1983

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Abstract
We have shown that serum, fibroblast growth factor, epidermal growth factor, and the tumour promotor, 12-O-tetradecanoylphorbol- 13-acetate (TPA), selectively induce the synthesis of certain secreted proteins in a variety of cell types. Similarly, a specific growth inhibitor protein selectively induces the synthesis of a secreted protein that is different from those induced by growth factors. In each case, the average increase in labeling by [3%]methionine of these secreted proteins after their induction is 7- to 10-fold. No correspondingly large increase is detected for intracellular proteins.

Disciplines
Biochemistry | Zoology

Comments
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SECRETED PROTEINS INDUCED BY GROWTH FACTORS AND INHIBITORS

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We have shown that serum, fibroblast growth factor, epidermal growth factor, and the tumour promotor, 12-O-tetradecanoylphorbol-13-acetate (TPA), selectively induce the synthesis of certain secreted proteins in a variety of cell types. Similarly, a specific growth inhibitor protein selectively induces the synthesis of a secreted protein that is different from those induced by growth factors. In each case, the average increase in labeling by [^{35}S]methionine of these secreted proteins after their induction is 7- to 10-fold. No correspondingly large increase is detected for intracellular proteins.

Fig. 1 shows the effect of the epidermal growth factor and fibroblast growth factor on the labeling by [^{35}S]methionine of both secreted and internal proteins in Swiss and Balb/c 3T3 cells and in African green monkey kidney epithelial (BSC-1) cells. BSC-1 cells also secrete an inhibitory protein that inhibits their own growth. This inhibitory protein also selectively induces a secreted protein. As for the growth factors--despite the dramatic extracellular changes induced by the growth inhibitor in the patterns of [^{35}S]methionine-labeled proteins after SDS polyacrylamide gel electrophoresis--there are no detectible changes in the pattern of labeling of the major internal (non-nuclear) proteins (Fig. 1).

The inductions of secreted proteins are transient; peak appearance of secreted proteins in the medium of stimulated cells varies from four to twenty four hours after addition of growth factor

