Int. J. Dev. Biol. 33: 63-70 (1989)

The expression of extracellular matrix and cytoskeleton in fused cells

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ABSTRACT. In the present paper results from our studies on the expression and regulation of the differentiated normal and transformed epithelial and fibroblastic phenotypes are reviewed. The expression of the extracellular matrix- and basement membrane-associated proteins, fibronectin and laminin, and different intermediate filament proteins were studied in different fused cells. Heterokaryons and cytoplasmic hybrids (cybrids) were formed by fusing normal or malignant epithelial cells with normal fibroblasts or malignant glial cells. No differences were observed in the expression of these phenotypic markers between unfused parental cells and the corresponding homokaryons. Thus, the fusion process itself does not cause changes in the expression of these phenotypic markers. In heterokaryons formed after fusion of normal or malignant epithelial cells with normal fibroblasts or malignant glial cells, two or even three different types of intermediate filaments could be co-expressed. Thus, no suppression of the expression of the various intermediate filaments is caused by the non-homologous parental cell. Expression of the extracellular matrix proteins, fibronectin and laminin, on the other hand, could be extinguished by cells and cytoplasts not expressing fibronectin matrix, such as transformed fibroblasts and HeLa cells. These results support the role of transacting regulatory factors controlling the expression of the different extracellular matrix proteins. Alternatively, the results can be explained by changes in the distribution and concentration of the extracellular matrix receptor proteins in the fused cells.

KEY WORDS: *Fibronectin, Laminin, Intermediate filaments, Heterokaryons, Phenotypic expression*

Introduction

The use of cell fusion in cell biology started in 1961 when Barski isolated the first spontaneous viable interspecific somatic cell hybrids. Later cell fusion has been induced by viruses (Harris and Watkins, 1965) and various chemical agents, such as PEG (Pontecorvo, 1976). The simplified techniques have made cell fusion a useful tool in the study of gene expression in somatic cells (Ringertz and Savage, 1976).

Cell fusion can result in *heterokaryons,* which contain two or more nuclei from different cells, or *homokaryons,* which contain only similar nuclei. After fusion the nuclei and cytoplasmic organelles are initially distributed at random within the heterokaryons, but reorganization of the nuclei and other cellular organelles takes place within a few hours (Gordon and Cohn, 1970). When heterokaryons enter cell division, the nuclei can fuse and mononucleated *hybrid cells* are formed.

Regulation of nuclear functions in heterokaryons

Metabolically inactive chick erythrocytes (CRBC) have been fused with somatic cells, as the chick red blood cell nuclei are easy to identify in heterokaryons because of their small size and condensed morphology. The CRBC nuclei are reactivated after fusion with metabolically active cells. The changes include increases in nuclear volume and mass, RNA and protein synthesis as well as DNA synthesis by the chick nucleus, and finally synthesis of globin polypeptides in the heterokaryons (Ringertz and Savage, 1976; Linder *et al.*, 1981).

The reactivation of the chick red blood cell nuclei also takes place in enucleated cytoplasts of mouse L cells, probably due to activating factors already present in the cells (Scheer *et al.*, 1983; Woodcock *et al.*, 1984). Furthermore, when mouse erythroleukemia cells or human erythroid K562 cells were fused with a variety of human and mouse non-erythroid cells, the globin genes of the inactive nuclei could be activated as detected by analysis of total cellular RNA. This indicated that in the active erythroid cells there were transacting regulatory factors capable of activating globin genes of non-erythroid cells (Baron and Maniatis, 1986). Thus cytoplasmic factors can clearly regulate nuclear functions in RBC heterokaryons.

