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Ets factors and regulation of the extracellular matrix

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Ets factors are critical mediators of extracellular matrix (ECM) remodelling. As the spectrum of Ets-regulated target genes widens, so does their role in various pathological and physiological processes. Regulation of matrix degrading proteases by Ets factors in tumor invasion and metastasis is well established. Emerging evidence suggests that they may also play a role in the pathology of autoimmune diseases. Newly characterized Ets target genes such as tenascin-C and collagen type I suggest their role in diseases characterized by aberrant collagen deposition (fibrosis). Ets function is also critical in bone and cartilage development. There is increasing knowledge of the complex regulatory mechanisms involved in transcription of Ets target genes. Ets factors may function as activators or as repressors via association with specific cofactors depending on the promoter context. Signaling pathways can modulate the activation status of Ets factors and their transcriptional partners. Precise understanding of the role of Ets factors in the complex cellular network governing the expression of ECM proteins and the enzymes that degrade them will be a focus of future studies. *Oncogene* (2000) **19,** 6464–6471.

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Introduction

The extracellular matrix (ECM) is a network of macromolecules in which cells are embedded. It provides not only structural support for tissue integrity but also an interactive environment for specialized cell functions. Although the composition of the ECM components varies in different tissues, the primary components are collagens, proteoglycans, and a variety of multiadhesive matrix proteins such as laminins and fibronectins. Physiological synthesis, deposition, and the degradation of the ECM occur during embryogenesis, female reproductive cycle, angiogenesis and wound repair. However, in the majority of normal adult tissues only limited turnover of the ECM takes place. In contrast, in many pathological conditions the balance between ECM synthesis and degradation is disrupted, leading to abnormal ECM remodeling. Excessive ECM deposition occurs in fibrotic diseases such as scleroderma, liver cirrhosis, and glomerulosclerosis; whereas, excessive breakdown of the ECM is associated with rheumatoid arthritis, osteoarthritis, periodontitis, as well as tumor invasion and metastasis.

There is increasing evidence that Ets factors play an important role in regulating ECM remodeling. This aspect of Ets function has been shown to contribute to epithelial-mesenchymal transition during development (reviewed by Remy and Baltzinger, this issue). On the other hand, abnormal Ets functions have been implicated in pathological processes such as tumor invasion (Wernert *et al*., 1994). The role of Ets factors in tumor invasion is mainly related to transcriptional activation of enzymes that are involved in ECM degradation such as serine proteases, matrix metalloproteinases (MMPs) and their inhibitors (TIMPs). Recent studies suggest, however, that the function of Ets factors may not be limited to regulating genes involved in the degradative pathways. Ets factors may regulate a wider spectrum of ECM related target genes including matrix proteins such as tenascin, collagen, and fibronectin as well as other cellular components involved in cell–matrix interactions. This review will discuss current knowledge on the role of Ets factors in regulating ECM remodeling during physiological and pathological processes.

Ets factors and ECM remodeling in tumors

Tumor invasion and metastasis depend on the ability of tumor cells to break through the surrounding connective tissue barriers (Stracke *et al*., 1994). Degradation of ECM by tissue serine proteases and the large family of MMPs plays a crucial role in this process (reviewed by: Johansson and Kahari, 2000; Curran and Murray, 1999; Forget *et al*., 1999). Immunohistochemical and *in situ* mRNA analyses of various human tumor tissues have demonstrated coexpression of Ets1 and matrix degrading proteases, particularly in invasive tumors. In contrast, in benign and non-invasive tumors, Ets1 transcripts are rarely detected (Calmels *et al*., 1995). The coexpression of Ets1 and collagenase 1 (MMP-1), urokinase-type plasminogen activator (u-PA), and in some cases stromelysin-1 (MMP-3) has been well documented in lung carcinomas (Bolon *et al*., 1995; Wernert *et al*., 1994) and angiosarcoma of the skin (Naito *et al*., 2000). Additional supporting evidence for the role of Ets1 in regulating MMPs in tumors came from the discovery of the single nucleotide polymorphism (SNP) in the MMP-1 promoter region, where an additional G creates an Ets binding site (Rutter *et al*., 1998). *In vitro* studies have shown that this polymorphism contributes to increased Ets1 binding and increased promoter activity (Rutter *et al*., 1998). Furthermore, endometrial carcinomas showed a significantly higher rate of 1G/2G and 2G/2G genotype than control individuals, which correlated with more frequent expression of MMP-1 protein (Nishioka *et al*., 2000).

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Interestingly, the cellular origin of abnormal Ets function and the source of elevated ECM remodeling enzymes are not consistent. For example, in the tumors mentioned above Ets1 mRNA has been primarily localized to endothelial and fibroblastic stromal cells (Wernert *et al*., 1994). Furthermore, coexpression of Ets1, collagenase-1 (MMP-1) and stromelysin-1 (MMP-3) has been almost exclusively localized to stromal fibroblast. These observations suggest that in these tumors stromal fibroblasts play an important role in 'landscaping' tumor invasion by synthesizing matrix proteases involved in ECM degradation. The role of stroma in supporting tumor growth is now well recognized (Wernert, 1997). In contrast, in human gastric carcinoma, elevated expression of Ets1 was detected in tumor cells, but not in stromal cells (Nakayama *et al*., 1996). Expression of a different member of Ets family, E1AF (hPEA3), by invading tumor cells was also observed in a large proportion of oral squamous cell carcinomas and is correlated with tumor invasion and metastasis (Hida *et al*., 1997b). On the other hand, in pancreatic and thyroid carcinomas, where Ets1 is expressed in tumor cells, there was no correlation between Ets1 expression and metastasis in these tumors (Ito *et al*., 1998b; Nakayama *et al*., 1999). The role of Ets1 in these tumors is presently unknown. Thus, *in vivo* studies of tumors indicate that abnormally expressed Ets factors are found in distinct compartments: tumor or stroma. While coexpression of Ets and MMPs in stroma is well documented, little is known about other genes controlled by Ets factors *in vivo* in stromal cells. The likely candidates based on *in vitro* studies include tenascin (Shirasaki *et al*., 1999) and various integrins (Rosen *et al*., 1994). As mentioned already, the role of Ets factors in tumor cells is presently unclear; this aspect of Ets function requires further investigation. Finally, the nature of the upstream factors responsible for expression of Ets in various tumour compartments remains to be elucidated.

