic epithelial cells induce more efficiently *c-ets-1* and *uPA* mRNA accumulation than do conditioned medium from normal cells. These results suggest that epithelial-mesenchymal interactions trigger *c-ets-1* and *uPA* expression in both compartments during mammary gland morphogenesis. The expression of the genes correlates with invasiveness of epithelial cells irrespective of their being normal or cancerous.

KEY WORDS: *mammary gland, tubulogenesis, metastasis, c-ets-1, uPA, interactions*

Introduction

Organs are complex structures composed of numerous types of tissues. The precise arrangement of tissues in these organs results from proximate interactions which direct changes in gene expression, cell shape and migration. Several transcription factors have been shown to be involved in the control of gene expression during these interactions.

Expression of the proto-oncogene *c-ets-1* has been detected when organogenesis takes place (reviewed in Vandenbunder *et al.*, 1995). This proto-oncogene is the cellular progenitor of the viral oncogene *v-ets* originally identified in the avian leukemia retrovirus E 26 (Leprince *et al.*, 1983; Nunn *et al.*, 1983). It encodes a transcription factor (Bosselut *et al.*, 1990; Gunther *et al.*, 1990; Ho *et al.*, 1990; Wasylyk *et al.*, 1990) which recognizes specific nucleotide sequences with a GGAA/T core sequence. *c-ets-1* transcripts have been detected as early as during gastrulation in the mesodermal layer of the embryo. Throughout the embryonic development, *c-ets-1* was shown to be expressed in situations involving cell movement. For example, *c-ets-1* transcripts have been detected in endothelial cells during the formation of new blood vessels (Vandenbunder *et al.*, 1989; Pardanaud and Dieterlen-Lièvre, 1993). *c-ets-1* transcripts are also abundant in mesenchymal cells adjacent to epithelial structures when inductive interactions occur (Quéva *et al.*, 1993) before the formation of cutaneous structures in dermis, limb bud, or during branching morphogenesis in kidney. In contrast, *c-ets-1* transcripts are absent in epithelia of the embryo whether they are derived from endoderm, mesoderm or ectoderm. The expression pattern of *c-ets-1* during pathological or normal development shares the same features, with *c-ets-1*

Abbreviations used in this paper: *UPA*, urokinase type plasminogen activator; *tPA*, tissue type plasminogen activator; PAI, plasminogen activator inhibitor; TIMP, tissue inhibitor of metalloproteinase; MMP9, 92 kDa collagenase IV; ECM, extracellular matrix; TNFα; transforming growth factor α; bFGF, basic fibroblast growth factor (type 2); HGF/SF, hepatocyte growth factor/scatter factor.

***Address for reprints:** Centre de Biologie Cellulaire, Unité Dynamique des Cellules Embryonnaires et Cancéreuses, Bâtiment SN 3, Université des Sciences et Technologies de Lille I, 59655 Villeneuve d'Ascq Cédex, France. FAX: 232434038. e-mail: desbiens@pop.univ-lille1.fr

0214-6282/96/$03.00

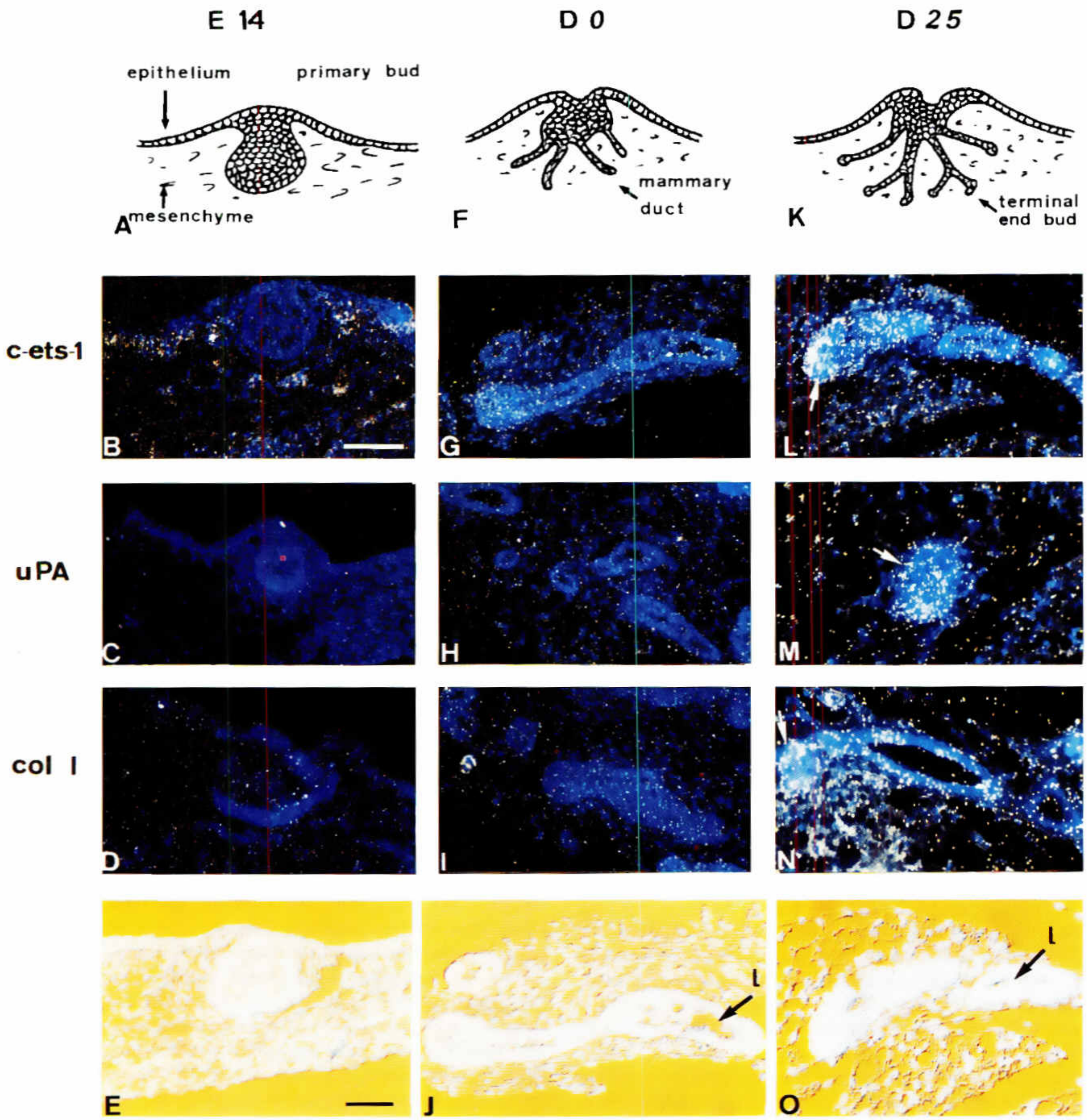


Fig. 1. *c-ets-1, uPA* and collagenase I (col I) expression during mouse mammary gland development. *At 14 days of embryonic development, i.e. E 14,* **(A-E)** *the mammary gland is constituted of an epithelial primary bud which invaginates into the subjacent mesenchyme. At birth, D0* **(F-J),** *the first epithelial mammary ducts are formed. At the onset of puberty, D25* **(K-O)** *the mammary gland shows growing and branching ducts with the first terminal end buds at their extremities. In situ hybridization performed on mouse mammary gland sections at E14 indicates that the primary buds are not labeled by either of the three probes* **(B,C,D)**. *The c-ets-1 signal initially clearly detected in the mesenchyme* **(B)** *decreases in this tissue and appears in the mammary epithelial ducts at birth* **(G)**. *c-ets-1 expression is particularly intense in the terminal end buds at D25* (**L**, arrow). *Both uPA and collagenase I expressions are detected at puberty* **(M,N)** *and serial sections performed on adjacent mammary ducts show a major expression in the terminal part of the ducts (arrows). E,J,O are enlarged Nomarski views of B,G,L respectively (l, lumen of the ducts). Bar, 35 μm.*

transcripts in the endothelium during tumor angiogenesis and in stromal fibroblasts surrounding invasive tumor formation (Wernert *et al.*, 1992). Using *in vitro* transactivation assays, several genes encoding proteinase precursors were shown to be Ets target genes. This is true for genes encoding pro-stromelysin 1 (Wasylyk *et al.*, 1991), the precursor of stromelysin 1 which degrades extracellular matrix (ECM) components (proteoglycans, laminin and fibronectin), pro-collagenase I (Gutman and Wasylyk, 1990),