When senescent or serum-deprived fibroblasts were fused with proliferating fibroblasts, the senescent phenotype dominated in the resulting heterokaryons (Stein and Yanishevski, 1979). Inhibition of protein and mRNA synthesis delayed the inhibition of DNA synthesis normally seen in the heterokaryons (Burmer and Norwood, 1982; Burmer *et al.*, 1984). On the other hand, when malignant cells such as HeLa cells or SV40-transformed fibroblasts were fused with senescent cells, the metabolically active phenotype dominated and the inactive nuclei reinitiated DNA synthesis (Stein and Yanishevski, 1981). It was postulated that senescent cells contain inhibitory factors which prevent cells from entering S phase, whereas malignant cells have acquired a dominant transforming factor enabling the cells to proliferate (Stein and Yanishevski, 1979, 1981). This is supported by the fact that human lymphocytes can be immortalized by fusion with enucleated cytoplasms from transformed mouse L929 cells (Abken *et al.*, 1986).

Enucleated cells and cybrids

Cells can be enucleated by centrifugation in the presence of cytochalasin B (Prescott *et al.*, 1972; Shay *et al.*, 1975; Laurila *et al.*, 1981). The resulting cell fragments are called *karyoplasts* or *minicells,* which contain the nucleus and a narrow rim of cytoplasm, and *cytoplasts,* which contain the rest of the cyto-

plasm (Ege *et al.*, 1974; Goldman and Pollack, 1974). These cell fragments remain viable for a limited period of time in culture, and they can perform a number of cellular functions (Goldman and Pollack, 1974; Goldman *et al.*, 1975; Clark and Shay, 1979). The karyoplasts, on the other hand, are able to attach but not spread on substratum, and they are usually not able to regenerate (Ege *et al.*, 1974; Zorn *et al.*, 1979).

The cell fragments can be fused with intact cells or with each other. When cytoplasts are fused with intact cells, *cytoplasmic hybrids* or *cybrids* are produced, whereas cytoplasts fused with karyoplasts form *reconstituted* cells (review, Ringertz and Savage, 1976). Cytoplasmic hybrids can obtain phenotypic characteristics from both the intact cell and the cytoplasmic fusion partner (review, Shay, 1983). The effects are, however, usually fairly short-lived, possibly due to the dilution of the factors obtained from the cytoplasmic donor (Kahn *et al.*, 1981). On the other hand, cytoplasmic inheritance has also been demonstrated in cybrids after fusion of intact cells with cytoplasts from cells resistant to chloramphenicol (Bunn *et al.*, 1974), erythromycin (Doersen and Stanbridge, 1979) and antimycin (Harris, 1978). Other long-term effects exerted by cytoplasts on intact cells include extinction of hemoglobin production of Friend erythroid cell cytoplasts (Gopalakrishnan *et al.*, 1977) and suppression of steroidogenesis of adrenal cells by fibroblast cytoplasts (Clark and Shay, 1982). Thus, both short-term and permanent effects can be obtained in cybrids.

The expression of the transformed phenotype in fused cells

Heterokaryons. When cells transformed by a tumorigenic virus such as SV40, Epstein-Barr virus (EBV), polyoma virus or Rous sarcoma virus are fused with normal cells, the resulting heterokaryons show characteristics of transformed cells. Virus-induced nuclear antigen, such as SV40 T-antigen and EBV-virus-induced nuclear antigen (EBNA), were expressed in heterokaryons of normal and transformed cells, and the antigens migrated into the normal nuclei of the heterokaryons (Rosenqvist *et al.*, 1975; Yamamoto *et al.*, 1976; Kellermayer *et al.*, 1978). The transformed phenotype was, therefore, considered dominant in these heterokaryons.

Hybrids. Hybrid cells formed between normal and transformed cells express transformed or non-transformed, as well as intermediate characteristics (reviews, Stanbridge *et al.*, 1982; Sager 1985, 1986). Several transformed characteristics, such as changes in morphology, growth to high saturation densities, anchorage-independent growth and the expression of SV40 T-antigen, were expressed in hybrids formed between normal and polyoma or SV40 virus-transformed cells (Basilico and Wang, 1971; Croce and Koprowski, 1974). The SV40 T-antigen expression was dominant in the hybrid cells and correlated with the presence of the human chromosome number 7, 8 or 17 (review, Croce, 1981).