Ets factors in autoimmune diseases

In addition to tumors, excessive expression of MMPs was also documented in other pathological conditions, including rheumatoid arthritis and osteoarthritis (Vincenti *et al*., 1994; Malemud and Goldberg, 1999). There are, however, very limited *in vivo* data regarding the possible role of Ets factors in these pathological processes. For example, Ets2 expression has been observed in 30% of patients with rheumatoid arthritis, but MMP expression has not been evaluated in this study (Dooley *et al*., 1996). In addition to rheumatoid arthritis, aberrant expression of Ets factors, particularly Ergb/Fli1 and Ets2, has been observed in other autoimmune diseases such as systemic lupus erythematosus (SLE) and Sjogren's syndrome (Georgiou *et al*., 1996). Elevated expression of Ergb/Fli1 was also present in infiltrating lymphocytes of the HTLV-I-Tax1 transgenic mice, considered an animal model of the autoimmune disease (Georgiou *et al*., 1996). Finally, transgenic mice that overexpress Fli1 develop progressive immunological renal disease and ultimately die of renal failure caused by tubulointerstitial nephritis and glomerulonephritis (Zhang *et al*., 1995). Massive accumulation of lymphocytes overexpressing Fli1 was detected in kidney tissues of the transgenic animals. The target genes that are controlled directly or indirectly by Fli1 are not known, but one can postulate that adhesion molecules as well as other genes that direct immune cells to the sites of inflammation are likely targets of Fli1 (Rosen *et al*., 1994). Thus, there is substantial evidence that elevated expression of Fli1 contributes to the invasive phenotype of lymphocytes and may play a major role in the development of autoimmune diseases (for review on Fli1 function in normal and transformed cells, see Truong and Ben-David, this issue).

Ets factors and bone formation

The role of Ets factors in bone and cartilage development has been implicated by several early *in situ* hybridization studies. Collectively, these studies demonstrate expression of Ets1 and Erg at various sites of bone and cartilage formation during murine and avian development (Kola *et al*., 1993; Maroulakou *et al*., 1994; Dhordain *et al*., 1995). Importantly, increased dosage of Ets2 has led to skeletal abnormalities characteristic of Down syndrome suggesting a specific role for Ets2 in skeletal development and in the pathology of Down syndrome (Sumarsono *et al*., 1996). Recent studies have begun to address the nature of the Ets target genes during osteogenesis, as well as the specificity of the Ets factors involved in this process. Using an osteoblast-like MC3T3-E1 cell line that recapitulates the specific stages of bone development *in vitro*, the expression of various Ets family members was compared to that of other stage-specific genes. Among the Ets genes assayed, only Ets1 and Ets2 were found to be expressed at significant levels in this system. Ets1 expression was mainly observed in the proliferation stage, while Ets2 expression was induced during differentiation and subsequent mineralization stages suggesting distinct functions for these two closely related Ets factors. Furthermore, evidence is presented that a non-collagenous ECM protein, osteopontin, is regulated by Ets2 during differentiation and mineralization stages (Vary *et al*., 2000).

The specific role for the two Erg isoforms, *ch*-Erg and a splice variant termed C-1-1, has been proposed in the process of chondrogenesis (Iwamato *et al*., 2000). Using the developing chick limb as a model, it has been observed that C-1-1 and *ch*-Erg have distinct expression patterns and different biological activities. Furthermore, ectopic expression of C-1-1, but not *ch*-ERG, led to induction of tenascin-C (TN-C), suggesting that TN-C may be directly regulated by C-1-1 *in vivo*. Consistent with this observation, a closely related subfamily member Fli1, was shown to activate TN-C expression in human dermal fibroblasts (Shirasaki *et al*., 1999).

Together, these studies support the role for Ets1 and Erg subfamilies in bone and cartilage development. It is becoming clear that at the specific stages of this tightly regulated process, individual Ets factors will play unique roles in regulating specific subsets of genes, including ECM genes. The experimental systems combining *in vitro* and *in vivo* approaches should lead to a better understanding of the role of Ets factors in bone and cartilage formation, not only during development, but also in pathologic human conditions.

A more detailed discussion of this topic is presented by Seth and Raouf in a separate article of this issue.

Ets expression in wound healing

Because of the active tissue remodeling that takes place during wound healing (Parks, 1999), one could also postulate a role for Ets factors herein. The stages of cutaneous wound repair are relatively well defined and include inflammation, tissue formation, and tissue remodeling (Singer and Clark, 1999). MMPs are largely undetectable in normal skin. Upon injury, tissue-type plasminogen activator (tPA) and uPA as well as specific subsets of MMPs are induced in various skin compartments (Martin, 1997). The migrating keratinocytes at the leading-edge express collagenase-1 (MMP-1), stromelysin-2 (MMP-10), and gelatinase-B (MMP-9) as well as tPA and uPA, which facilitate their movement through the fibrin clot and along the basal lamina. Once reepithelialization is complete, MMP expression by keratinocytes is turned off (Parks, 1999). Dermal cells, including fibroblasts, endothelial cells, and perivascular cells express gelatinase-A (MMP-2), MT1-MMP (MMP-14), stromelysin-1 (MMP-3), collagenase-1 (MMP-1), and collagenase-3 (MMP-13). Infiltrating immune cells, predominantly neutrophils and macrophages, secrete collagenase-2 (MMP-8) and gelatinase-B (MMP-9). Expression of MMPs by dermal cells continues during late stages of repair where it contributes to collagen remodeling during the transition from granulation tissue to scar. Thus during repair, specific subsets of MMPs are produced by specific cell types in different wound compartments. Further work is required to determine the physiological role of these specific enzymes. Moreover, little is known about how their expression is regulated *in vivo*, in particular the nature of the 'turn-on' and 'turn-off' signals. Growth factors and matrix signals are undoubtedly involved. Because of the redundancy and cross-talk between different pathways, a clear view of the *in vivo* regulation of gene expression during wound healing is currently lacking.

