Control of intermediate filament protein synthesis by cell-cell interaction and cell configuration

Avri Ben-Ze'ev

Department of Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

Received 29 March 1984

The expression of cytokeratins and vimentin was investigated in epithelial cells under conditions of varied cell spreading and cell-cell contact. When extensive cell-cell contact was achieved in dense monolayer cultures, or in suspension in multicellular aggregates, the cells synthesized high levels of cytokeratins and low levels of vimentin. In contrast, sparse monolayer and suspension cultures, with minimal cell-cell contact, synthesized low levels of cytokeratins and high levels of vimentin. The ratio of cytokeratin to vimentin synthesis was independent of the cell cycle and was also reflected at the level of mRNA translational activity in vitro. Thus control of cytokeratin synthesis involves cell-cell contact, while vimentin synthesis responds to cell shape changes.

Intermediate filament *Cytokeratin* *Vimentin* *Cell-cell interaction* *Cell shape*

1. INTRODUCTION

A variety of important cellular activities including cell proliferation [1] and differentiation [2] require the attachment and spreading of cells on extracellular matrices, which result in cell shape changes (review [3]). Such changes bring about responses at the level of macromolecular metabolism [4–8]. Since the subcellular cytoskeletal network is implicated primarily in determining cell shape, our studies on cell configuration-related macromolecular metabolism were directed toward the investigation of cytoskeletal protein gene expression. These studies strongly suggest that cytoskeletal protein gene expression responds to alterations in cell morphology [9–11]. This study examined the coexpression of intermediate filament protein genes in epithelial cells in culture in response to cell shape changes and the extent of cell-cell contact, as a model system for cell morphology-related regulation of gene expression.

2. MATERIALS AND METHODS

MDBK cells were grown in Dulbecco's modified Eagle medium plus 10% fetal bovine serum. Cells were seeded either on tissue culture plates or on plates coated with poly(2-hydroxyethylmethacrylate) [poly(HEMA)] as in [5]. Proteins were labeled with 100 μCi/ml of [^{35}S]methionine for 2 h. Total cell protein or Triton X-100-soluble and -insoluble fractions were prepared as in [9] and the proteins were analyzed on 10% or 7–17% acrylamide slab gels, or by two-dimensional gel electrophoresis as in [8]. Poly(A)-containing cytoplasmic RNA was prepared [7] and translated in vitro in a commercially available reticulocyte lysate [8].

3. RESULTS

3.1. *Vimentin* vs *cytokeratin synthesis in monolayer and suspension cultures of epithelial cells*

Recently we found that a variety of transformed and non-transformed mesenchymal cells displayed a reversible down regulation of vimentin synthesis during suspension culture (3T3, SV3T3, CHO; see [10]). Therefore, it was of interest to investigate the control of the cytokeratin-type intermediate filament protein synthesis in cultured epithelial cells that express in addition to the cytokeratin type filaments, also the vimentin-type mesenchymal intermediate filaments [12]. Epithelial cells

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

(MDBK) were seeded either as a monolayer culture (fig.1A) or in suspension culture on poly(HEMA)-coated culture dishes for 3 days (fig.1B). In suspension, the cells formed large, tightly packed aggregates that contain epithelial specific junctional complexes of adherence and desmosomal type as revealed by electron microscopic analysis (not shown). Such monolayer (fig.1C) and suspension (fig.1D) cell cultures were labeled for 2 h with [^{35}S]methionine and the Triton X-100-insoluble cytoskeletal fraction containing the intermediate filaments was analyzed by two-dimensional gel electrophoresis. Fig.1C,D demonstrates that the two cytokeratins nos 8 and 18, characteristic to MDBK cells [13], are equally expressed in both monolayer and suspension culture, while vimentin synthesis was dramatically reduced in suspension culture (fig.1D) as also obtained in mesenchymal cells [10]. Thus unlike vimentin synthesis the level of cytokeratin synthesis is not affected by changes in cell shape. In the in vitro translation assay, mRNA isolated from monolayer (fig.1E) and suspension cultures (fig.1F) of MDBK cells is equally active in directing the synthesis of cytokeratins nos 8 and 18, but the mRNA from suspension cultures is less active in directing the synthesis of actin and vimentin (fig.1F).