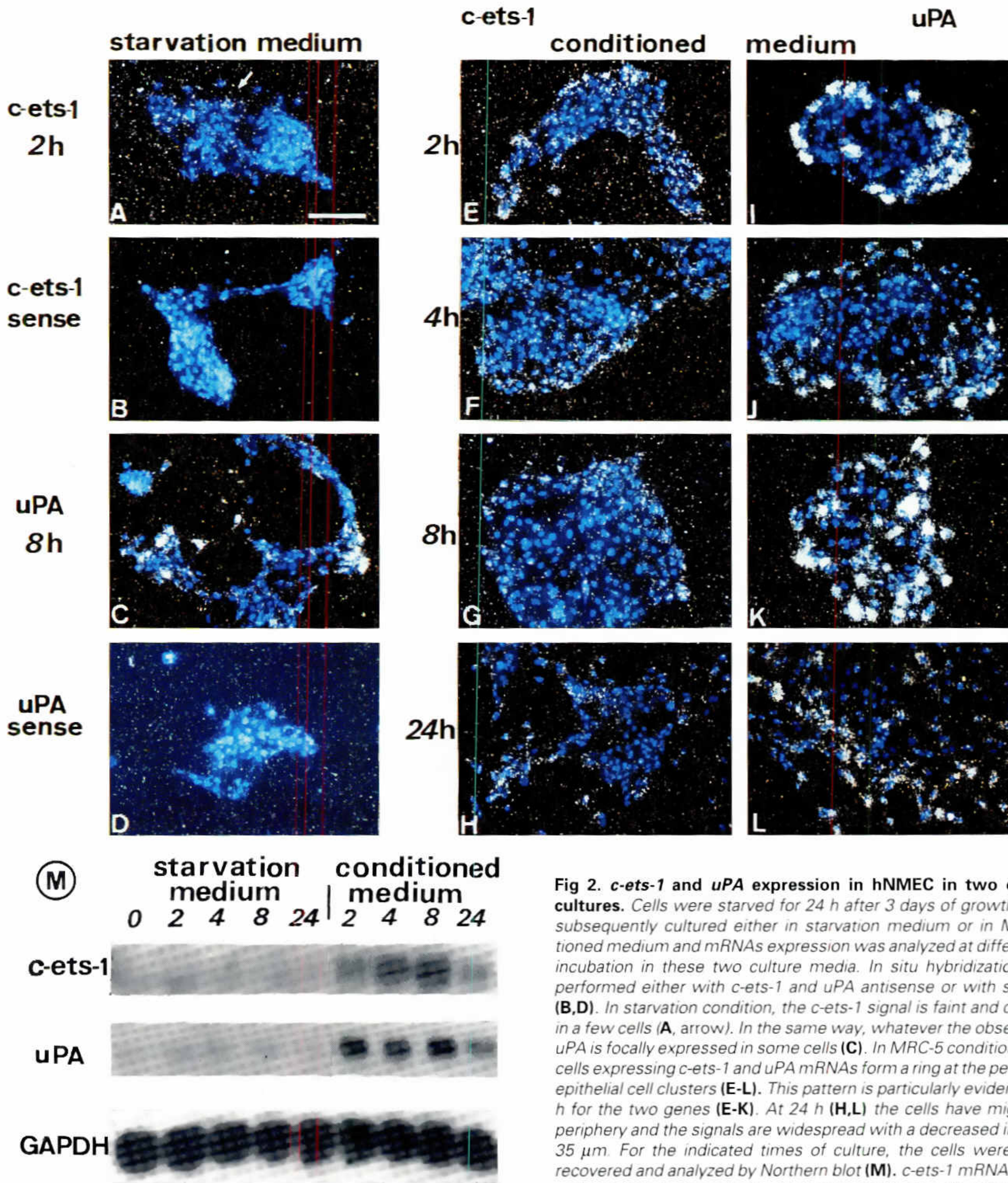
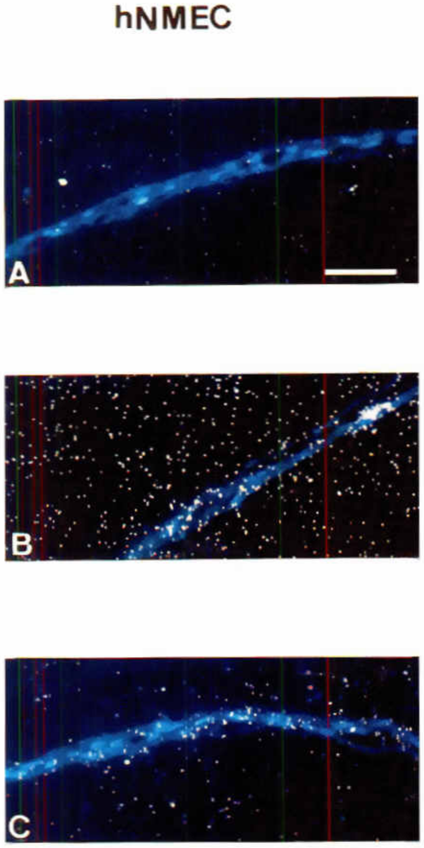


Fig 2. *c-ets-1* and *uPA* expression in hNMEC in two dimensional cultures. *Cells were starved for 24 h after 3 days of growth. They were subsequently cultured either in starvation medium or in MRC-5 conditioned medium and mRNAs expression was analyzed at different times of incubation in these two culture media. In situ hybridization* **(A-L)** *was performed either with c-ets-1 and uPA antisense or with sense probes* **(B,D)**. *In starvation condition, the c-ets-1 signal is faint and detected only in a few cells* (**A**, *arrow). In the same way, whatever the observation time, uPA is focally expressed in some cells* **(C)**. *In MRC-5 conditioned medium, cells expressing c-ets-1 and uPA mRNAs form a ring at the periphery of the epithelial cell clusters* **(E-L)**. *This pattern is particularly evident from 2 to 8 h for the two genes* **(E-K)**. *At 24 h* **(H,L)** *the cells have migrated to the periphery and the signals are widespread with a decreased intensity. Bar, 35 μm. For the indicated times of culture, the cells were lysed, RNA recovered and analyzed by Northern blot* **(M)**. *c-ets-1 mRNA level is quite undetectable in starved cells. In these conditions, uPA mRNA expression exhibits a minimum level by 0 to 24 h. In MRC-5 conditioned medium the level of c-ets-1 expression increases as soon as 2 h and reaches its maximum at 4 to 8 h while uPA mRNA levels also increase as early as 2 h after the beginning of stimulation and peak at 8 h. A GAPDH probe was used as control for equal loading.*

the precursor of collagenase I responsible for cleavage of the collagen triple helix, and pro-urokinase type plasminogen activator, pro-uPA, (Rorth *et al.*, 1990) the precursor of uPA which converts plasminogen into plasmin after fixation to its receptor and activation (Stephens *et al.*, 1989). Plasmin is able not only to degrade ECM components but also to activate pro-collagenase and pro-stromelysin (He *et al.*, 1989).

It is well established that proteinases are involved in cellular movement processes of morphogenesis. During organogenesis, proteinases would allow the correct development of embryonic



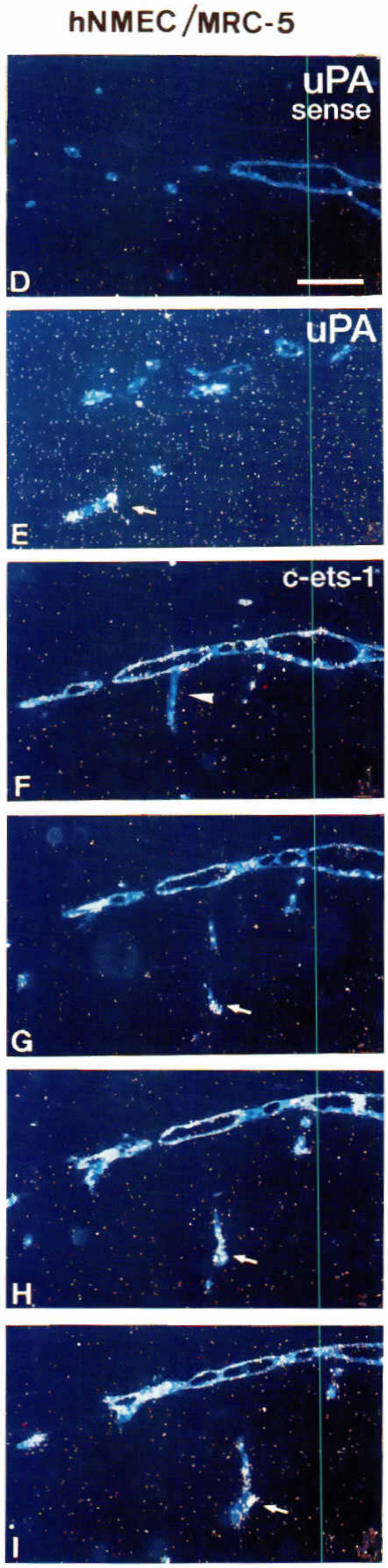
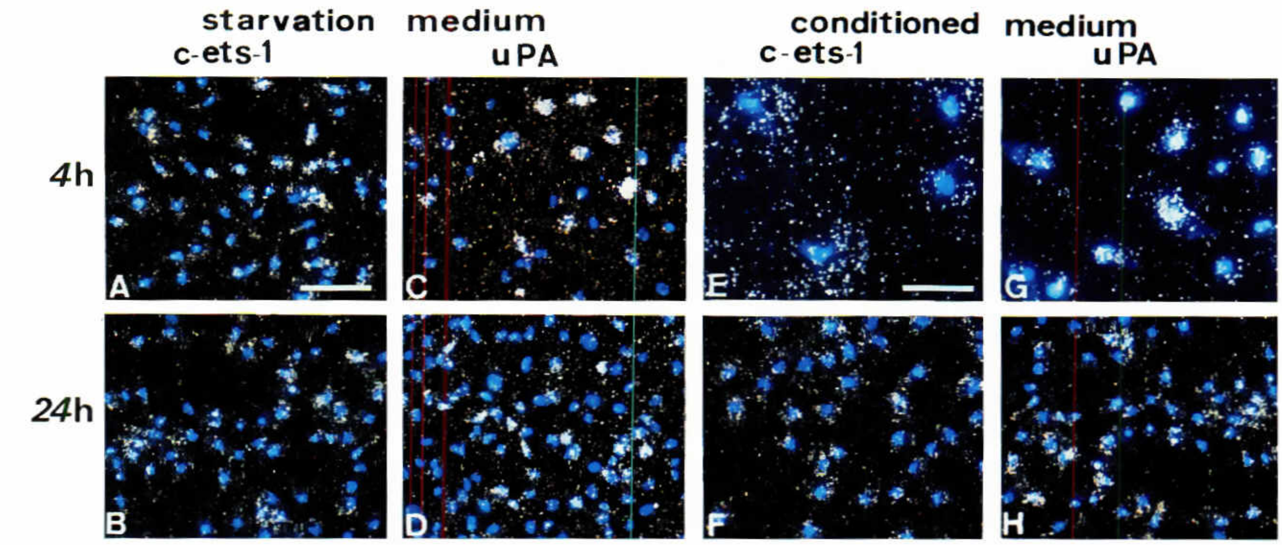