So far, there are only limited studies that address the role of Ets factors during wound healing. For example, elevated expression of Ets1 and collagenase-1 (MMP-1) was observed in fibroblasts and endothelial cells in the early healing stage of gastric ulcer before declining to control levels at later stages (Ito *et al*., 1998a). Rapid induction of Ets1 was also reported in smooth muscle cells after balloon injury preceding smooth muscle cell migration and proliferation (Hultgardh-Nilsson *et al*., 1996). It is significant that in these two models Ets expression is transient. One can speculate that the stringent regulation of Ets expression during physiological processes and its dysregulated expression in tumors may be one of the early critical points that distinguishes between controlled and uncontrolled matrix remodeling.

Ets and the regulation of MMPs in experimental models

In vitro experimental models using cultured cells allow elucidation of the molecular and cellular mechanisms that lead to dysregulated expression of Ets. These experiments also provide more direct evidence for the role of Ets in regulating specific genes. Despite the progress that has been made in these areas, many unresolved issues remain. For example, how do specific signaling pathways regulate expression and function of Ets genes? What are the tissue-specific target genes regulated by a particular Ets factor? What are the molecular mechanisms that regulate tissue and target gene specificity?

As described above, *in vivo* studies of human carcinomas have shown that Ets is correlated with invasive phenotype. Furthermore, expression of Ets1 was limited to stromal cells in the vicinity of the tumors, whereas surrounding uninvolved tissue was negative for Ets1 expression (Bolon *et al*., 1995; Wernert *et al*., 1994). The mechanism of the *in vivo* activation of Ets1 gene expression in stromal fibroblasts is not known. It could be induced by factors secreted by tumor cells or could result from the host immune response to the tumor. Co-culture experiments of the human keratinocyte squamous cell carcinoma cell line II4 with primary human fibroblasts suggest that cell–cell contact as well as tumor cell-derived soluble factors may play a role in Ets1 induction in fibroblasts. Increased expression of uPa and the 92 kDa type IV collagenase (MMP-9) were also observed under these experimental conditions (Borchers *et al*., 1994). Possible cytokines involved in this induction include IL-1α, TNFα, PDGF, bFGF and EGF (Gilles *et al*., 1996).

As discussed in the previous section, in some tumors Ets1 is also expressed in tumor cells that are derived from the epithelia (Nakayama *et al*., 1996). While it was originally felt that Ets1 is not expressed in the epithelial cells, recent studies have shown that Ets1 is transiently expressed in the epithelial structures during migration of neural crest cells and dispersion of somites (Fafeur *et al*., 1997). Furthermore, the authors of this study have demonstrated that scatter factor/hepatocyte growth factor (SF/HGF) induces Ets1, uPa, and collagenase-1 (MMP-1) *in vitro* in epithelial MDCK cells, suggesting that Ets1 may be an effector of SF/HGF during epithelial mesenchymal transition. In fact, SF/HGF and its receptor c-Met are also expressed during emigration of neural crest cells (Fafeur *et al*., 1997). Thus, re-expression of Ets1 in epithelial tumor cells may be a reversal to the embryonic genetic program and may be dependent on the stromal fibroblast derived SF/HGF.

Evidence that ETS genes are necessary for the invasive phenotype

A direct link between Ets1 induced production of proteolytic enzymes and tumor invasiveness also requires demonstrating the reversal of tumor invasion phenotypes by reducing Ets function. Two *in vitro* strategies have been successfully employed recently, using antisense constructs or overexpressing dominant-interference Ets mutants. It has been shown that treatment of a glioma cell line with antisense Ets1 oligonucleotides concurrently inhibited the expression of Ets1 and uPa, and blocked glioma cell migration and invasion (Kitange *et al*., 1999). Similarly, inhibition by antisense oligonucleotides of a different

member of Ets family, E1AF, in a squamous cell carcinoma cell line has led to inhibition of basal and HGF-induced MMP-9 expression and invasive potential of these cells (Hida *et al*., 1997a; Hanzawa *et al*., 2000). Blocking of Ets function by overexpressing a mutant of Ets1 lacking its activation domain (dominant-interference) has also led to similar phenotypic changes. Delannoy-Courdent *et al*., (1998) have demonstrated that stable expression of Ets1-DBD (contains only DNA binding domain) in epithelial cell lines derived from normal and cancerous mouse mammary tissues inhibited expression of uPA and decreased cell migration and invasion of both cell types. It also impaired the capacity to form tubules by normal mammary cells. In a different experimental system, Kim *et al*., (2000) have shown that overexpression of Epstein–Barr virus latent membrane protein 1 (LMP1) in MDCK epithelial cells leads to a transformed phenotype which correlates with increased expression of Ets1, PAI-1 and uPA. Expression of a dominant-interference Ets1 mutant in LMP1-transformed cells resulted in decreased of uPA expression and cell motility, suggesting that the transformed phenotype in this experimental system is at least partially mediated by Ets1. These experiments demonstrate that Ets function is necessary for expression of certain matrix degrading enzymes and invasive phenotype in *in vitro* experimental models. It will be critical to extend these approaches to the animal models and demonstrate that inhibition of ets function would affect tumor invasion *in vivo* (see also review on Ets target genes by Sementchenko and Watson, this issue).