3.2. *Variations in vimentin and cytokeratin synthesis in sparse and dense epithelial cultures*

The semiconfluent and suspension conditions described in fig.1A,B enabled the establishment of extensive cell-cell contact, conditions favourable for normal epithelial function, i.e., structural and functional polarization. Therefore, the synthesis of cytokeratins and vimentin was also analyzed

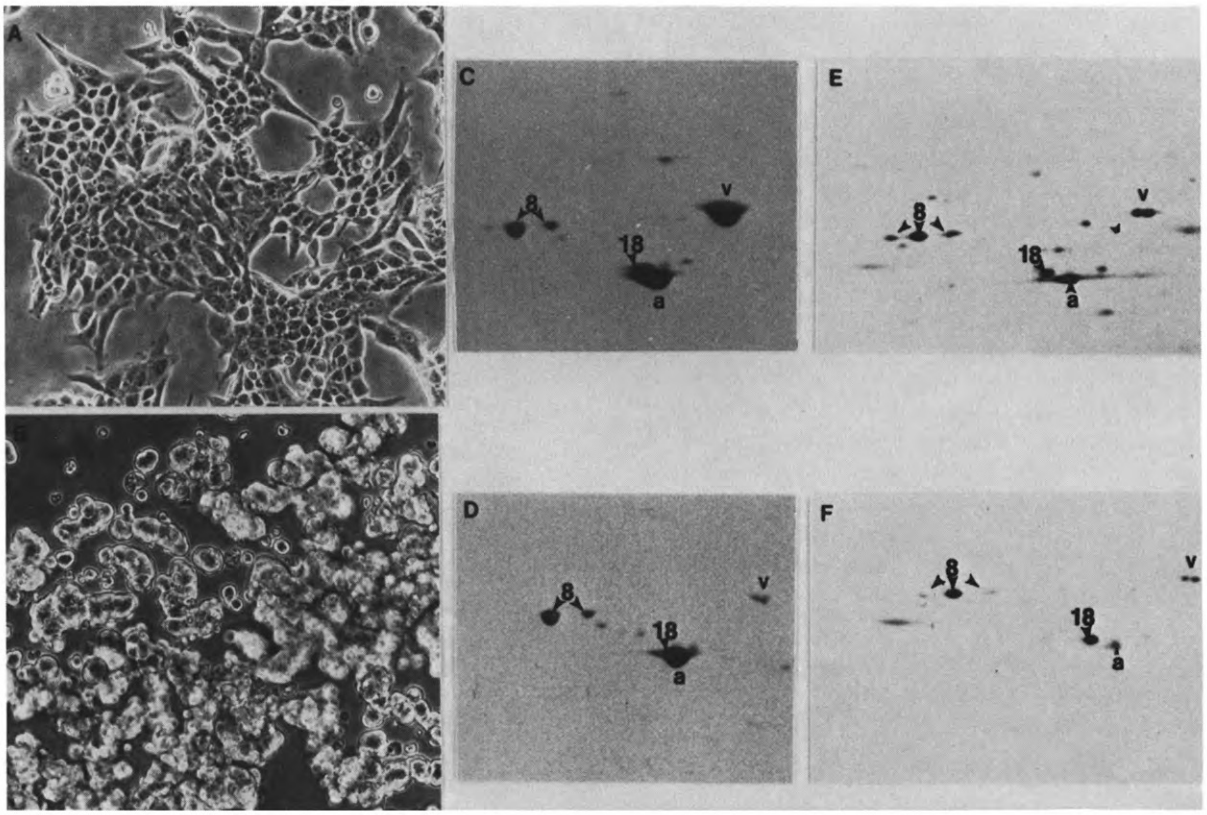

Fig.1. Vimentin *vs* cytokeratin synthesis in monolayer (A) and 3 day suspension cultures (B) of MDBK cells. [^{35}S]Methionine-labeled Triton-cytoskeleton from monolayer (C) and suspended cell cultures (D) and in vitro translation products of mRNA from monolayer (E) and suspension cultures (F). (a) Actin, (v) vimentin, (8,18) cytokeratins.

under conditions that limit to a minimum the extent of cell-cell interaction such as in very sparse cell cultures (fig.2B) and was compared to very dense monolayer cultures (fig.2A) that facilitate cell-cell contact. Dense monolayer cultures were found to synthesize low levels of vimentin and high levels of cytokeratins (fig.2C,G) as compared to sparse monolayer cultures that synthesize high levels of vimentin and low levels of cytokeratins (fig.2D,H). The Triton X-100-soluble fractions from these cultures (fig.2E,F) do not contain detectable amounts of intermediate filament proteins, but show a reduced level of actin synthesis in dense cultures (fig.2E). Translation activity in vitro with mRNA isolated from these cultures reflects the shift from a high vimentin low keratin content in sparse monolayer culture (fig.2J), to a high cytokeratin low vimentin content in dense monolayer culture (fig.2I). Furthermore, in single cell suspension cultures, where methylcellulose was added to the medium to prevent cell-cell contact, the cells synthesized low levels of cytokeratins as in sparse monolayer cultures (not shown). In addition, when semiconfluent MDBK cultures (fig.1A) were treated with inhibitors of DNA or protein synthesis for 24–48 h, or when the cells were arrested by starvation in low serum and then stimulated into growth with 10% serum, there was no change in the ratio of cytokeratins to vimentin synthesis (similar to fig.1C). The results from fig.1,2 thus suggest that

