Modulation of the Signal Recognition Particle 54-kDa Subunit (SRP54) in Rat Preosteoblasts by the Extracellular Matrix*

(Received for publication, May 23, 1995, and in revised form, July 5, 1995)

Kathy Traianedes, David M. Findlay, T. John Martin, and Matthew T. Gillespie
From St. Vincent's Institute of Medical Research and The University of Melbourne, Department of Medicine, St. Vincent's Hospital, 41 Victoria Parade, Fitzroy, Victoria 3065, Australia

Rat preosteoblastic cells, UMR201, develop a more mature phenotype when subcultured onto a type I collagen gel when compared with their growth on plastic. Basal osteopontin mRNA expression is up-regulated, whereas retinoic acid-induced alkaline phosphatase expression is reduced in cells on collagen when compared with cells plated onto plastic. We have used differential display polymerase chain reaction (PCR) of mRNA to identify other mRNA species that are regulated by collagen and/or retinoic acid in UMR201 cells. A number of differentially expressed PCR products were isolated, whose sequences did not correspond to known sequences in the data bank. However, one species which was up-regulated by growth on collagen showed 95 and 94% homology to the murine and canine 54-kDa subunit of the signal recognition particle (SRP54), respectively. In time course experiments, using reverse transcription PCR, it was found that SRP54 mRNA was up-regulated in UMR201 cells as early as 1 h after subculture onto collagen, when compared with cells subcultured onto plastic, and levels remained elevated after 48 h. The increased expression of SRP54 paralleled the increased expression of a known secreted protein, osteopontin. SRP54 recognizes signal sequences of proteins destined for secretion and retards them for further elongation in the endoplasmic reticulum. The increased expression may correlate with the synthesis of specific extracellular matrix molecules in differentiating osteoblasts.

It is well recognized that the extracellular matrix environment can regulate gene expression of cells in a tissue-specific manner (for review, see Ref. 1). Differentiation has been demonstrated in mammary gland epithelial cells cultured onto type I collagen gels or a reconstituted basement membrane material (2), in hepatocytes cultured on a basement membrane-like matrix (3) or laminin (4), in sertoli cells cultured onto laminin-rich substrata (5, 6), and more recently in bone cells cultured onto type I collagen (7, 8). We have shown that the preosteoblastic cells, UMR201 (9), were directed to a more mature phenotype when cells were plated onto a matrix of gelized type I collagen (7). Growth on type I collagen was shown to decrease growth rate, down-regulate RA1-stimulated alkaline phosphatase mRNA and enzyme levels, as well as increase the basal and RA-stimulated expression of OP. Alkaline phosphatase and OP are among the genes whose expression is known to be modulated during osteoblast differentiation (10–12). Since previous studies from our group have demonstrated that plating cells on a collagen substratum gave definitive and reproducible changes (7) in the differentiation profile of rat osteoblastic cells, we used differential display-polymerase chain reaction (dd-PCR) to identify other genes whose expression is regulated either by collagen substratum and/or RA. The technique of differential display of mRNA species (13) has recently been used to determine differentially regulated mRNA species between comparative populations of cells (14), tissues (15), and tumors (16).

Using this technique, we have identified a regulated mRNA species that codes for a protein involved in the protein secretory pathway. Most proteins destined for secretion first pass through the endoplasmic reticulum (for reviews, see refs. 17–19). Identification and targeting of nascent polypeptide chains is apparently achieved by the binding of the 54-kDa subunit of the signal recognition particle (SRP54). Evidence suggests that SRP54 binds to the signal sequence as it emerges from the ribosome, thereby forming a complex and retarding further elongation of the polypeptide chain. This ribosome-SRP complex then binds to the SRP receptor on the endoplasmic reticulum membrane. The ribosome forms a complex with the "translocon," translation elongation resumers, and SRP54 is released for another round of targeting (17, 19). The regulation of this mRNA species has not been reported previously. We have identified a 267-bp sequence of the rat homologue of SRP54 using dd-PCR and show that this mRNA species was up-regulated when preosteoblastic cells, UMR201, were plated on a physiological substratum. The up-regulation of this mRNA species may be related to the differentiation state of the preosteoblasts. A more differentiated state of these cells in response to plating on type I collagen is demonstrated with concomitant up-regulation of OP mRNA, a secreted protein which is a recognized marker of osteoblast differentiation.