Ets transcription factors function in a cell context-dependent manner

Unlike other families of transcription factors such as homeobox proteins, whose members bind to diverse DNA sequences, all Ets factors recognize a core DNA motif GGAA/T (reviewed by Wasylyk *et al*., 1993; Graves and Peterson, 1998). However, each Ets factor in its unique *in vivo* context must be able to recognize a specific subset of targets. The GGAA/T motif has been functionally characterized in many target genes including those for MMPs (Crawford and Matrisian, 1996) and other matrix related proteins. Historically, this motif has been termed PEA3 (polyomavirus enhancer A-binding protein-3). More recently the term EBS (Ets Binding Site) has generally been adopted. The section below provides an overview of the promoter studies for which the role of Ets factors has been established. Specific examples are used to illustrate cellular mechanisms that may afford the specificities of Ets-mediated gene regulation. Collectively, these studies show that there is a high degree of selectivity between different Ets family members for specific target genes. The complexity involved in gene regulation by Ets factors has only started to be unraveled. The future challenge will be to understand how this specificity is achieved.

Ets factors as the effectors of Ras–MAP kinase and other signaling pathways

The studies of transcriptional regulation of the uPA gene nicely illustrate the involvement of Ets factors as the effectors of Ras–MAP kinase signaling pathway. A composite PEA3/AP1 site was characterized in the murine uPA enhancer and shown to play a major role in the activity of this enhancer. Interestingly, the relative contribution of this element varied among different transformed cell lines (Nerlov *et al*., 1991). An additional functional PEA3/AP1 site was also found in the far upstream region of this gene (D'Orazio *et al*., 1997). One of the early studies has also shown that the PEA3/AP1 element mediates the EGF response in murine keratinocytes (Rorth *et al*., 1990). In the subsequent studies, the signal transduction pathway involved in regulation of this gene by RTK (receptor tyrosine kinase) and TPA have been delineated. Besser *et al*., (1995) have utilized wild-type and dominant-negative mutants of various signaling molecules to demonstrate the involvement of the Ras/Raf-1/MEK/ERK-2 pathway in the stimulation of the uPA gene by TPA and FGF-2 in NIH3T3 cells. Using a similar approach, the involvement of c-Ha-*ras*, c-*raf*, and ERKs in induction of the uPA promoter has also been demonstrated in ovarian adenocarcinoma cell line (OVCAR-3) by Lengyel *et al*., (1995b). Additional components of this pathway that may also contribute to regulation of the uPA promoter via the composite EBS/AP1 element have been characterized in the response of NIH 3T3 cells stably overexpressing c-Met receptor to HGF/SF (Ried *et al*., 1999). Besides the previously characterized mediators such as c-Ha-Ras/c-Raf, Mek1, and Erk, this study has identified Grb2, Sos1, and RhoA as critical mediators of this response. A role for RhoA in regulating uPA gene expression is of special interest because it may help to explain the mechanism of the Rho-mediated cell transformation. A different member of the Ras superfamily, Rac1, had no effect on uPA expression. Interestingly, although PI3-kinase is induced by HFG/SF, it had no effect on the expression of the uPA promoter (Ried *et al*., 1999).

Ets2, as well as Ets1 has been shown to mediate induction of the uPA promoter in response to growth factors. Ectopic expression of wild-type Ets2 potentiated stimulation of this promoter by TPA and FGF-2, while a dominant-interference Ets2 abrogated growth factor responses (D'Orazio *et al*., 1997). In a different study, a breast cancer cell line that overexpresses Erb-2/neu protein was used to demonstrate the specificity among different Ets family members in activating the uPA promoter (Watabe *et al*., 1998). The cotransfections of either Ets1 or Ets2 potentiated EGF stimulation of this promoter, while cotransfection with two other members of the Ets family, Fli1 and E1AF/PEA3, did not have a stimulatory effect. Ets1 and Ets2 contain the conserved Pointed domain also present in *Drosophila* Ets-like protein, Pointed P2 (see Hsu and Schultz, this issue). A conserved threonine within the Pointed domain is a direct target for MAP kinase (McCarthy *et al*., 1997). Importantly, it was shown that Ets2 carrying a mutation in the MAP kinase phosphorylation site failed to activate the uPA promoter in response to EGF (Watabe *et al*., 1998). Stimulation of uPA promoter by CSF-1 (colony stimulating factor-1) also involves phosphorylation of Ets2 by MAP kinase (Fowles *et al*., 1998). These results demonstrate a role for Ets2 as an effector of the MAPK pathway cascade initiated by the activation of

RTK. Significantly, it was shown that ovarian carcinoma cell lines with activated Ras/MAP kinase pathway were also characterized by the presence of phosphorylated Ets2 and increased expression levels of uPA gene (Patton *et al*., 1998).

While the above studies strongly support the role of EBS/AP1 as a MAPK target in the TPA and RTK induced signaling cascades, there is evidence that other signaling pathways may converge at this site. For example, it has been shown that Ets2 in cooperation with ATF-2 and c-Jun mediates activation of the uPA promoter by IL-1 in HepG2 cells (Cirillo *et al*., 1999). The activation of the Jun N-terminal kinase (JNK) was required for stimulation of the uPA gene by IL-1 as well as by TPA in these cells. The PEA3/Ap1 element has also been shown to mediate the TNF-α response in a squamous cell carcinoma cell line, but the specific signaling pathway involved in this stimulation has not been examined (Lengyel *et al*., 1995a). More detailed discussion on the Ets response to signal transduction pathways is provided in a separate review in this issue (Yordy and Muise-Helmericks).