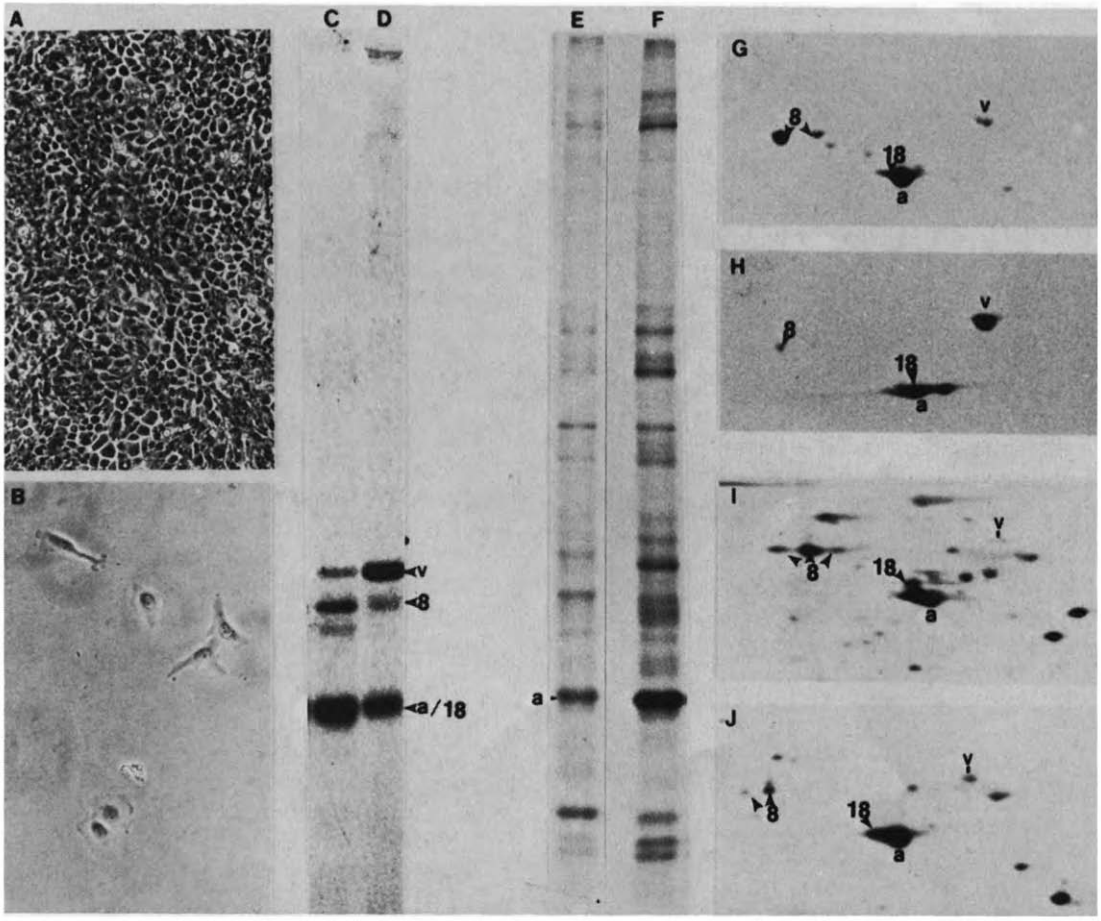

Fig.2. Vimentin *vs* cytokeratin synthesis in dense (A) *vs* sparse (B) cell cultures. [^{35}S]Methionine-labeled proteins from Triton X-100-insoluble fractions from dense (C,G) and sparse (D,H) monolayer cultures and from the Triton X-100-soluble fraction of dense (E) and sparse (F) cultures. In vitro translation products from dense (I) and sparse (J) cultures.

the regulation of intermediate filament protein synthesis does not simply correlate with the rate of growth, but rather cytokeratin synthesis is mediated by cell-cell contact while vimentin synthesis is sensitive to cell shape changes.