Fig. 3. *c-ets-1* and *uPA* expressions in hNMEC in three dimensional cultures. *The cells were either cultured alone or cocultured with MRC-5 fibroblasts in a collagen gel during 7 days. ISH of serial sections through these gels showed that cultured alone* **(A-C)**, *hNMEC form a sheet where c-ets-1* **(C)** *and uPA* **(B)** *are poorly expressed. In coculture* **(D-I)**, *a network of ducts is constituted.* **(F-I)** *Views corresponding to 4 adjacent sections, showing different parts of the same duct from its basis* (**F**, arrowhead) *to the terminal end bud* (**I**, arrow). *The c-ets-1* **(F-I)** *and uPA* **(E)** *signals (arrows) are both located in the extremities of the ducts penetrating the gel.* **(A, D)** *Control sections hybridized with uPA sense probe. Bar: A-C, 35 μm; D-I, 70 μm.*

tissues by monitoring the amount of ECM components. For example, stromelysin 1, by degrading basement membrane, allows branching of epithelial ducts during mammary gland morphogenesis (Sympson *et al.*, 1994). The urokinase-type plasminogen activator, *uPA* is expressed in the maternal vessel walls during uterine neo-vascularization (Grévin *et al.*, 1993). On the other hand, cancer cells in invasive carcinomas induce neighboring stromal cells to express proteinases such as *uPA* (Pyke *et al.*, 1991), collagenase I (Polette *et al.*, 1993) as well as the 72 kDa collagenase IV (Ballin *et al.*, 1991) and stromelysin 3 (Basset *et al.*, 1990). Moreover, in both normal and cancerous rodent mammary glands, Ossowski *et al.* (1979) showed the association of plasminogen activator production with tissue remodeling. It has been suggested that the degradation of ECM components by one or combinations of these proteinases facilitates either epithelial or endothelial invasion through the surrounding stroma.

In endothelial cells and in mesenchymal tissues of the uterus colonized by aggressive trophoblastic cells during implantation of the embryo (Grévin *et al.*, 1993) as well as in stromal fibroblasts adjacent to invasive neoplastic cells (Wernert *et al.*, 1992, 1994), the expression of *c-ets-1* correlated with the accumulation of *uPA* and/or collagenase and stromelysin transcripts. In contrast, in embryonic tissues during morphogenesis, in endothelial cells during angiogenesis or in mesenchymal cells expressing *c-ets-1*, neither *uPA* nor collagenase I transcripts were detected. The fact that *uPA* transcripts were not detected in the early stages of development agree with the proposal of Carmeliet *et al.* (1994) that *uPA* is not required for normal embryonic development.

In the present studies, we focused our attention on the mammary gland whose organogenesis takes place in the embryo and resumes during puberty. Previous results from Heuberger *et al.* (1982) using tissue recombinations have elegantly demonstrated the primordial role of epithelial-mesenchymal interactions in this development. In order to characterize the migration processes induced by these interactions, we set up *in vitro* reconstitution models in which normal or cancerous mammary epithelial cells interact or not with fibroblasts. Our results show for the first time, that epithelial mammary cells are able to express both *c-ets-1* and *uPA*. They also demonstrate that the expression of these genes is associated either with organized tubulogenesis by normal epithelial cells or with unregulated invasive processes by cancerous cells. These expressions would lead to a modification of epithelial organization and allow mesenchymal ECM to be degraded.



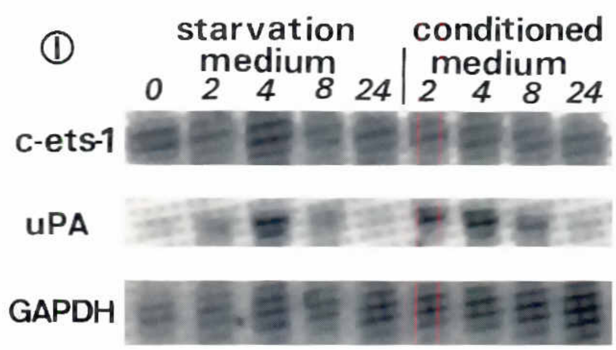


Fig 4. *c-ets-1* and *uPA* expressions in MDA-MB-231 in two dimensional cultures. *Cells which expressed c-ets-1 or uPA are distributed in the whole seeded region either in starvation or in MRC-5 conditioned medium and at any times of analysis. However, c-ets-1 expressing cells* **(A,B,E,F)** *appear more numerous than uPA expressing cells* **(C,D,G,H)**. *Views* **E** *and* **G** *allow us to observe the distinct aspect of the two signals. While the c-ets-1 signal is located in the whole cell, uPA signal appears more focally localized just near the nucleus. Moreover, the uPA signal decreases in 24 h. cultured cells (compare* **C** *and* **D**). *Bars: A to D,F,H, 35 μm; E and G, 17.5 μm. Northern blots* **(I)** *point out that the time courses of expression, for each of the two genes, are the same either in starvation or in MRC-5 conditioned medium.*

Results

c-ets-1 and proteinases are expressed in vivo during morphogenesis of mouse mammary gland

Sections of mouse mammary glands at different stages of development were hybridized with murine *c-ets-1*, *uPA* and collagenase I probes. At E14, the primary epithelial buds were not labeled by the *c-ets-1* probe whereas a signal was detected in the surrounding mesenchyme (Fig. 1B). Collagenase I and *uPA* transcripts were not detected, either in epithelial or in mesenchymal cells (Fig. 1C,D). At D0, the *c-ets-1* signal decreased in the mesenchyme and clearly appeared in epithelial mammary ducts (Fig. 1G). Again, proteinase RNAs were not detected (Fig. 1H,I). At D25, the onset of puberty, (and D35, data not shown), epithelial cells in the tips of growing ducts expressed *c-ets-1* as well as *uPA* and collagenase I transcripts (Fig. 1L,M,N). The ISH signals were higher in these extremities than in epithelial cells forming the more proximal collecting tubules. For each probe, focal signals were detected in the loose mesenchyme.

Thus, during mammary gland morphogenesis, *c-ets-1* was strongly expressed in the mesenchymal component at the onset of primary epithelial bud formation. Later, *c-ets-1* expression was detected in the epithelial component where it correlated with proteinase expression, at the tips of the ducts. The features of these expression patterns prompted us to investigate whether diffusible signals released by either compartment could mediate the induction of *c-ets-1* and *uPA* expressions.

c-ets-1 and uPA expressions are inducible in normal mammary epithelial cells (hNMEC)

In a first set of experiments, we tested the effects of conditioned medium by MRC-5 fibroblasts on hNMEC in two dimensional cultures. Northern blot analysis (Fig. 2M) revealed the presence of low levels of *c-ets-1* and *uPA* mRNAs in starvation conditions for each time. As early as 2 hours after stimulation by MRC-5 conditioned medium, *c-ets-1* mRNAs levels increased and reached a maximum from 4 to 8 h. In the same conditions, *uPA* mRNA levels increased from 2 to 8 h. In two dimensional cultures, hNMEC grew as clusters that dissociated upon incubation with MRC-5 conditioned medium. Interestingly, ISH analysis revealed that *c-ets-1* and *uPA* signals were localized at the periphery of the clusters formed by hNMEC. Cultured in starvation conditions, very few peripheral cells were weakly labeled by the *c-ets-1* riboprobe for each time of the experiment (Fig. 2A). In the same way, *uPA* was focally expressed in a few peripheral cells (Fig. 2C). In MRC-5 conditioned medium, from 2 to 8 h after stimulation, both the number of peripheral cells expressing *c-ets-1* and *uPA* transcripts, and the signal intensity, increased (Fig. 2E to G, I to K). After 24 h of treatment, *c-ets-1* and *uPA* transcripts were detected in peripheral spread cells which had migrated. At this time, signals appeared lower than those observed after 2 or 8 h (Fig. 2H,L).