Partnership of Ets and Jun in regulation of MMPs and TIMP

The Ets factors require cofactors for optimal activity. Members of the Jun family cooperate with Ets in regulation of matrix degrading proteases. The most fully characterized model systems are stromelysin-1 (MMP-3) and collagenase-1 (MMP-1) promoters. Early studies have demonstrated the presence of the EBSs in close proximity to the AP1 sites in the stromelysin-1 (MMP-3) and collagenase-1 (MMP-1) promoters. These sites contributed to both basal activity and TPA induction of these promoters (Wasylyk *et al*., 1991; Auble and Brinckerhoff, 1991; Wasylyk and Wasylyk, 1992; Buttice and Kurkinen, 1993). Subsequent studies have revealed that distinct Ets family members modulate AP-1 dependent regulation of these promoters differently (Buttice *et al*., 1996; Westermarck *et al*., 1997). Interestingly, depending on the promoter context, Erg elicits dual function. It is a potent activator of the collagenase-1 (MMP-1) promoter, while it inhibits Ets2 mediated activation of the stromelysin-1 (MMP-3) promoter (Buttice 1996). The molecular basis of this promoter dependent repressor versus activator mode of Erg is presently not known, however several recent studies have begun to address this issue. The study by Basuyaux *et al*., (1997) has demonstrated that Ets2 physically interacts with the Fos/Jun complex via its DNA binding domain (DBD). More significantly, this interaction was stabilized by the presence of DNA fragments specific for the stromelysin-1 (MMP-3) promoter. It was also shown that Ets2 interacts with Ets1 and Erg. Unlike its interaction with Fos/Jun (Basuyaux *et al*., 1997), the interaction with Erg was DNA-independent and involved transactivation (TA) domain of Ets2, in addition to the DBD domain. The authors of this study propose that Erg may directly interfere with Ets2 either by masking the TA domain or by blocking the DBD to prevent Ets2 from binding to DNA.

Recruitment of rate-limiting coactivators such as CBP/p300 is an important regulatory mechanism for a large number of transcription factors (Goodman and Smolik, 2000). Recent studies have shown that CREB binding protein (CBP) and p300 bind to Ets1 and facilitate Ets1 trans-activating function (Yang *et al*., 1998). CBP/p300 has also been shown to cooperate with Ets1 and Ets2 in activating the stromelysin-1 (MMP-3) promoter (Jayaraman *et al*., 1999). Consistent with the earlier findings (Buttice *et al*., 1996), Erg2 and PEA3 did not cooperate with p300 in the context of the stromelysin-1 (MMP-3) promoter. Significantly, Erg2 and PEA3 were able to cooperate with p300 in the context of a different promoter. This result clearly underscores the importance of the promoter-specific sequences in assembling the 'appropriate' combination of transcription factors. These recent studies begin to unravel the complex protein-protein interactions between specific members of the Ets family and other components of the transcriptional machinery involved in regulation of Ets target genes.

Other recently characterized members of the Ets family may also contribute to regulation of collagenase-1 (MMP-1), stromelysin-1 (MMP-3) and other MMPs *in vivo*. For example, E1AF/hPEA3 is a potent activator of the stromelysin-1 (MMP-3), collagenase-1 (MMP-1), and type IV collagenase (MMP-9) promoters in transient transfection assays (Higashino *et al*., 1995). The adjacent PEA3 and AP1 motifs were characterized in the MMP-9 promoter and shown to mediate the induction of this promoter by *ras* (Gum *et al*., 1996). A functional EBS adjacent to the AP1 site has also been characterized in the TIMP-1 promoter (Edwards *et al*., 1992; Logan *et al*., 1996). Whereas overexpression of Ets1 alone did not activate this promoter in F9 embryonic carcinoma cell line, c-Ets1 synergized with AP1 in activating transcription of the TIMP-1 promoter in these cells (Logan *et al*., 1996). On the other hand, a newly characterized member of the Ets subfamily, EHF (ets homologous factor), represses Ets2 stimulation of the stromelysin-1 (MMP-3) and collagenase-1 (MMP-1) promoters in transient transfections. The pattern of EHF expression in normal versus tumor tissues suggests that it may function as a tumor suppressor gene (Kleinbaum *et al*., 1999).

Sp1 and Sp3 are cofactors of Ets in regulation of ECM genes

While ECM degrading proteases have been well recognized as target genes for Ets factors, recent studies suggest that ECM proteins as well as other cellular components involved in matrix regulation may also be directly regulated by Ets factors.

As mentioned in the previous section, TN-C is regulated by the member of Erg subfamily *in vivo* (Iwamoto *et al*., 2000). Regulation of TN-C by Ets factors has been investigated in human fibroblasts (Shirasaki *et al*., 1999). Four functional EBSs were identified in the human TN-C promoter. Two of these EBS contained a tandem repeat of the GGA motif, characteristic of the GABPα/β binding sites. *In vitro* binding assays have demonstrated that in human fibroblasts GABPα/β interact with these motifs. Three of the EBS sites, including both GABPα/β binding sites, contributed to the basal activity of the promoter in human fibroblasts, while all four sites were important for the transactivation of this promoter by

exogenous Fli1. Fli1 strongly activated the TN-C promoter, while Ets1 and Ets2 were weak activators. Furthermore, experiments in *Drosophila* Schneider SL cells have shown that either Fli1 or GABPα/β cooperate with Sp1 in activation of TN-C promoter (Shirasaki *et al*., 1999).

Recent work from our laboratory has demonstrated that Fli1 and Ets1 contribute to regulation of the human collagen type I gene in dermal fibroblasts. A functional EBS was identified in the collagen α2(I) promoter in close proximity to the Sp1 sites. Ets1 and Fli1 had opposite effects on the activity of this promoter: Ets1 stimulated while Fli1 inhibited the promoter activity. Significantly, Sp1 binding was essential for the inhibitory effect of Fli1. Thus, in the collagen promoter context, Sp1 functions as a corepressor of Fli1, while in the TN-C promoter context Sp1 functions as a coactivator of Fli1. Given the prominent role of Sp1 factors in regulation of the various ECM genes (Trojanowska *et al*., 1998), their interactions with Fli1 and possibly other Ets factors may provide important clues for the mechanisms controlling ECM gene expression. Consistent with the promoter data, overexpression of Fli1 in dermal fibroblasts led to a dramatic decrease in collagen mRNA and protein levels (Czuwara-Ladykowska *et al*., submitted).