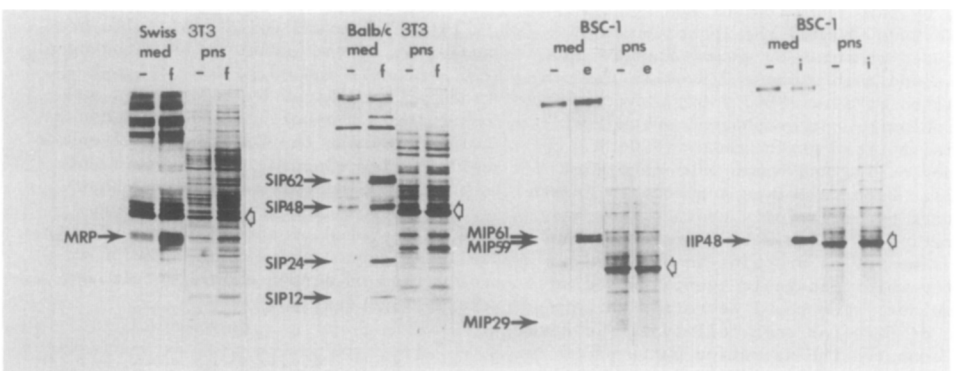

Fig. 1. *Effect of growth factors and a growth inhibitor on the synthesis of secreted and intracellular proteins in three cell lines. Cells were labeled for four hours with [^{35}S]methionine after treatment with FGF, EGF, or growth inhibitor protein, as in Nilsen-Hamilton et al. (1980). For Balb/c 3T3 cells, the induction was enhanced by including cycloheximide (1 ug/ml) before, but not during, labeling as in Nilsen-Hamilton et al. (1982). The labeling pattern of proteins secreted into the medium (Med) by stimulated and control cells are compared with the labeling pattern of proteins in a post-nuclear supernatant (PNS) of an NP40 lysate prepared as in Nilsen-Hamilton et al. (1980). Fluorograms of [^{35}S]methionine-labeling patterns on 7.5-15% gradient SDS polyacrylamide gels are shown. Additions are control (-); 5 ng/ml EGF (e), 100 ng/ml FGF (f); growth inhibitor (i). Proteins identified are "mitogen-regulated protein" (MRP), "superinducible proteins" (SIP 12, SIP 24, SIP 48, and SIP 62); "mitogen-induced proteins" (MIP 29, MIP 59, and MIP 61) and "inhibitor-induced protein" (IIP 48). The position of ovalbumin (M_r, 42,300) is marked for each gel by the open arrow.*

0309-1651/83/070527-02/$03.00/0

Table I. Effect of actinomycin D on the induction of four secreted proteins.

	Area (arbitrary units)			
	Control		Actinomycin D	
	—	Stimulated	—	Stimulated
MEP	0.15	0.96 (FGF)	0.23	0.14 (FGF)
SIP 24	0.03	0.59 (FGF)	0.02	0.02 (FGF)
MRP	0.52	4.0 (EGF)	0.15	0.12 (EGF)
IIP 48	3.1	8.8 (inhibitor)	2.8	2.3 (inhibitor)

The cells, described in the legend to Fig. 1, were treated with growth factor or inhibitor, as described for Fig. 1, except that to some cultures actinomycin D (0.5 µg/ml) was added with the stimulant. The amount of [^{35}S]methionine incorporated into the proteins secreted into the medium was determined from densitometric scans of fluorograms of gels similar to those shown in Fig. 1. MEP = major excreted protein.

or growth inhibitor. The increases in appearance of these proteins in the medium precede the increase (or decrease) in the rate of DNA synthesis that occurs in response to the addition of growth factors (or growth inhibitor). The inductions by growth factors and growth inhibitor are inhibited by actinomycin D (Table I). The basal levels of synthesis of these and other proteins are, in most cases, only marginally affected, if at all, by this inhibitor of RNA synthesis.

We have studied the induction of secreted proteins by growth factors in Swiss and Balb/c mouse 3T3 cells (Nilsen-Hamilton et al., 1980, 1982), human skin fibroblasts, rat esophageal epithelial cells, African green monkey (BSC-1) kidney cells, and human adenocarcinoma cells. Each cell type responds to growth factors by selectively synthesizing and secreting a unique set of proteins. And the same set of proteins is produced no matter which mitogenic agent is used as stimulant. The basal secretion pattern also differs for each cell type. We have now compared the secretion patterns of ten different cell types and can readily distinguish each cell type by their secretion pattern. This difference in secretion pattern does not seem to be due to clonal variation. A comparison of the secretion patterns of human skin fibroblasts from 9 different individuals, both male and female, ranging from 3 to 53 years of age, showed virtually identical patterns for all 9 cell strains.

For BSC-1 cells, the specific growth inhibitor has a negative effect on DNA synthesis. Epidermal growth factor has a positive effect on DNA synthesis. Together, the effects of the growth factor and inhibitor are additive, canceling each other out. The growth inhibitor induces the synthesis of only one secreted protein, whereas epidermal growth factor induces three other proteins. The combination of inhibitor and growth factor are also additive in inducing the synthesis of secreted proteins. This result suggests that the early biochemical pathways initiated by epidermal growth factor and the growth inhibitor do not interact.

These inductions of secreted proteins in fibroblasts and epithelial cells are analogous to the induction by mitogens of the synthesis and secretion of lymphokines and monokines by lymphocytes and monocytes. Lymphokines and monokines help coordinate the functions of lymphoid cells during elaboration of the immune response. Similarly, we propose that growth factors and growth inhibitors induce secreted proteins that coordinate cellular growth and differentiation in solid tissues during embryogenesis and tissue regeneration.

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