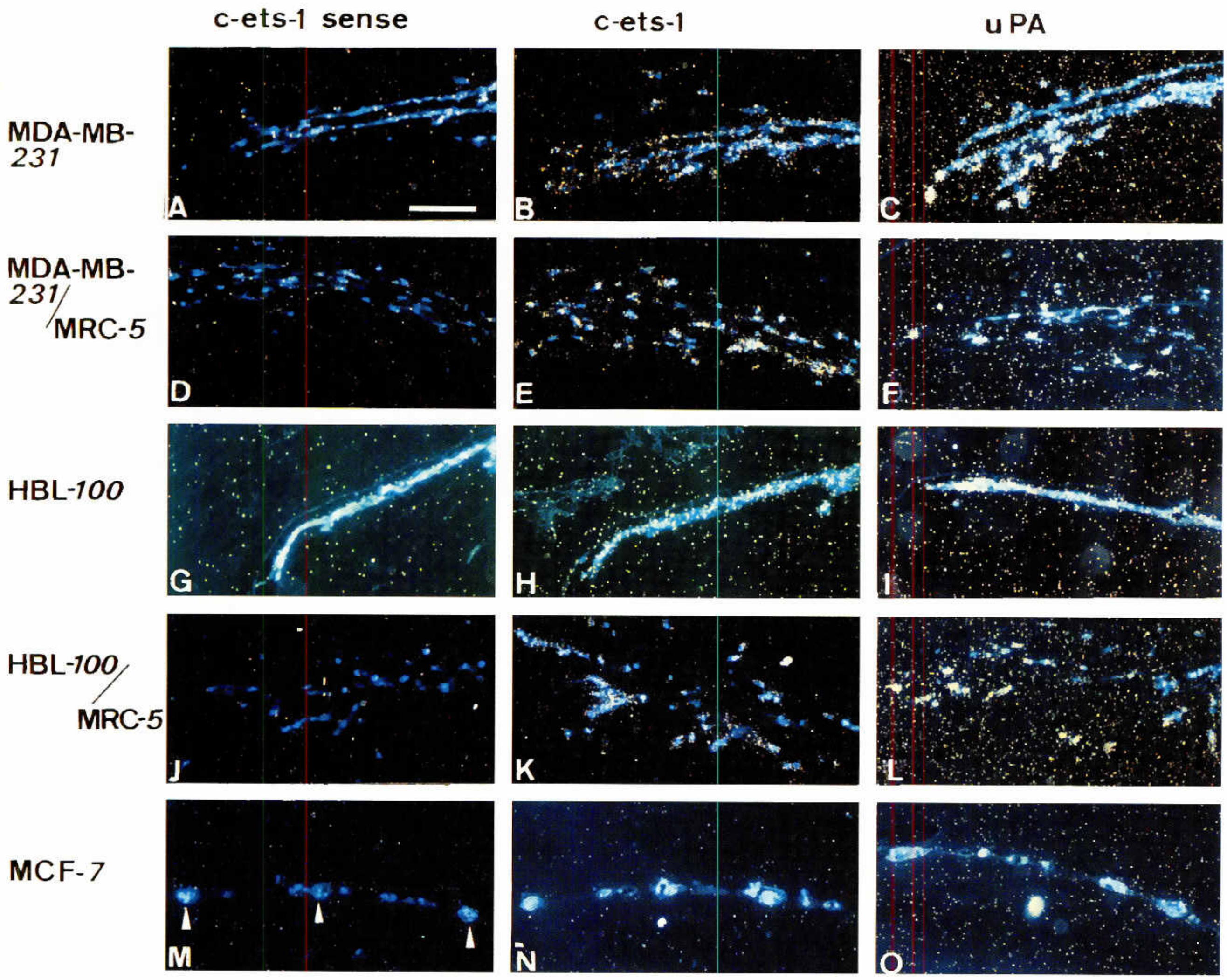


Fig. 5. *c-ets-1* and *uPA* expressions in MDA-MB-231, HBL-100 and MCF-7 in three dimensional collagen gels. *Culture conditions were the same as these used for hNMEC in three dimensional cultures. Cultured alone, MDA-MB-231 cells constitute parallel layers in the center of the seeded zone and scatter in the periphery of this zone* **(A-C)**. *When cocultured, they scattered all along the seeded region* **(D-F)**. *In both cases, the most invasive cells infiltrating the gel clearly express c-ets-1* **(B,E)** *and uPA* **(C,F)**. *In a fibroblast free gel, HBL-100 form a compact layer* **(G-I)** *where the genes are weakly expressed* **(H,I)**. *In coculture, the cells scattering and invading the gel all along the seeded zone* **(J-L)** *distinctly expressed c-ets-1* **(K)** *and uPA* **(L)**. *Cultured alone or in coculture, MCF-7 grew as adjacent cysts* (arrowheads in **M**). *c-ets-1 and uPA signals are never detected* **(M to O)**. *Views A,D,G,J,M are control sections hybridized by c-ets-1 sense probe. Bar, 70 μm.*

The fact that *c-ets-1* and *uPA* were expressed at the periphery of the epithelial cell clusters suggested a role in morphogenetic processes. We next set up three dimensional cultures that more closely mimic physiological situations.

A 10 μl drop containing 25000 hNMEC was laid between two collagen gels in absence of fibroblasts. Seven days later the cells formed a pluristratified compact sheet without lumen formation. The antisense *c-ets-1* or *uPA* probes detected a weak signal in these cells (Fig. 3B,C) when compared to the sense probes (Fig. 3A). When cocultured with MRC-5, hNMEC began to produce branching tubules after 3-4 days of culture. After 7 days, these tubules formed a three dimensional network which infiltrated the gel and extended from the initial center where cells were initially laid down to the periphery. Sections of these gels clearly revealed the presence of lumen within the tubules. It is worthy of note that migrating cells located at the tips of invasive ducts showed particularly intense *c-ets-1* and *uPA* signals before lumen establishment (Fig. 3E to I).

These experiments demonstrated that *c-ets-1* and *uPA* gene expression was associated with migration and morphogenetic processes in normal mammary epithelial cells and could be induced by cytokines released by neighboring fibroblasts.

Invasive cancerous epithelial cells express high levels of c-ets-1 and uPA-transcripts in the absence of stimulation

In MDA-MB-231 cancerous cells, incubated either in starvation medium or in MRC-5 conditioned medium, *c-ets-1* transcripts detected by Northern blot analysis (Fig. 4I) were abundant for each time, and mRNA levels were similar in both culture conditions. In the same way, the time courses of *uPA* expression were similar in

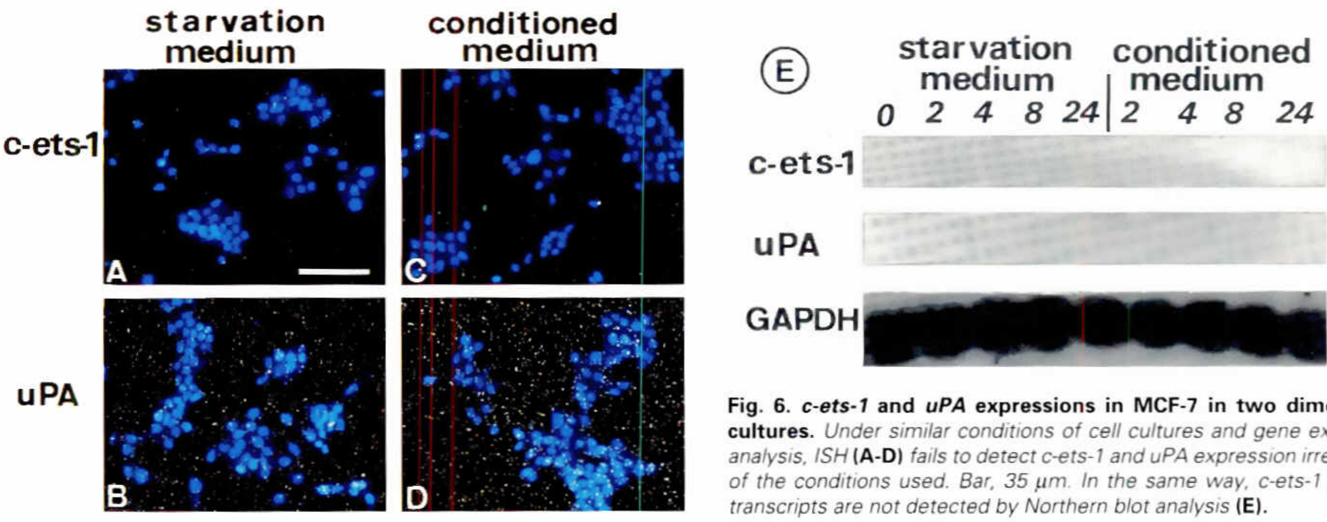


Fig. 6. *c-ets-1* and *uPA* expressions in MCF-7 in two dimensional cultures. *Under similar conditions of cell cultures and gene expression analysis, ISH* **(A-D)** *fails to detect c-ets-1 and uPA expression irrespective of the conditions used. Bar, 35 µm. In the same way, c-ets-1 and uPA transcripts are not detected by Northern blot analysis* **(E).**

the two conditions. MDA-MB-231 cells did not aggregate and rather grew as spindle-like cells. In starvation medium as well as in MRC-5 conditioned medium, ISH showed that the *c-ets-1* and *uPA* expressing cells were distributed throughout the culture at any time of treatment. However, while most of the MDA-MB-231 cells expressed *c-ets-1* (Fig. 4A,B,E,F) only a few dispersed cells expressed *uPA* (Fig. 4C,D,G,H). Similar results were obtained with HBL-100 cells.

We subsequently investigated the behavior of these cells in our three dimensional assay. After 3 days of culture in a fibroblast-free gel, MDA-MB-231 began to invade the gel around the initial cell deposit, in the three dimensions, producing a lot of tight cytoplasmic extensions. At the end of the experiment, an anarchic network of spindle-shaped cells was obtained. Sections revealed that cells were organized in parallel layers in the center of the seeded zone but scattered at its periphery (Fig. 5A). Cells obviously invading the surrounding gel expressed *c-ets-1* and *uPA* more conspicuously than did the cells in the central area (Fig. 5B,C). When MDA-MB-231 were co-cultured with fibroblasts, the limits of the initial cell deposit were rapidly overstepped. After 7 days, the area covered by MDA-MB-231 cells in co-culture was twice as large as the area covered in fibroblast-free cultures. Cells were scattered all along the seeded zone but they were unable to form tubules. *c-ets-1* and *uPA* signals were preferentially detected in invasive cells infiltrating the gel (Fig. 5E,F). When HBL-100 were cultured alone, these cells migrated only close to the limits of the initial deposit. They formed a compact layer with poor scattering and showed a weak *c-ets-1* signal (Fig. 5H). *uPA* labeling was more evident at the extremity of the layer (Fig. 5I). After one week of co-culture, HBL-100 extensively colonized the gel with anarchic scattering similar to that of MDA-MB-231. Invading HBL-100 conspicuously expressed *c-ets-1* and *uPA* (Fig. 5K,L).