Other experimental models support the role of Ets factors in control of the ECM genes. For example, overexpression of Tel in NIH3T3 cells resulted in unusual morphological changes, which partially resembled tube formation characteristic of endothelial cells. This phenotype was associated with upregulation of matrix components, including entactin/nidogen, collagen type III, and fibronectin, but down-regulation of collagen type I. Interestingly, Smad5, a downstream effector of BMP signaling was also upregulated (Van Rompaey *et al*., 1999). Extracellular deposition of collagen was also inhibited in NIH3T3 cells upon exogenous expression of EWS/FLI and EWS/ETV (Teitell *et al*., 1999).

Conclusions and future perspective

The role of the Ets family of transcription factors in regulation of ECM remodeling has now been firmly established. While traditionally the emphasis was on the role of Ets in regulating degradative enzymes in tumorogenesis, the expanding spectrum of target genes, especially collagens, suggest that Ets factors may also contribute to the aberrant synthesis of ECM in stroma of solid tumors. In fact, active synthesis of type I and III collagen has been correlated with aggressive forms of ovarian, breast, and uterine cancers (Santala *et al*., 1998; Kaupilla *et al*., 1998, 1999). Although at present experimental evidence is lacking, it is reasonable to postulate that Ets factors would also contribute to the excessive collagen deposition in fibrotic diseases. While the ultimate outcomes of tumor invasion and organ fibrosis appear to be very different, destruction versus overproduction of ECM, in both cases the activated fibroblast (myofibroblast) seem to play a major role. In tumors the balance is tilted towards MMP production, while in fibrosis collagen synthesis predominates. It is intriguing that Ets1 is involved in regulation of both types of genes. However, regulation of MMPs requires cooperation between AP1 and Ets. Although data are limited, it appears that Sp1 is an Ets partner in regulating matrix genes. Thus, in the same cell Ets may differentially regulate MMPs and collagen via association with different co-factors on the respective promoters. The nature of the factors that affect the balance between synthetic and degradative pathways are yet to be determined. We can also postulate that the preferential expression of one class of genes (MMPs) versus another (collagens) depends on the activation of different signaling pathways. For example, TGFβ induced signaling dominates in fibrosis, while RTK and TNF-α in tumor invasion and arthritis. In turn, these signaling pathways can modulate relative expression levels of the specific Ets factors, as well as the activation status of Ets factors and their transcriptional partners (Figure 1).

In this article, we have focused on the role of Ets factors in regulating ECM remodeling. It should be noted that Ets factors only represent one link in the complex cellular network governing the expression of ECM proteins and enzymes that degrade them. Such a network is likely initiated by cell interaction with cytokines, various matrix molecules, as well as neighboring cells. These interactions then lead to activation of specific signaling pathways that need to be integrated in a precise manner. Disruption of these pathways will result in an imbalance in the regulation of ECM components, which in turn leads to various pathological processes. Since excessive production and excessive degradation are both pathological, it is imperative to carefully consider these issues when designing therapeutic strategies.

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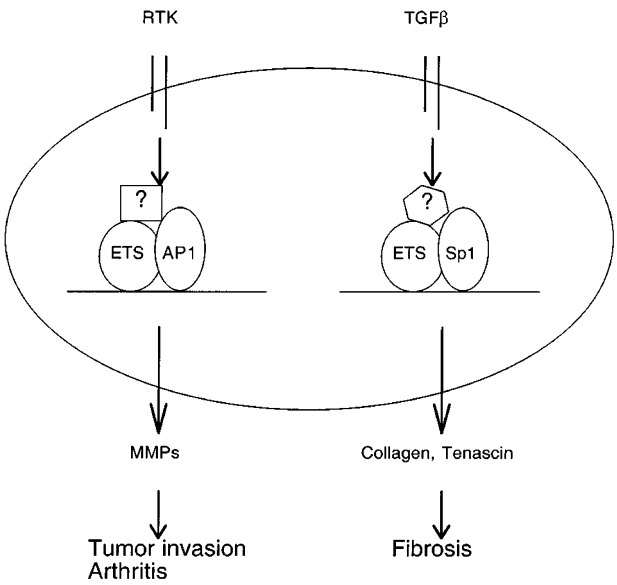

Figure 1 Ets proteins are mediators of signal transduction pathways. A model for the role of Ets factors as the effectors of the RTK and TGF-β signaling pathways. The promoter specific transcription complexes are formed in response to activation of specific signal transduction pathways

References

Auble DT and Brinckerhoff CE. (1991). *Biochem.,* **30,** 4629–4635.

Basuyaux JP, Ferreira E, Stehelin D and Buttice G. (1997). *J. Biol. Chem.,* **272,** 26188–26195.

Besser D, Presta M and Nagamine Y. (1995). *Cell Growth Diff.,* **6,** 1009–1017.

Bolon I, Gouyer V, Devoassoux M, Vandenbunder B, Wernert N, Moro D, Brambilla C and Brambilla E. (1995). *Am. J. Pathol.,* **147,** 1298–1310.

Borchers AH, Powell MB, Fusenig NE and Bowden GT. (1994). *Exp. Cell Res.,* **213,** 143–147.

Buttice G and Kurkinen M. (1993). *J. Biol. Chem.,* **268,** 7196–7204.

Buttice G, Duterque-Coquillaud M, Basuyaux JP, Carrere S, Kurkinen M and Stehelin D. (1996). *Oncogene,* **13,** 2297–2306.

Calmels TPG, Mattot V, Wernert N, Vandenbunder B and Stehelin D. (1995). *Biol. Cell,* **84,** 53–61.