4. DISCUSSION

This study demonstrates a differential control of cytokeratin and vimentin synthesis in epithelial cells where both intermediate filaments are expressed. The cell shape-dependent control of vimentin synthesis is thus common to both mesenchymal cells [10] and epithelial cells (here) and the presence of the cytokeratin-type network in the same cell does not affect the cell configuration-related control of vimentin synthesis. Thus in suspension culture (fig.1B) of most cell types, or in very dense monolayers of epithelial cells, where the projected cell area is drastically reduced (fig.2A), vimentin synthesis is inhibited. Similarly, the cell-cell interaction related control of cytokeratin synthesis is also observed in epithelial cells that lack vimentin such as MCF-7 cells [14], suggesting that the two intermediate filament-type proteins are differentially regulated whether expressed in different cell types or coexpessed in the same cell. While coexpression of vimentin and cytokeratins in epithelia in vivo is very rare, recent studies on the parietal endoderm of the mouse embryo [15] and on certain neoplastic cells of epithelial origin [16] demonstrated such coexpression in individual populations and/or in motile cells. The desmosome-type intercellular junctions observed in dense monolayer and suspension cultures of epithelial cells are most relevant to this study, since cytokeratin fibrils often terminate in the desmosomal plaques at the intercellular boundary [17]. The formation of desmosomes in epithelia and in embryonal development is usually accompanied by the formation of cytokeratin-type intermediate filaments that anchor at the desmosomal plaques [18]. This is compatible with the present study where extensive cell-cell interaction induces the synthesis of cytokeratins.

The recently molecularly characterized desmosomal plaque proteins [19] connecting the cytokeratin filaments to the plasma membrane constitute together with the cytokeratins a useful system for the study of structurally linked cytoskeletal elements that respond to cell-cell interaction and cell shape changes, and a model system for investigation cytoarchitecture and differentiation-related gene expression.

ACKNOWLEDGEMENT

This study was supported in part by a grant from the Israel-USA Binational Foundation, Jerusalem, Israel.

REFERENCES

[1] Folkman, J. and Moscona, A. (1978) Nature 273, 345–349.
[2] Gospodarowicz, D., Greenburg, G. and Birdwell, C.R. (1978) Cancer Res. 38, 4155–4171.
[3] Ben-Ze'ev, A. (1984) in: Cell and Muscle Motility, vol. 6 (Shay, J.W. ed.) Plenum Press, New York, in press.
[4] Ben-Ze'ev, A. (1983) J. Cell. Physiol. 114, 145–152.
[5] Ben-Ze'ev, A., Farmer, S.R. and Penman, S. (1980) Cell 21, 365–372.
[6] Ben-Ze'ev, A. and Raz, A. (1981) Cell 26, 107–115.
[7] Benecke, B.J., Ben-Ze'ev, A. and Penman, S. (1978) Cell 14, 931–939.
[8] Farmer, S.R., Ben-Ze'ev, A., Benecke, B.-J. and Penman, S. (1978) Cell 15, 627–637.
[9] Ben-Ze'ev, A., Farmer, S.R. and Penman, S. (1979) Cell 17, 319–325.
[10] Ben-Ze'ev, A. (1983) J. Cell Biol. 97, 858–865.
[11] Farmer, S.R., Wan, K.M., Ben-Ze'ev, A. and Penman, S. (1983) Mol. Cell. Biol. 3, 182–189.
[12] Lazarides, E. (1982) Annu. Rev. Biochem. 51, 219–250.
[13] Moll, R., Franke, W.W., Schiller, D., Geiger, B. and Krepler, R. (1982) Cell 31, 11–24.
[14] Gigi, O., Geiger, B., Eshhar, Z., Moll, R., Schmid, E., Winter, S., Schiller, D.L. and Franke, W.W. (1982) EMBO J. 1, 1429–1437.
[15] Lane, E.B., Hogan, B.L.M., Kurkinen, M. and Garrels, J.I. (1983) Nature 701–704.
[16] Ramaekers, F.C.S., Haag, D., Kant, A., Moesker, O., Jap, P.H.K. and Vooijs, G.P. (1983) Proc. Natl. Acad. Sci. USA 80, 2618–2622.
[17] Farquhar, M.G. and Palade, G.E. (1963) J. Cell Biol. 17, 375–412.
[18] Jackson, B.W., Grund, C., Schmid, E., Bürki, K., Franke, W.W. and Illmensee, K. (1980) Differentiation 17, 161–189.
[19] Muller, H. and Franke, W.W. (1983) J. Mol. Biol. 163, 647–671.