Expression of c-ets-1 and uPA is not detected in non-invasive cancerous epithelial cells

In two dimensional cultures, MCF-7 grew as little clones and never showed *c-ets-1* or *uPA* expression. ISH or Northern blot failed to detect the transcripts whatever the culture conditions used (Fig. 6A to E). These cells cultured with or without fibroblasts in three dimensional cultures grew as individual cysts and never formed tubular structures. In both conditions, MCF-7 never expressed *c-ets-1* and *uPA* (Fig. 5N,O).

Normal mammary epithelial cells are less efficient than cancerous cells in inducing c-ets-1 and uPA expression in fibroblasts

In order to evaluate whether diffusible signals released by epithelial cells could induce *c-ets-1* and *uPA* expression in neighboring fibroblasts, we cultured MRC-5 fibroblasts in control medium or in media conditioned by either hNMEC, MCF-7, MDA-MB-231 or HBL-100. After treatment by hNMEC conditioned medium, many fibroblasts were labeled by *c-ets-1* and *uPA* probes (Fig. 7C,D). The labeling intensities were slightly higher than that observed in control cultures (Fig. 7A,B). Most fibroblasts treated with MCF-7 (Fig. 7E,F), MDA-MB-231 (Fig. 7G,H) or HBL-100 conditioned media contained a higher amount of transcripts for the two genes than did the cells treated by control or hNMEC conditioned medium. Under the same conditions, normal breast fibroblasts showed the same expression than MRC-5 fibroblasts (data not shown).

Consistent with these results, in three dimensional cultures, MRC-5 located close to the most invasive epithelial cells strongly exhibited enhanced *c-ets-1* and *uPA* expressions (data not shown).

Discussion

c-ets-1 expression has been widely documented in mesenchymal tissues during invasive processes and under epithelial-mesenchymal interactions. Here, we describe for the first time *c-ets-1* expression pattern in invasive epithelial cells during normal *in vivo* mammary gland development. Using *in vitro* reconstitution models we show that *c-ets-1* expression is associated either with mesenchymal-induced tubulogenesis by normal mammary epithelial cells or with epithelial scattering by cancerous mammary cells. Furthermore, the expression pattern of *uPA*, a putative target gene for c-Ets-1, parallels both *c-ets-1* expression pattern and the morphological feature of epithelial cell in movement.

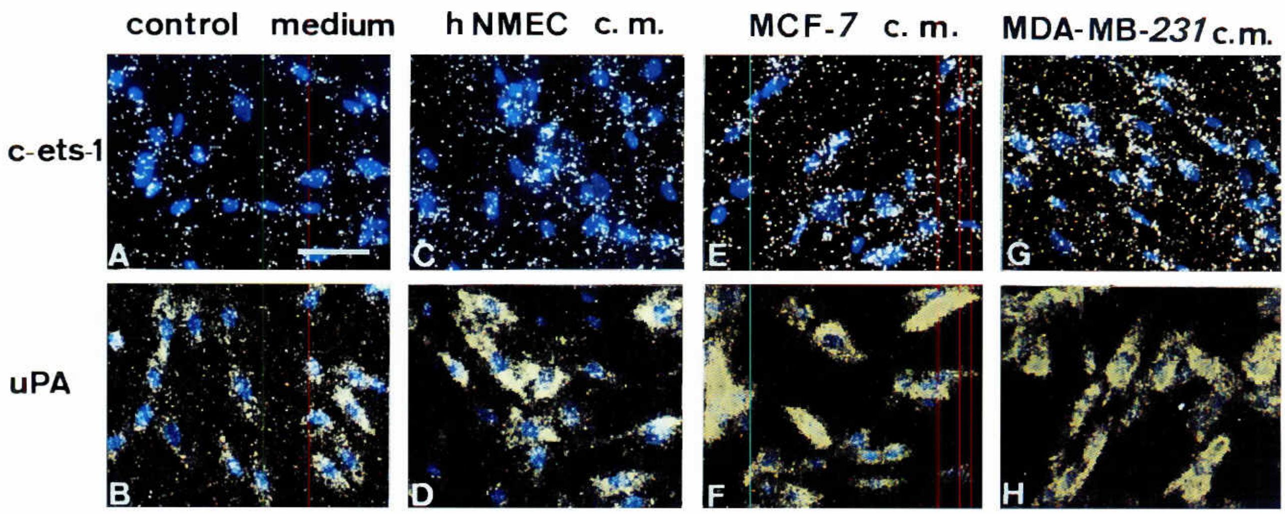


Fig. 7. *c-ets-1* and *uPA* expression in MRC-5 fibroblasts treated by epithelial mammary cells conditioned media. *After growth and starvation, MRC-5 were cultured in media conditioned by hNMEC, MCF-7or MDA-MB-231 (c.m.). ISH* **(A-H)** *shows that after 24 h of treatment by hNMEC c.m., MRC-5 cells expressed c-ets-1* **(C)** *and uPA* **(D)** *more intensely than in control medium* **(A,B)**. *Note that, for the two genes, the density of silver grains drastically increases under cancerous epithelial cell c.m. The uPA signal intensity increases such as cell nuclei are quite invisible* **(E to H)**. *Bar, 17.5 μm.*

Expression of c-ets-1 and uPA in invaded mesenchymal tissue

During mammary gland morphogenesis, we show that *c-ets-1* is clearly expressed at E14 in the mammary mesenchyme close to the invaginating epithelial primary bud. Later, at birth and at puberty, the loose mesenchyme in the vicinity of epithelial ducts displays focal expression of *c-ets-1*. Similar features of the expression patterns have been observed in situations where epithelial layers invade various mesenchymal compartments such as the uterine wall during mouse implantation or the stromal part of invasive carcinomas (reviewed in Vandenbunder *et al.*, 1995). At puberty, mesenchymal cells located near the mammary ducts also expressed *uPA* and collagenase. The late expression of these proteinases suggests either that they are not required for embryonic tissue remodeling, or that our probes are not suitable to detect a very low level of expression as previously reported for stromelysin 3 expression (Lefebvre *et al.*, 1995). Indeed, using the same mouse *uPA* probe, Grévin *et al.* (1993) never observed *uPA* expression in the embryo proper at least until day 10.5 of development while, as previously demonstrated by Sappino *et al.* (1989), trophoblastic cells strongly expressed this gene as early as day 6.5. In the same way, Mattot *et al.* (1995) using the same collagenase I probe, detected collagenase I mRNAs solely in chondrocytes in the E17 mouse fetus. Consistent with our data, the finding that *tPA* (tissue type plasminogen activator) and *uPA* double-deficient mice appear normal at birth suggests that neither *tPA* nor *uPA* is required for normal embryonic development (Carmeliet *et al.*, 1994). As previously shown in adult tissues (Grévin *et al.*, 1993; Wernert *et al.*, 1994), *c-ets-1*, *uPA* and collagenase expressions are detected in mesenchyme facing invasive epithelial tissue. Taken together, these *in vivo* observations suggest that epithelial cells send signals to mesenchymal cells which react by expressing these genes. According to *in vitro* transactivation assays (Gutman and Wasylyk, 1990; Rorth *et al.*, 1990; Wasylyk *et al.*, 1991; Stacey *et al.*, 1995), it is tempting to think that, during active growing phases of mammary gland development, *c-ets-1* expression takes part in events that trigger *uPA* and collagenase I expression in the invaded mesenchyme. Thus, the mesenchyme, under the stimulation of epithelial cells, would be able to control ECM degradation and to become permissive to epithelial invasion.

Our *in vitro* cultures were designed to compare the stromal reaction triggered either by normal epithelial mammary cells or by cancerous epithelial cells. It clearly appears that normal mammary epithelial cells, which migrate as sprouting ducts, induce *c-ets-1* and *uPA* expression in fibroblasts with a lower efficiency than cancerous cells. These results provide support for the hypothesis that instructive inductions monitored by epithelial cells via secreted factors are specific of epithelial cell status; these factors, in turn, induce *c-ets-1* and the proteinase gene expression. In the same way, human breast fibroblasts cultured with epithelial MDA-MB-231 conditioned medium increase their production of collagenase IV (Noël *et al.*, 1994); stromelysin 3 is specifically expressed by fibroblasts in the vicinity of neoplasic cells of mammary carcinomas (Basset *et al.*, 1990). TNFα and bFGF, both potent stimulators of *uPA* expression which trigger *c-ets-1* expression in fibroblasts, appear as good candidates for epithelial signaling (Wernert *et al.*, 1994; Gilles *et al.*, 1996).

Expression of c-ets-1 and uPA in invasive epithelial cells

In addition, our work shows that *c-ets-1* can be expressed in migrating epithelial cells themselves. Indeed, during mammary gland morphogenesis, at day 0 *c-ets-1* expression appears in the epithelial cells of primary ducts. This observation is the first evidence of *c-ets-1* expression in an epithelium during development. During morphogenetic processes, epithelial cells disrupt their specific organization to allow local repositioning (Gumbiner, 1992). Therefore, we suggest that *c-ets-1* plays a role in cell rearrangement inside an epithelial sheet at this stage. Later, at puberty, when the growth of the gland is reactivated, epithelial cells display a conspicuous signal for *c-ets-1*, *uPA* and collagenase I. It is worthy of note that the three signals are particularly intense in the growing ends of the ducts where cells are probably most invasive. At this stage epithelial cells have to force their way through the surrounding mesenchyme. *uPA*, collagenase I and *c-ets-1* expressions superimpose in the epithelial cells at the invasive extremity of the ducts during mammary development. These proteinases, known to be expressed in many migrating cell types, are here involved in the normal invasive epithelium. In the same way, *c-ets-1*, *uPA* and collagenase were recently detected in lung carcinoma cells and were suspected to be involved in tumor invasion (Bolon *et al.*, 1995).