On the other hand, non-transformed characteristics, such as loss of T-antigen from hybrids of normal and SV40-transformed cells, as well as different intermediate characteristics have also been reported (Weiss, 1970; Pereira-Smith and Smith, 1981). Furthermore, other characteristics of transformed cells, such as anchorage-independent growth and low serum requirement, were suppressed in hybrids of normal and transformed Chinese hamster cells. The presence of the terminal portion of Chinese hamster chromosome 1 was associated with the anchorage requirement for these hybrid cells (Marshall *et al.*, 1982). Later, complementation groups for these transformation parameters were detected and the different characteristics were independently re-expressed (Smith and Sager, 1985).

The expression of cytoskeletal structures has also been studied in hybrids of normal and transformed cultured cells. Initially, cytoplasmic microfilaments were observed in the non-tumorigenic hybrid cells. The reappearance of tumorigenicity was associated with the loss of microfilaments (Gowing *et al.*, 1984) and lack of collagen-containing extracellular matrix (Harris, 1985).

The expression of tumorigenicity in cell hybrids

The expression of tumorigenicity in cell hybrids has been a subject of continuous controversy over the past 20 years (review, Stanbridge *et al.*, 1982). In the early studies the loss of parental cell chromosomes was a very common phenomenon. Harris and Klein and co-workers studied the relationship between chromosome loss and tumorigenicity in hybrids of malignant and normal mouse cells (review, Harris, 1971). All hybrids showed a reduced frequency of tumor induction in syngeneic or irradiated newborn mice (Wiener *et al.*, 1974). Occasionally, however, tumors were obtained after injection of hybrid cells to syngeneic animals. Cells derived from these tumors showed a marked loss of chromosomes. This suggested that malignancy behaved as a recessive characteristic and that the loss of specific chromosomes was required for the re-expression of malignancy (review, Harris, 1971). Furthermore, tumorigenicity reappeared in hybrid cells formed between murine melanoma or lymphoma cells and normal fibroblasts after the hybrid cells had lost either one or both copies of chromosome 4 of the normal parental cell (Evans *et al.*, 1982).

Hybrids involving rodent cells have an unstable karyotype, and this makes the interpretation of the results from these studies difficult. Therefore, Stanbridge and co-workers fused malignant human cells (HeLa) with fibroblasts or other normal cells. These intraspecific hybrids had a relatively stable karyotype and showed suppression of malignancy (Stanbridge *et al.*, 1982), although some rare tumorigenic revertant cells were isolated (Der and Stanbridge, 1980). In these cells certain patterns of fibronectin matrix expression were associated with tumorigenicity (Der *et al.*, 1981). It was also suggested that specific chromosomes may play a role in the suppression of tumorigenicity (Stanbridge *et al.*, 1982).

In subsequent experiments the loss of chromosome 11 was found to correlate with the re-expression of tumorigenicity (Srivatsan *et al.*, 1986). Accordingly, when a normal human chromosome 11 was introduced into Wilms' tumor cells, the tumor-forming ability of these cells was completely suppressed. The cells continued, however, to express transformed characteristics *in vitro*. This indicates that genetic information in chromosome 11 can control the malignant expression of Wilm's tumor cells (Weissman *et. al.*, 1987). This is of special interest since chromosome 11 carries the Ha-ras-gene (Yunis, 1983) and a gene that regulates fibronectin synthesis (Eun and Klinger, 1980).