Cirillo G, Casalino L, Vallone D, Caraciolo A, de Cesare D and Verde P. (1999). *Mol. Cell. Biol.,* **19,** 6240–6252.

Crawford HC and Matrisian LM. (1996). *Enzyme Protein.,* **49,** 20–37.

Curran S and Murray GI. (1999). *J. Pathol.,* **189,** 300–308.

Delannoy-Courdent A, Mattot V, Fafeur V, Fauquette W, Pollet I, Calmels T, Vercamer C, Boilly B, Vandenbunder B and Desbiens X. (1998). *J. Cell Science.,* **111,** 1521–1534.

Dhordain P, Dewitte F, Desbiens X, Stehelin D and Duterque-Coquillaud M. (1995). *Mech. Dev.,* **50,** 17–28.

Dooley S, Herlitzka I, Hanselmann R, Ermis A, Henn W, Remberger K, Hopf T and Welter C. (1996). *Ann. Rheum. Dis.,* **55,** 298–304.

D'Orazio D, Besser D, Marksitzer R, Kunz C, Hume DA, Kiefer B and Nagamine Y. (1997). *Gene,* **201,** 179–187.

Edwards DR, Rocheleau H, Sharma RR, Wills AJ, Cowie A, Hassell JA and Heath JK. (1992). *Biochim. Biophys. Acta,* **1171,** 41–55.

Fafeur V, Tulasne D, Queva C, Vercamer C, Dimster V, Mattot V, Stehelin D, Desbiens X and Vandenbunder B. (1997). *Cell Growth Differ.,* **8,** 655–665.

Forget MA, Desrosiers RR and Beliveau R. (1999). *Can. J. Physiol. Pharmacol.,* **77,** 465–480.

Fowles LF, Martin ML, Nelsen L, Stacey KJ, Redd D, Clark YM, Nagamine Y, McMahon M, Hume DA and Ostrowski MC. (1998). *Mol Cell Biol.,* **18,** 5148–5156.

Georgiou P, Maroulakou I, Green JE, Dantis P, Romano-Spica V, Kottaridis S, Lautenberger JA, Watson DK, Papas TS, Fischinger PJ and Bhat NK. (1996). *Int. J. Oncol.,* **9,** 9–18.

Gilles F, Raes M-B, Stehelin D, Vandenbunder B and Fafeur D. (1996). *Exp. Cell Res.,* **222,** 370–378.

Goodman RH and Smolik S. (2000). *Genes Dev.,* **14,** 1553–1577.

Graves BJ and Petersen JM. (1998). *Adv. Cancer Res.,* **75,** 1–55.

Gum R, Lengyel E, Juarez J, Chen JH, Sato H, Seiki M and Boyd D. (1996). *J. Biol. Chem.,* **271,** 10672–10680.

Hanzawa M, Shindoh M, Higashino F, Yasuda M, Inoue N, Hida K, Ono M, Kohgo T, Nakamura M, Notani K, Fukada F, Totsuka Y, Yoshida K and Fujinaga K. (2000). *Carcinogenesis,* **21,** 1079–1085.

Hida K, Shindoh M, Yasuda M, Hanzawa M, Funaoka K, Kohgo T, Amemiya A, Totsuka Y, Yoshida K and Fujinaga K. (1997a). *Am. J. Pathol.,* **150,** 2125–2132.

Hida K, Shindoh M, Yoshida K, Kudoh A, Furaoka K, Kohgo T, Fujinaga K and Totsuka Y. (1997b). *Oral Oncol.,* **33,** 426–430.

Higashino F, Yoshida K, Noumi T, Seiki M and Fujinaga K. (1995). *Oncogene,* **10,** 1461–1463.

Hultgardh-Nilsson A, Cercek B, Wang J-W, Naito S, Lovdahl C, Sharifi B, Forrester JS and Fagin JA. (1996). *Circ. Res.,* **78,** 589–595.

Ito M, Nakayama T, Naito S, Matsuu M, Scichijo K and Sekine I. (1998a). *Biochim. Biophys. Res. Com.,* **246,** 123–127.

Ito T, Nakayama T, Ito M, Naito S, Kanematsu T and Sekine I. (1998b). *Mod. Pathol.,* **11,** 209–215.

Iwamoto M, Higuchi Y, Koyama E, Enomoto-Iwamoto M, Kurisu K, Yeh H, Abrams WR, Rosenbloom J and Pacifici M. (2000). *J. Cell Biol.,* **150,** 27–39.

Jayaraman G, Srinivas R, Duggan C, Ferreira E, Sathyamangalam S, Somasundaram K, Williams J, Hauser C, Kurkinen M, Dhar R, Weitzman S, Buttice G and Thimmapaya B. (1999). *J. Biol. Chem.,* **274,** 17342–17352.

Johansson N and Kahari V-M. (2000). *Histol. Histopathol.,* **15,** 225–237.

Kauppila S, Stenback F, Risteli J and Risteli L. (1998). *J. Pathol.,* **186,** 262–268.

Kauppila S, Stenback F, Kacinski BM, Carcangiu M-L, Risteli J and Risteli L. (1999). *Cancer,* **86,** 1299–12306.

Kim K-R, Yoshizaki T, Miyamori H, Hasegawa K, Horikawa T, Furukawa M, Harada S, Seiki M and Sato H. (2000). *Oncogene,* **19,** 1764–1771.

Kitange G, Shibata S, Tokunaga Y, Yagi N, Yasunaga A, Kishikawa M and Naito S. (1999). *Lab. Invest.,* **79,** 407–416.

Kleinbaum LA, Duggan C, Ferreira E, Coffey GP, Buttice G and Burton F. (1999). *Biochem. Biophys. Res. Com.,* **264,** 119–126.

Kola I, Brookes S, Green AR, Garber R, Tymms M, Papas TS and Seth A. (1993). *Proc. Natl. Acad. Sci. USA,* **90,** 7588–7592.