Northern blot analysis indicates that *c-ets-1* and *uPA* expression can be induced in hNMEC in response to MRC-5 conditioned medium. Moreover, for the first time, our *in vitro* experiments on two dimensional or three dimensional cultures reveal that *c-ets-1* and *uPA* transcripts are expressed in migrating hNMEC stimulated by soluble factors released by MRC-5 fibroblasts. On the contrary, HBL-100 and MDA-MB-231 cells expressed high levels of *c-ets-1* and *uPA* either constitutively or under MRC-5 stimulation. These data suggest that hNMEC are dependent upon migrating signals to significantly express these genes whereas HBL-100 and MDA-MB-231 cells, which exhibit a natural invasive power, do not need such signals to express *c-ets-1* and *uPA*. The correlation between these gene expressions and the migration process is furthermore reinforced by MCF-7 behavior. These cells grow tightly aggregated as clones or cysts, share abundant surface E cadherin (Révillion *et al.*, 1993) known to act as an invasion suppressor molecule (Vleminckx *et al.*, 1991), and never express *c-ets-1* and its putative target proteinase gene. These cells, when transfected with a member of the Ets family, E1AF, strongly express the metalloproteinase MMP9 and furthermore scatter both *in vitro* and *in vivo* (Kaya *et al.*, 1996).

Under the influence of both MRC-5 conditioned medium and three dimensional matrix, hNMEC cells form tubular structures whereas MDA-MB-231 and HBL-100 scatter. Irrespective of their own status, the most invasive cells express both *c-ets-1* and *uPA*. Recently, involvement of Ets factors, including c-Ets-1, was confirmed in the transactivation of the *uPA* promoter *in vitro* (Stacey *et al.*, 1995). However, our ISH experiments on two dimensional cultures show that the *c-ets-1* signal is rather homogeneously distributed in cell populations while the *uPA* labeled cells appear more focally in the population concerned with *c-ets-1* expression. The distribution of *c-ets-1* and *uPA* signals during placentation share the same features (Grévin *et al.*, 1993). Thus, there may exist a threshold of *c-ets-1* expression from which *uPA* expression would be triggered. Alternatively, we can also propose that the activation of *uPA* expression needs additional Ets family proteins or, one or several cofactor(s). Ets transcription factors cooperate with factors as Jun/Fos (Wasylyk *et al.*, 1990). Experiments are currently in progress to go further in the demonstration of the inductive role of *c-ets-1* in our model.

Remodeling the ECM

uPA plays a central role in ECM proteolysis as the starter of a proteinase cascade: it leads to pro-collagenase activation in human mammary carcinoma cells (Paranjpe *et al.*, 1980). It can also activate stromelysin (Wolf *et al.*, 1994). As a consequence, *uPA* has been highly correlated with cell invasiveness and metastatic power for example in melanoma cells (Mignatti *et al.*, 1986) and in lung colonies (Hearing *et al.*, 1988). It has been suggested that epithelial cells can degrade the matrix using uPA linked to its surface receptors (Pyke *et al.*, 1991). These receptors are overexpressed in breast cancer cells (Jankun *et al.*, 1993) and this overexpression has been correlated with the high invasiveness capacity of the cells. Among cancerous epithelial cells, the most invasive MDA-MB-231 clearly exhibit the *uPA* signals whereas the non-invasive MCF-7 do not show these signals. These results agree with the finding of Funahashi *et al.* (1994): proteinase activities are proportional to the metastatic potentials of the cells in culture. Of course, cellular invasion depends on the balance between the levels of structural proteins and their proteinases, between the activators and the inhibitors of these proteinases like PAI 1, PAI 2 and TIMPs. The breakdown of this balance leading to high levels of plasminogen activator or metalloproteinases may be responsible for the abnormal tumoral progression. In this view, we recently performed uPA activity estimations in our three dimensional cultures and the results showed an increased uPA activity in epithelial cells under MRC-5 stimulation while PAI-1 levels were not affected (Fauquette *et al.*, in preparation).

In conclusion, the description of *c-ets-1* expression in migrating epithelial cells is a new finding. Furthermore, we show that in adult tissues and in our *in vitro* systems the expression pattern of *c-ets-1* in invasive cells correlates with the expression pattern of *uPA*, keeping in mind the fact that the expression and the activity of uPA have been associated with the invasiveness capacity of epithelial cells. The comparison between our results concerning the normal mammary gland tubulogenesis *in vivo* and *in vitro* and the scattering of cancerous mammary epithelial cells suggests that the transient and inducible expression of *c-ets-1* and *uPA* during normal development can be constitutively activated under pathological conditions. We now aim at inhibiting *c-ets-1* expression with the expectation to block the mechanism which leads to invasion and metastasis.

Materials and Methods

In vivo model

Mammary glands of E14 mouse embryos as well as those of young mice at birth (D0) or at puberty (D25 and D35) were collected and prepared for hybridization: they were fixed at 4°C for 16 h in 4% paraformaldehyde in PBS containing 5 mM $MgCl_2$, washed in PBS, dehydrated, embedded in paraffin and serially cut (7 µm). Sections were transferred to 3 amino-propyl-triethoxysilane (TESPA, Aldrich) coated slides and incubated at 37°C for three days. Slides were stored at 4°C until use.

In vitro model

Cell types and their maintenance in culture

All cell lines were routinely maintained in plastic flasks (Falcon 75 cm^2) fed with 15 ml of cell type specific medium containing serum (see below), and incubated in a humid atmosphere of 5% CO_2 in air, at 37°C. Medium was changed every three days.

Human Normal Mammary Epithelial Cells (hNMEC) were isolated from mammary reduction (generous gift from Dr Pellerin) and cultured as previously described by Berthon *et al.* (1992). hNMEC were maintained at low Ca^{++} concentration (20 µM) in DMEM/Ham F12 (Eurobio) with 100 ng/ml cholera toxin (Sigma), 2 ng/ml EGF (Genzyme), 5×10^{-6} M cortisol (Sigma), 2 mM glutamine (Eurobio), 10 µg/ml insulin (Endopancrine, Organon), 40 U/40 µg/ml penicillin-streptomycin (Eurobio), 4 µg/ml gentamycin (Sigma), 0,25 ng/ml fungizone (Eurobio), 5% calcium free Fetal Calf Serum (FCS, Eurobio). In these conditions, cells produced by mitosis stay in suspension, and can be recovered by centrifugation (15 min at 1200 rpm). For each experiment, cells were resuspended in the same medium supplemented with ordinary FCS allowing cell attachment after seeding.

The other epithelial cells were obtained from the American Type Culture Collection. HBL-100 cells were initially reported as non-tumorigenic in nude mice but, recently some clones including the clones we used in these studies, exhibited a transformed phenotype and an increased motility (data not shown). MDA-MB-231 cancerous cells are tumorigenic in nude mice and are hormone-independent for growth. In contrast, MCF 7 cancerous cells are hormone-dependent. These cells were cultured in Epithelial cell Medium (EM) consisting of MEM (Eurobio) supplemented with 10% Fetal Calf Serum (FCS, Eurobio), 2 mM glutamine (Eurobio), 100 U/100 μg/ml penicillin-streptomycin (Eurobio), 1% non essential amino acids (Eurobio) and 5 μg/ml insulin (Endopancrine, Organon).

MRC-5 fibroblasts are derived from normal lung tissue of a 14 week-old male fetus (Eurobio). Montesano *et al.* (1991a) showed that these cells produce HGF/SF capable of inducing tubulogenesis in Madin Darby Canine Kidney (MDCK) three dimensional cell cultures. MRC-5 were maintained in Fibroblast Medium (FM) consisting of EM deprived of non-essential amino acids and insulin.

Two-dimensional cultures

Epithelial cells and fibroblasts were collected either according to standard conditions with 0.25% trypsin-EDTA or by centrifugation of cell suspensions for hNMEC. The cells were resuspended in appropriate medium, numbered and seeded at the following densities: MDA-MB-231 were plated at 10,000 cells/ml, HBL-100 at 15,000 cells/ml, MCF-7 and hNMEC at 30,000 cells/ml and fibroblasts at 60,000 cells/ml. These densities allowed us to obtain subconfluent cultures after three days of growth. In order to perform *in situ* hybridization, cells were seeded with 250 μl of medium in 8-well tissue culture chambers of Lab Tech slides (Nunc) previously coated with collagen to provide the cells with an artificial matrix. For Northern blot analysis, epithelial cells were plated with 20 ml of medium in 150 mm dishes. The cells were grown for three days before replacing complete medium by starvation medium (EM or FM without serum but supplemented with 2 μg/ml fibronectin and 30 μg/ml transferrin). After 24 h, cells were submitted to the following conditions. Epithelial cells were cultured either in starvation medium (reference culture) or in MRC-5 conditioned medium. This conditioned medium was recovered from a subconfluent MRC-5 culture after 2 days of growth in serum-free medium. The fibroblasts were either cultured in complete medium as control, or treated with 50% complete medium and 50% conditioned media recovered from subconfluent cultures of each epithelial cell line after 2 days of growth in serum free medium.