Extracellular matrix in fused cells

Fibronectin is a high molecular weight glycoprotein phylogenetically conserved in structure and function (review, Ruoslahti, 1988). It was initially discovered as a surface protein distinguishing between normal and transformed fibroblasts

(Hynes, 1973; Vaheri and Ruoslahti, 1974). It is found both in soluble form in plasma, as well as in soluble form in the extracellular matrix of various tissues (Stenman and Vaheri, 1978; Vaheri *et al.*, 1980). Fibronectin is a heterodimer with M, 500,000-550,000 consisting of two subunits with specific binding sites for a number of different molecules, such as major matrix components collagens, heparin, heparan sulfate, staphylococci, actin, fibrinogen, fibrin and for fibronectin-fibronectin interaction. Fibronectin also interacts specifically with the cell surface through the RGDS-attachment site (review, Buck and Horwitz, 1987). The interaction of matrix fibronectin with the cell surface is disrupted after malignant transformation of fibroblastic cells (e.g. Alitalo and Vaheri, 1982; Ruoslahti, 1988).

Laminin (M, 900,000) is a non-collagenous glycoprotein and a major specific component of basement membranes (Timpl *et al.*, 1979). Laminin promotes cell attachment, migration, and differentiation, it interacts with basement membrane collagens, with heparin, heparan sulfate and with cell surfaces (reviews, Timpl and Dziadek, 1986; Martin and Timpl, 1987). A receptor for laminin has also been isolated from a rat myoblast cell line, murine fibrosarcoma cells and human mammary carcinoma cells, and the laminin receptor appears to be distinct although closely related to the integrin family of proteins (review, Buck and Horwitz, 1987; Gehlsen *et al.*, 1988).

Expression of fibronectin and laminin in heterokaryons

We have studied the regulation of expression of the extracellular fibronectin matrix by using heterokaryons as a our model-system. Before fusion the parental cells were labelled with cytoplasmic polystyrene particles of two different sizes (Fig. 1). This allowed us to reliably identify multinucleated heterokaryons as opposed to the unfused parental cells and homokaryons (Laurila *et al.*, 1979).

To investigate the possible dominance or suppression of the transformed phenotype, as expressed by the composition of the extracellular matrix, normal human fibroblasts were fused with SV40-transformed human fibroblasts, and the expression of fibronectin matrix was studied in the resulting heterokaryons. Normal fibroblasts express an extensive extracellular fibronectin matrix, which is not seen in transformed cells. Homokaryons of normal fibroblasts had a fibrillar fibronectin matrix similar to that of the unfused parental cells. Analogously, homokaryons of transformed cells lacked such a matrix (Fig. 2). Thus, cell fusion itself did not cause changes in the expression of the extracellular matrix. Heterokaryons, on the other hand, had lost most of their matrix fibronectin within 3 to 24 hours after fusion (Figs. 2 and 3). Yet they showed a strong intracellular fibronectin

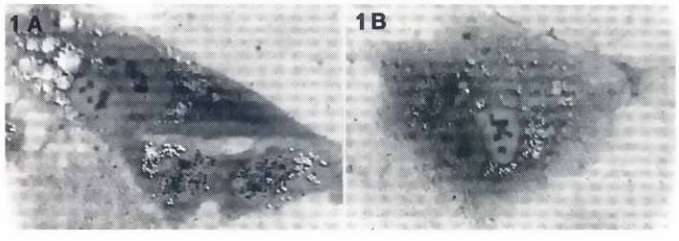

Fig. 1. *Autoradiogram of fused normal and SV40-transformed human fibroblasts. (A) A fibroblast homokaryon (above) containing two unlabeled nuclei and only small cytoplasmic polystyrene particles. Two unfused transformed cells (below), labeled with large cytoplasmic particles and ^{3}H-thymidine. (B) A heterokaryon with both large and small cytoplasmic particles. Accordingly both one unlabeled and two ^{3}H-thymidine-labeled nuclei can be seen (from Laurila* et al., *1979).*