EXPERIMENTAL PROCEDURES

The rat preosteoblast cell line, UMR201 (9), was used in all experiments as described previously (7).Briefly, cells were routinely grown in α-modified minimal essential medium (α-MEM) (Life Technologies, Inc.) containing 10% fetal bovine serum (Cyto systems, Castle Hill, New South Wales, Australia). All media contained gentamicin (80 mg/ml) (David Bull Laboratories, Mulgrave, Victoria, Australia) and minocycline (1 mg/ml) (Sigma). Incubations were carried out at 37°C and equilibrated with 5% CO2 in humidified air. Cells were used between passages 9 and 11 (9). Cells on plastic were harvested using phosphate-buffered saline with 5 mM EDTA. All chemicals were purchased from...
BDH (Kilsyth, Victoria, Australia) unless otherwise specified.

**Results**

In order to identify genes potentially regulated by cell-matrix interactions, the technique of dd-PCR was used to compare RNA derived from UMR201 preosteoblastic cells grown on a gel of rat tail type I collagen and compared with RNA derived from cells plated onto tissue culture plastic. Comparison was also made between RNA derived from cells that were either untreated or treated with RA, a known osteoblast differentiating agent (9). To confirm differentially regulated bands, and to eliminate false positives, dd-PCR was performed at least twice on RNA isolated from experiments conducted months apart. To exclude the possibility of primer or genomic DNA contamination, PCR was performed on non-reverse-transcribed RNA samples and in the absence of template. No PCR products were detected after 40 cycles of the PCR reaction in these controls (not shown).

Many PCR bands appeared differentially regulated between RNA samples from cells that were plated on plastic or collagen and that were either untreated or treated with RA (Fig. 1A). A number of these differentially expressed bands were excised from the sequencing gel, re-amplified, and cloned. Ten bands were subsequently sequenced, two of which were identical, two proved to be smaller fragments of longer sequences, indicating that the 5' primer could hybridize to multiple sites within the same sequence, and six were unique sequences sharing no homology to sequences in the EMBL or GenBank databases. One band of 267 bp (Fig. 1A) was consistently up-regulated in cells plated on collagen and was determined to be the rat homolog of the 54-kDa subunit of the signal recognition particle (SRP54). This sequence had a 95 and 94% homology, at the nucleotide level, with mouse (27) and canine (28) SRP54, respectively, with only one amino acid difference in the 86-amino acid sequence (Fig. 1B). Interestingly, the PCR fragment corresponding to the rat homologue of SRP54 resulted from a 5'-primer-5' primer amplification and not a 5'-primer-3' primer amplification.

To confirm regulation of SRP54 in response to growth of UMR201 cells on collagen, specific oligonucleotides (540F and 540B) internal to the initial PCR fragment were synthesized (not shown). A graphical
A rapid increase in SRP54 mRNA is suggestive of transcriptional activation by collagen. Growth of the UMR201 cells on collagen had no effect on the stability of SRP54 mRNA, which we determined to have an average half-life of 12.4 ± 0.9 h in cells grown on plastic or 14.4 ± 1.4 h in cells grown on collagen (S.E., n = 3). These results support the notion that collagen induces transcriptional activation of SRP54.