Lengyel E, Klostergaard J and Boyd D. (1995a). *Biochem. Biophys. Acta,* **1268,** 65–72.

Lengyel E, Stepp E, Gum R and Boyd D. (1995b). *J. Biol. Chem.,* **270,** 23007–23012.

Logan SK, Garabedian MJ, Campbell CE and Werb Z. (1996). *J. Biol. Chem.,* **271,** 774–782.

Maroulakou IG, Papas TS and Green JE. (1994). *Oncogene,* **9,** 1551–1565.

Martin P. (1997). *Science,* **276,** 75–81.

Malemud CJ and Goldberg VM. (1999). *Front. Biosci.,* **4,** D762–D771.

McCarthy SA, Chen D, Yang BS, Ramirez JJG, Cherwinski H, Chen XR, Klagsbrun M, Hauser CA, Ostrowski MC and McMahon M. (1997). *Mol. Cell. Biol.,* **17,** 2401–2412.

Naito S, Sgimizu K, Nakashima M, Nakayama T, Ito T, Ito M, Yamashita S and Sekine I. (2000). *Pathol. Res. Pract.,* **196,** 103–109.

Nakayama T, Ito M, Ohtsuru A, Naito S, Nakashima M, Fagin JA, Yamashita S and Sekine I. (1996). *Am. J. Pathol.,* **149,** 1931–1939.

Nakayama T, Ito M, Ohtsuru A, Naito S, Nakashima M and Sekine I. (1999). *Mod. Pathol.,* **12,** 61–68.

Nerlov C, Rorth P, Blasi F and Johnsen M. (1991). *Oncogene.,* **6,** 1583–1592.

Nishioka Y, Kobayashi K, Sagae S, Ishioka Si, Nishikawa A, Matsushima M, Kanamori Y, Minaguchi T, Nakamura Y, Tokino T and Kudo R. (2000). *Jpn. J. Cancer Res.,* **91,** 612–615.

Patton SE, Martin ML, Nelsen LL, Fang X, Mills GB, Bast RC and Ostrowski MC. (1998). *Cancer Res.,* **58,** 2253–2259.

Parks WC. (1999). *Wound Rep. Reg.,* **7,** 423–432.

Ried S, Jager C, Jeffers M, Vande Woude GF, Graeff H, Schmitt M and Lengyel E. (1999). *J. Biol. Chem.,* **274,** 16377–16386.

Rosen GD, Barks JL, Iademarco MF, Fisher RJ and Dean DC. (1994). *J. Biol. Chem.,* **269,** 15652 – 15660.
Rorth P, Nerlov C, Blasi F and Johnsen M. (1990). *Nucl. Acids Res.,* **18,** 5009 – 5017.
Rutter JL, Mitchel TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ and Brinckerhoff CE. (1998). *Cancer Res.,* **58,** 5321 – 5325.
Santala M, Risteli J, Risteli L, Puistola U, Kacinski BM, Stanley ER and Kaupilla A. (1998). *British J. Cancer,* **77,** 1825 – 1831.
Shirasaki F, Makhluf HA, LeRoy C, Watson DK and Trojanowska M. (1999). *Oncogene,* **18,** 7755 – 7764.
Singer AJ and Clark RAF. (1999). *New England. J. Med.,* **341,** 738 – 746.
Stracke ML, Murata J, Aznavoorian S and Liotta LA. (1994). *In vivo,* **8,** 49 – 58.
Sumarsono SH, Wilson TJ, Tymms MJ, Venter DJ, Corrick CM, Kola R, Lahoud MH, Papas TS, Seth A and Kola I. (1996). *Nature,* **379,** 534 – 537.
Teittell MA, Thompson AD, Sorensen PHB, Shimada H, Triche TJ and Denny CT. (1999). *Lab. Invest.,* **79,** 1535 – 1543.
Trojanowska M, LeRoy EC, Eckes B and Krieg T. (1998). *J. Molec. Med.,* **76,** 266 – 274.
Van Rompaey L, Dou W, Buijs A and Grosveld G. (1999). *Neoplasia,* **1,** 526 – 536.
Vary CPH, Li V, Raouf A, Kitching R, Kola I, Franceschi C, Venanzoni M and Seth A. (2000). *Exp. Cell Res.,* **257,** 213 – 222.
Vincenti MP, Clark IM and Brinckerhoff CE. (1994). *Arthritis Rheum.,* **37,** 1115 – 1126.
Wasylyk C, Gutman A, Nicholson R and Wasylyk B. (1991). *EMBO J.,* **10,** 1127 – 1134.
Wasylyk C and Wasylyk B. (1992). *Cell Growth Differ.,* **3,** 617 – 625.
Wasylyk B, Hahn SL and Giovane A. (1993). *Eur. J. Biochem.,* **211,** 7 – 18.
Watabe T, Yoshida K, Shindoh M, Kaya M, Fujikawa K, Sato H, Seiki M, Ishii S and Fujinaga K. (1998). *Int. J Cancer,* **77,** 128 – 137.
Wernert N, Gilles F, Fafeur V, Bouali F, Raes M-B, Pyke C, Dupressoir T, Seitz G, Vandenbounder B and Stehelin D. (1994). *Cancer Res.,* **54,** 5683 – 5688.
Wernert N. (1997). *Virchows Arch.,* **430,** 433 – 443.
Westermarck J, Seth A and Kahari V-M. (1997). *Oncogene,* **14,** 2651 – 2660.
Yang C, Shapiro LH, Rivera M, Kumar A and Brindle PK. (1998). *Mol. Cell. Biol.,* **18,** 2218 – 2229.
Zhang L, Eddy A, Teng Y-T, Fritzler M, Kluppel M, Melet F and Bernstein A. (1995). *Mol. Cell. Biol.,* **15,** 6961 – 6970.