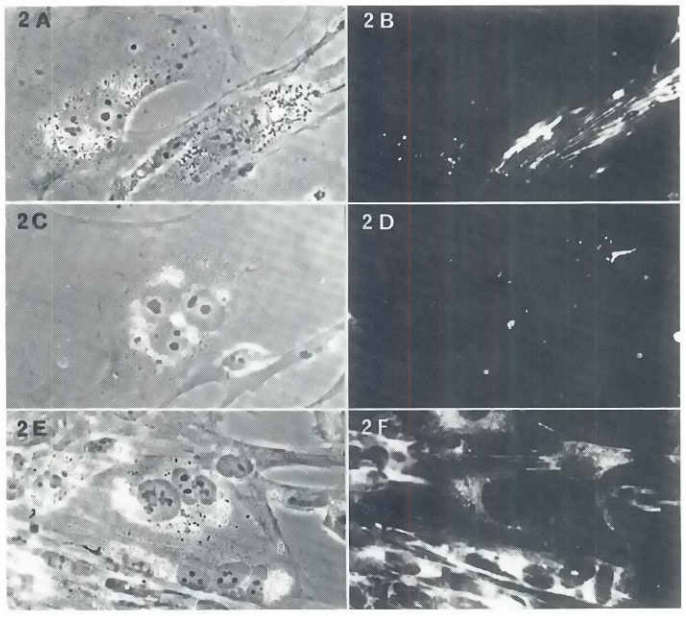

Fig. 2. *Fibronectin expression in homo- and heterokaryons. (A) Phase contrast micrograph of a fibroblast homokaryon (right), and a heterokaryon of normal and SV40-transformed fibroblasts (left). (B) The homokaryon contains a dense pericellular fibronectin matrix, whereas the heterokaryon shows only spotty fluorescence. (C) A homokaryon of SV40-transformed fibroblasts. (D) Only spotty fibronectin fluorescence can be seen in the homokaryon (from Laurila et al.,* 1979). (E) A fibroblast homokaryon (below), and a trinucleated heterokaryon of normal fibroblasts and HeLa cells (center, large and small cytoplasmic particles). (F) Both the fibroblast homokaryon and the heterokaryon show an intracellular fibronectin fluorescence (from Laurila, 1981).

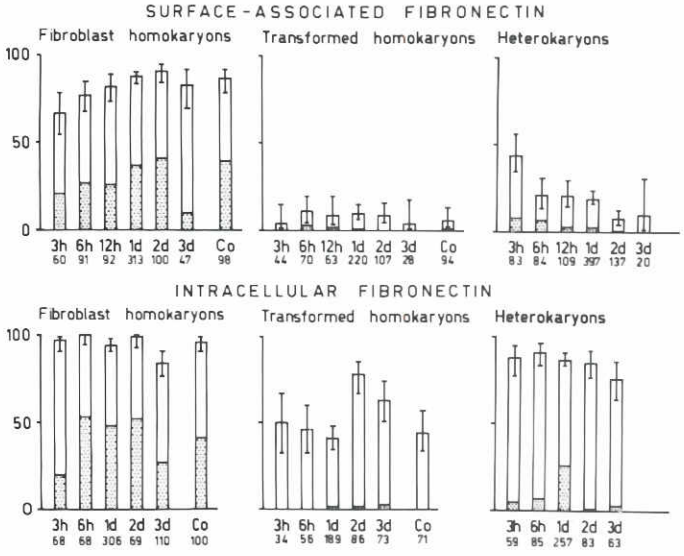

Fig. 3. *A histogram showing the expression of fibronectin in different homo- and heterokaryons at different time points after fusion. The height of the bars indicates the percentages of cells expressing moderate or strong (dotted part of the bars) fibronectin fluorescence. Surface-associated fibronectin fluorescence (top) and intracellular fibronectin fluorescence (bottom). The number of cells studied and the 95 % confidence limits, based on a binomial distribution (negative or weak vs. moderate or strong), are indicated (from Laurila* et al., *1979).*

fluorescence, corresponding to the area of the endoplasmic reticulum (Laurila *et al.*, 1978). This indicated that the heterokaryons continued to produce fibronectin after fusion (Laurila *et al.*, 1979). Thus, in line with earlier results from heterokaryons of SV40-transformed and normal cells (review, Ringertz and Savage, 1976), the transformed phenotype, i.e. the lack of extracellular fibronectin matrix, dominated in our heterokaryons.