Since SRP54 is involved in the protein secretory pathway, it was of interest to determine whether up-regulation of a known secreted protein paralleled the increase in SRP54. We have previously shown that osteopontin mRNA was up-regulated in UMR201 cells plated onto collagen for 24 h (7). We therefore measured OP mRNA levels over the time course period described above for SRP54. The same RNA samples for the experiment of Fig. 2 were subjected to Northern blot analysis using a rat osteopontin cDNA probe. Fig. 2B shows that osteopontin mRNA, like that of SRP54, was up-regulated by 1 h after plating cells onto collagen, and this up-regulation was also maintained over the experimental period. A graphical representation of OP mRNA normalized to GAPDH levels is shown in Fig. 2C. Quantification of SRP54 and OP mRNA levels showed that both these species were up-regulated in cells plated onto collagen compared with cells on plastic. SRP54 mRNA remained 2- to 4-fold higher in cells on collagen compared with cells plated onto plastic, whereas OP mRNA in cells on collagen accumulated over the same period and at 24 h was 6-fold above levels in cells on plastic.

To determine whether the induction of SRP54 mRNA was specific to collagen, UMR201 cells were plated on several non-collagenous matrices containing various cell-binding motifs, including the RGD sequence. In UMR201 cells plated onto fibronectin, laminin, or vitronectin, expression of neither SRP54 nor OP mRNA was regulated differently from cells on plastic (not shown). This suggests that the presence of an RGD motif in the exogenous substrate cannot alone account for the above observations.

**DISCUSSION**

The technique of differential display of mRNA, or mRNA fingerprinting, is a powerful tool for comparing the abundance of mRNA species between RNA populations. Here we have shown that this technique was valuable in identifying mRNA species that were regulated in preosteoblastic cells when grown in contact with a physiological substrate. We have previously demonstrated the regulation of gene expression of known osteoblastic markers of differentiation when UMR201 cells were plated on collagen or matrigel, a reconstituted basement membrane preparation (7).

A number of regulated mRNA species were identified using this procedure, some of which had no corresponding sequence recorded in the EMBL or GenBank data banks. This may...
have been the result of amplified sequences being confined mainly to the 3'-untranslated region or they might in fact represent cDNA species for novel mRNA transcripts. One mRNA species was of particular interest, since it was consistently up-regulated in cells on collagen. Sequence analysis identified this mRNA as the rat homologue of SRP54. The sequence obtained was the result of the 5'-primer acting as both sense and antisense primer, without the involvement of the anchored 3'-primer, fortuitously resulting in a sequence of the coding region being obtained.

Regulation of SRP54 mRNA species has not been reported previously. Although the mRNA species was identified in preosteoblast-like cells, this protein is ubiquitous and has been identified in mammals (27, 28), yeasts (29, 30), prokaryotes (27, 28, 31), and chloroplasts (32). The regulation of this species may be related to the differentiated state of the cell and its synthetic activity. It is likely that cells actively involved, for example, in organogenesis or repair require up-regulation of components of the synthetic machinery, whereas quiescent cells could down-regulate the expression of these components. Plating of UMR201 cells onto collagen appears to favor a more differentiated state of these cells when compared with cells on plastic (7). Osteopontin is a known secreted protein of osteoblasts, and the markedly increased expression of this mRNA species may require up-regulation of many components of the protein secretory pathway, including SRP54, to accommodate the throughput of mRNA transcripts of proteins destined for secretion. Thus, cells may require a certain level of SRP54 protein expression to accommodate increased mRNA translation of secreted proteins. This may explain the expression profile found in the present study where SRP54 mRNA expression was rapidly increased, perhaps until a sustainable level of SRP54 protein was attained, followed by reduced steady-state mRNA levels thereafter. The rapid and concordant increase in SRP54 mRNA and OP mRNA after UMR201 cells were plated on to collagen would imply that the matrix evokes downstream signaling pathways to induce transcriptional activation of these genes. The inability of other RGD-containing peptides, such as fibronectin, laminin, or vitronectin, to mimic the induction of mRNA suggests that the presence of an RGD sequence alone is insufficient to elicit such a response. Thus, the collagen-induced activation of these genes may occur via an RGD-independent integrin interaction. It will be intriguing to investigate the signaling pathways that mediate these responses as well as identifying other secreted proteins or other components of the protein secretory pathway where expression is regulated by physiological substrate.