After each culture assay, cells were fixed for ISH in 4% paraformaldehyde in PBS containing 5 mM $MgCl_2$, washed in PBS and dehydrated. Slides were stored at 4°C until ISH. To perform Northern blot analysis, total RNAs of epithelial cells were extracted in guanidium isothiocyanate according to Chirgwin *et al.* (1979).

Three-dimensional cultures

Rat tail collagen gels were prepared according to Montesano *et al.* (1991b). Briefly, 8 volumes of a 2 mg/ml collagen solution were melted at 4°C to one volume of 10xMEM and one volume of 22.2 g/l sodium bicarbonate. We performed either cocultures with epithelial cells and MRC-5 fibroblasts in a way that reproduces the *in vivo* situation of epithelial-mesenchymal interactions, or control cultures of epithelial cells alone.

In the first case, 300 μl of collagen gel containing 500,000 MRC-5 cells/ml were dispensed in 16 mm wells of 24-well plates (Nunc). Then, a 10 μl-drop containing 25,000 epithelial cells was laid at the center of the collagen gel surface. In the other case, the 10 μl-drop was put down onto a fibroblast free collagen gel. The epithelial cells were allowed to attach to the substrate during about one hour in the incubator. Then, a cell free collagen gel (300 μl) was applied to cover the cell deposit. 500 μl of complete medium were added. The cultures were maintained during one week, and subsequently the gels were prepared for hybridization.

Preparation of the probes

The antisense and sense ^{35}S RNA probes for ISH and the ^{32}P probes for Northern blot analysis were transcribed from the following cDNA fragments: the 1.6 Kb Sac I/Kpn I fragment of the mouse *c-ets-1* cDNA (Chen *et al.*, 1990) cloned into the Bluescript KS (Stratagene); the 660 bp Pst I/Hind III fragment of the mouse *uPA* cDNA (Belin *et al.*, 1985) cloned into pSP64 and pSP65 (Promega); the 2.7 Kb Hind III/Not I fragment of the mouse collagenase I cDNA (Henriet *et al.*, 1992) cloned into the Bluescript KS; the 825 bp fragment of the human *c-ets-1* cDNA (Watson *et al.*, 1988) cloned into pSP64 and pSP65; the 600 bp Eco R1/Pst I fragment of the human *uPA* cDNA (Wernert *et al.*, 1994) cloned into the Bluescript KS.

In situ hybridization

ISH were performed as previously described (Quéva *et al.*, 1992). Briefly, after deparaffinization (for sections) and hydration, slides were treated with 1 μg/ml proteinase K (Boehringer, Mannheim) for 15 min at 37°C, post-fixed in 4% paraformaldehyde, washed in PBS, acetylated by 0.25% acetic anhydride in 0.1 M triethanolamine, washed in 2xSSC and dehydrated by ethanol. Probe, in hybridization mixture, was applied to slides and hybridization was performed at 60°C for 18 h. The slides were subsequently washed several times and incubated with 20 μg/ml RNase A (type III A, Sigma) for 1h at 37°C. Final washes were performed for 15 min at 60°C in 2xSSC and for 15 min at 60°C in 0.1xSSC. Sections and cells were dehydrated in ethanol, dried and dipped in a nuclear track emulsion (Kodak NTB2). The slides were exposed for 2.5 weeks at 4°C. After development they were stained by the intercalating dye Hoechst 33258, mounted in glycergel (Dako) and observed under a double illumination using an Olympus BH2 photo-microscope with epifluorescence for Hoechst staining and a dark-field condenser for silver grain detection.

Northern blot analysis

RNAs (15 μg) were separated on 1.2% agarose/formaldehyde gels and transferred overnight onto nitrocellulose membranes (Hybond-C-extra, Amersham). Membranes were baked at 80°C for 2 h and hybridized at 42°C with probes prepared by the Megaprime labeling system (Amersham).

Acknowledgments

We are extremely grateful to Dr. Pellerin for generously providing mammary explants. We also thank Drs. H. Hondermarck and S. Flament for critical reviews of the manuscript, Dr. R. Montesano for his valuable help in three dimensional reconstitutions, members of the "In situ" group of URA 1160 for their technical help and discussion, and Dr. D. Stéhelin for taking an interest in our research. This work was supported by the Region Nord-Pas de Calais, the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (EA 1033) and by the Association pour la Recherche sur le Cancer. A. Delannoy-Courdent is the recipient of a fellowship from the "Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche".

References

BALLIN, M., MACKAY, A.R., HARTZLER, J.L., NASON, A., PELINA, M.D. and THORGEIRSSON, U.P. (1991). Ras levels and metalloproteinase activity in normal versus neoplasic rat mammary tissues. *Clin. Exp. Metastasis. 9:* 179-189.

BASSET, P., BELLOCQ, J.P., WOLF, C., STOLL, I., HUTIN, P., LIMACHER, J.M., PODHAJCER, O.L., CHENARD, M.P., RIO, M.C. and CHAMBON, P. (1990). A novel metalloprotéinase gene specifically expressed in stromal cells of breast carcinomas. *Nature 34:* 699-704.

BELIN, D., VASSALI, J.D., COMBEPINE, C., GODEAU, F., NAGAMINE, Y., REICH, E., KOSHER, H.P. and DUVOISIN, R.M. (1985). Cloning, nucleotide sequencing and expressions of cDNAs encoding urokinase-type plasminogen activator. *Eur. J. Biochem. 148:* 225-232.

BERTHON, P., PANCINO, G., DE CREMOUX, P., ROSETO, A., GESPACH, C. and CALVO, F. (1992) Characterization of normal breast epithelial cells in primary cultures: differentiation and growth factor receptors studies. *In vitro Cell. Dev. Biol. 28A:* 716-724.

BOLON, I., GOUYER, V., DEVOUASSOUX, M., VANDENBUNDER, B., WERNERT, N., MORO, D., BRAMBILLA, C. and BRAMBILLA, E. (1995). Expression of c-ets-1, collagenase 1, and urokinase-type plasminogen activator genes in lung carcinomas. *Am. J. Pathol. 147:* 1298-1310.

BOSSELUT, R., DUVAL, J.F., GÉGONNE, A., BAILLY, M., HÉMAR, A., BRADY, J., and GHYSDAEL, J. (1990). The product of c-ets-1 proto-oncogene and the related c-ets-1 protein act as transcriptional activators of the long terminal repeat of human T cell leukemia virus HTLV-1. *EMBO J. 9:* 3315-3322.

CARMELIET, P., SCHOONJANS, L., KIECKENS, L., REAM, B., DEGEN, J., BRONSON, R., DE VOS, R., VAN DEN OORD, J.J., COLLEN, D. and MULLIGAN, R.C. (1994). Physiological consequences of loss of plasminogen activator gene function in mice. *Nature 368:* 419-424.

CHEN, J.H. (1990). Cloning and sequencing of mouse c-ets-1 cDNA in baculovirus expression system. *Oncogene Res. 5:* 277-285.

CHIRGWIN, J.M., PRZYBYLA, A.E., MACDONALD, R.J. and RUTTER, W. (1979). Isolation of biologically activeribonucleic acid from sources enrichedin ribonuclease. *Biochemistry 18:* 5294-5299.

FUNAHASHI, T., SHIMAMURA, M., KOCHA, T., FUKUDA, T. and AOYAGI, T. (1994). Proportionality of protease activities in malignant cells to their metastatic potentials. *Biol. Pharmacol. Bull. 17:* 1118-1120.

GILLES, F., RAES, M.B., STÉHELIN, D., VANDENBUNDER, B. and FAFEUR, V. (1996). The c-ets-1 proto-oncogene is a new early-response gene differentially regulated by cytokines and growth factors in human fibroblasts. *Exp. Cell Res. 222:* 370-378.

GRÉVIN, D., CHEN, J.H., RAES, M.B., STÉHELIN, D., VANDENBUNDER, B. and DESBIENS, X. (1993). Involvement of the proto-oncogene c-ets-1 and the urokinase plasminogen activator during mouse implantation and placentation. *Int. J. Dev. Biol. 37:* 519-529.

GUMBINER, B.M. (1992). Epithelial morphogenesis. *Cell 69:* 385-387.

GUNTHER, C.V., NYE, J.A., BRYNER, R.S. and GRAVES, B.J. (1990). Sequence-specific DNA binding of the proto-oncoprotein Est-1 define a transcriptional activator sequence within the long terminal repeat of the Moloney murine sarcoma virus. *Genes Dev. 4:* 667-679.

GUTMAN, A. and WASYLYK, C. (1990). The collagenase gene promoter contains a TPA and oncogene responsive unit encompassing the PEA3 and AP-1 binding sites. *EMBO J. 9:* 2241-2246.

HE, C., WILHEM, S.M., PENTLAND, A.P., MARMER, B.L., GRANT, G.A., EISEN, A.Z. and GOLDBERG, G.I. (1989). Tissue cooperation in proteolytic cascade activating human interstial collagenase. *Proc. Natl. Acad. Sci. USA 86:* 2632-2636.

HEARING, J.V., LAW, L.W., CORTI, A., APPELLA, E. and BLASI, F. (1988). Modulation of metastatic potential by cell surface urokinase of murine melanoma cells. *Cancer Res. 48:* 1270-1278.

HENRIET, P., ROUSSEAU, G.G. and EECKOUT, Y. (1992). Cloning and sequencing of mouse collagenase cDNA. Divergence of mouse and rat collagenase from the other mammalian collagenases. *FEBS Lett. 310:* 175-178.

HEUBERGER, B., FIZTKA, I., WASNER, G. and KRATOCHWIL, K. (1982). Induction of androgen receptor formation by epithelium-mesenchyme interaction in embryonic mouse mammary gland. *Dev. Biol. 79:* 2957-2961.

HO, I.C., BHAT, N.K., GOTTSCHALK, L.R., LINDSTEN T., THOMPSON, C.B., PAPAS, T.S. and LEIDEN, J.M. (1990). Sequence specific binding of human c-ets-1 to T cell receptor α gene enhancer. *Science 250:* 814-818.

JANKUN, J., MERRICK, H.W. and GOLDBLATT, P.J. (1993). Expression and localization of elements of the plasminogen activator system in benign breast disease and breast cancers. *J. Cell. Biochem. 53:* 135-144.

KAYA, M., YOSHIDA, K., HIGASHINO, F., MITAKA, T., ISHII, S. and FUJINAGA, K. (1996). A single *ets*-related transciption factor, E1AF, confers invasive phenotype on human cancer cells. *Oncogene 12:* 221-227.

LEFEBVRE, O., RÉGNIER, C., CHENARD, M.P., WENDLING, C., CHAMBON, P., BASSET, P. and RIO, M.C. (1995). Developmental expression of mouse stromelysin-3 mRNA. *Development 121:* 947-955.

LEPRINCE, D., GÉGONNE, A., COLL, J.,DE TAISNE, C., SCHNEEBERGER, A., LAGROU, C. and STÉHELIN, D. (1983). A putative second cell-derived oncogene of the avian leukemia virus E-26. *Nature 306:* 395-397.

MATTOT, V., RAES, M.B., HENRIET, P., EECKHOUT Y., STÉHELIN, D., VANDENBUNDER, B. and DESBIENS, X. (1995). Expression of interstitial collagenase is restricted to skeletal tissue during mouse embryogenesis. *J. Cell Sci. 108:* 529-535.

MIGNATTI, P., ROBBINS, E. and RIFKIN, D.B. (1986). Tumor invasion through the human amniotic membrane: requirement for a proteinase cascade. *Cell 47:* 487-498.

MONTESANO, R., MATSUMOTO, K., NAKAMURA, T., and ORCI, L. (1991a). Identification of a fibroblast derived epithelial morphogen as hepatocyte growth factor. *Cell 67:* 901-908.

MONTESANO, R., SCHALLER, G., and ORCI, L. (1991b). Induction of epithelial tubular morphogenesis *in vitro* by fibroblast-derived soluble factors. *Cell 66:* 697-711.

MOSCATELLI, D., PRESTA, M. and RIFKIN, D.B. (1986). Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis and migration. *Proc. Natl. Acad. Sci. USA 83:* 2091-2095.

NOËL, A.C., POLETTE, M., LEWALLE, J.M., MUNAUT, C., EMONARD, H.P., BIREMBAUT, P. and FOIDART, J.M. (1994). Coordinate enhancement of gelatinase A mRNA and activity levels in human fibroblasts in response to breast-adenocarcinoma cells. *Int. J. Cancer 56:* 331-336.

NUNN, M.F., SEEBURG, P.H., MOSCOVI, C. and DUESBERG, P.H. (1983). Tri partite structure of the avian erythroblastosis virus E 26 transforming gene. *Nature 306:* 391-395.

OSSOWSKI, L., BIEGEL, D. and REICH, E. (1979). Mammary plasminogen activator: correlation with involution, hormonal modulation and comparison between normal and neoplastic tissue. *Cell 16:* 929-940.

PARANJPE, M., ENGEL, L., YOUNG, N. and LIOTTA, L.A. (1980). Activation of human breast carcinoma collagenase through plasminogen activator. *Life Sci. 26:* 1223-1231.

PARDANAUD, L. and DIETERLEN-LIÈVRE, F. (1993). Expression of c-ets-1 in early chick embryo mesoderm: relationship to the hemangioblastic lineage. *Cell Adhesion Commun. 1:* 151-160.

POLETTE, M., CLAVEL, C., COCKETT, M., GIROD DE BENTZAMM, S., MURPHY, S. and BIREMBAUT, P. (1993). Detection and localisation of mRNA encoding matrix metalloproteinases and their tissue inhibitor in human breast pathology. *Invas. Metast. 13:* 31-37.

PYKE, C., KRISTENSEN, P., RALFKIAER, E., GRONDAHL-HANSEN, J., ERIKSEN, J., BLASI, F. and DANO, K. (1991). Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invasive foci in human colon adenocarcinomas. *Am. J. Pathol. 138:* 1059-1067.

QUÉVA, C., NESS, S.A., GRÄSSER, F.A., GRAF, T., VANDENBUNDER, B. and STÉHELIN, D. (1992). Expression patterns of c-myb and of v-myb induced myeloid-1 (mim-1) gene during the development of the chick embryo. *Development 114:* 125-133.

QUÉVA, C., LEPRINCE, D. STÉHELIN, D. and VANDENBUNDER, B. (1993). p54c-ets-1 and p68c-ets-1 the two transcription factors encoded by the c-ets-1 locus are differentially expressed during the development of the chick embryo. *Oncogene 8:* 2511-2520.

RÉVILLION, F., VANDEWALLE, B., HORNEZ, L. and LEFÈBVRE, J. (1993). Influence of cAMP on E-cadherin expression and cell surface heparan sulfate proteoglycan synthesis in human breast cancer cells. *Anticancer Res. 13:* 1625-1630.

RORTH, P., NERLOV, C., BLASI, F. and JONHNSEN M. (1990). Transcription factor PEA3 participates in the induction of urokinase plasminogen activator transcription in murine keratinocytes stimulated with epidermal growth factor or phorbol-ester. *Nucleic Acids Res. 18:* 5009-5017.

SAPPINO, A-P., HUARTE, J., BELIN, D. and VASSALI, J-D. (1989). Plasminogen activators in tissue remodeling and invasion: mRNA localization in mouse ovaries and implanting embryos. *J. Cell Biol. 109:* 2471-2479.

STACEY, K.J., FOWLES, L.S., COLMAN, M.S., OSTROWSKI, M.C. and HUME, D.A. (1995). Regulation of urokinase-type plasminogen activator gene transcription by Macrophage Colony Stimulating Factor. *Mol. Cell. Biol. 15; 6:* 3430-3441.

STEPHENS, R.W., PÖLLÄNEN, J., TAPIOVAARA, H., LEUNG, K.C., SIM, P.S. SALONEN, E.M., RONNE, E., BEHRENDT, N., DANO, K. and VAHERI, A. (1989). Activation of pro-urokinase and plasminogen on human sarcoma cells: a proteolytic system with surface bound reactants. *J. Cell Biol. 108:* 1987-1995.

SYMPSON, C.J., TALHOUK, R.S., ALEXANDER, C.M., CHIN, J.R., CLIFT, S.M., BISSEL, M.J. and WERB, Z. (1994). Target expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue specific gene expression. *J. Cell Biol. 125:* 681-693.

VANDENBUNDER, B., PARDANAUD, L., JAFFREDO, T., MIRABEL, M.A. and STÉHELIN, D. (1989). Complementary patterns of expression of c-ets-1, c-myb and c-myc in the blood forming system of the chick embryo. *Development 107:* 265-274.

VANDENBUNDER, B., QUÉVA, C., DESBIENS, X., WERNERT, N. and STÉHELIN D. (1995). Expression of the transcription factor c-ets-1 correlates with the occurence of invasive processes during normal and pathological development. *Invas. Metast. 14:* 198-209.

VLEMINCKX, K., VAKAET, L. Jr., MAREEL, M., FIERS, W. and VAN ROY, F. (1991). Genetic manipulation of E-Cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell 66:* 107-119.

WASYLYK, C., GUTMAN, A., NICHOLSON, R. and WASYLYK, B. (1991). The c-Ets oncoprotein activates the stromelysin promoter through the same element as several non-nuclear oncoproteins. *EMBO J. 10:* 1127-1134.

WASYLYK, B., WASYLYK, C., FLORES, P., BEGUE, A., LEPRINCE, D. and D. STEHELIN, D. (1990). The c-ets proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation. *Nature 346:* 191-193.

WATSON, D.K., MACWILLIAMS, M.J., LAPIS, P., LAUTENBERGER, J.A., SCHWEINFEST, C.W. and PAPAS, T.S. (1988). Mammalian ets-1 and ets-2 genes encode highly conserved proteins. *Proc. Natl. Acad. Sci. USA 85:* 7862-7866.

WERNERT, N., GILLES, F., FAFEUR, V., BOUALI, F., RAES, M.B., PYKE, C., DUPRESSOIR, T., SEITZ, G., VANDENBUNDER, B. and STÉHELIN, D. (1994). Stromal expression of c-ets-1 transcription factor correlates with tumor invasion. *Cancer Res. 54:* 5683-5688.

WERNERT, N., RAES, M.B., LASSALLE, B., DEHOUCK, M.P., GOSSELIN, B., VANDENBUNDER, B. and STÉHELIN, D. (1992). C-ets-1 proto-oncogene is a transcription factor expressed in endothelial cells during tumor vascularization and other forms of angiogenesis in humans. *Am. J. Pathol. 138:* 111-117.

WOLF, C., LEFEBVRE, O., ROUYER, N., CHENARD, M.P., BELLOCQ, J.P., RIO, M.C., CHAMBON, P. and BASSET, P. (1994). Protéases d'origine stromale et progression tumorale. *Med. Sci.10:* 507-515.

Received: July 1996
Accepted for publication: September 1996