Many epithelial cells and cell lines synthesize fibronectin, although they cannot produce or retain an extensive pericellular matrix (Smith *et al.*, 1979; Yang *et al.*, 1980). To investigate whether such normal or malignant cells can also suppress the formation of fibronectin matrix in heterokaryons, malignant human cervical carcinoma cells (HeLa) and normal canine kidney epithelial cells (MDCK) were fused with normal human fibroblasts. In the resulting heterokaryons, the expression of the fibronectin matrix was suppressed to a considerable extent by both the normal and the malignant epithelial cells within one day (Laurila, 1981). Thus, the normal fibroblastic phenotype can be suppressed not only by different malignant cells but also by non-malignant epithelial cells which lack fibronectin matrix. This suppression could be due to a change in the density of the integrin-type of fibronectin receptors in the cell membrane of the heterokaryon, as mixing of intramembranous receptor molecules is known to occur in heterokaryons rapidly after cell fusion (Edidin *et al.*, 1982). Alternatively, transacting diffusible substances present in the cytoplasm of the non-fibroblastic parental cell could affect the expression of fibronectin or the integrin-type of receptor proteins in the heterokaryons.

Recently, two groups using heterokaryons as their model-system have shown that diffusible substances can affect the state of differentiation or even induce differentiation in undifferentiated cells. In heterokaryons of myoblastic cells and human amnion epithelial cells, induction of human-specific muscle proteins was observed (Blau *et al.*, 1983). The induction was dependent on gene dosage in the heterokaryons (Pavlath and Blau, 1986). The accumulation of human actin transcripts in the heterokaryons resembled that seen in human primary muscle cell cultures and the usual decline in the level of mouse cardiac actin, seen in control mouse muscle cell cultures, was not observed in these heterokaryons. Such findings suggest that the activated human nuclei produce their own muscle-type regulatory factors in these heterokaryons. These factors can direct the expression of the human muscle phenotype and may also alter the expression of mouse muscle-specific genes (Hardeman *et al.*, 1986). Analogous results have also been obtained by Wright and his co-workers (Wright 1981, 1984).

The expression of the epithelial and fibroblastic matrix phenotypes was further studied by us in heterokaryons, by fusing mouse parietal yolk sac tumor cells (PYS-2), which express a prominent laminin matrix and do not produce fibronectin, with normal mouse and human fibroblasts. The heterokaryons expressed both fibronectin and laminin matrices in a dose-dependent manner. Moreover, both matrices showed different morphological patterns, indicating that the matrices were formed independently of one another. Thus, both the epithelial and fibroblastic matrix characteristics could be co-expressed in an independent gene-dose-dependent manner (Laurila and Leivo, unpublished results).

Expression of fibronectin in cybrids

To study the relative role of the nucleus and the cytoplasm in the regulation of the extracellular matrix expression, enucleated HeLa (Fig. 4) or non-tumorigenic epithelial cells (MDCK) were fused with intact human fibroblasts. The expression of fibronectin matrix in the resulting cybrids (cytoplasmic hybrids) was initially clearly reduced (Fig. 5; see also Laurila and Stenman, 1982). Within one day, however, the level of the extracellular fibronectin increased and reached the level of the fibroblastic parental cell. Thus, both the normal and the malignant epithelial cell cytoplasts exerted only a temporary influence on the expression of the extracellular matrix of the fibroblastic cell (Laurila and Stenman, 1982). These results can be interpreted to support the idea of transacting cytoplasmic factors, which temporarily suppress the expression matrix-forming proteins.

Several other studies have also reported cytoplasmic influence on the expression of phenotype in fused cells. The direction of differentiation was altered in cybrids of embryonal carcinoma cells (PCC4) and cytoplasts from rat myoblasts (Iwakura *et al.*, 1985), suggesting that cytoplasmic factors can have a prolonged or permanent influence on the fused cells. The

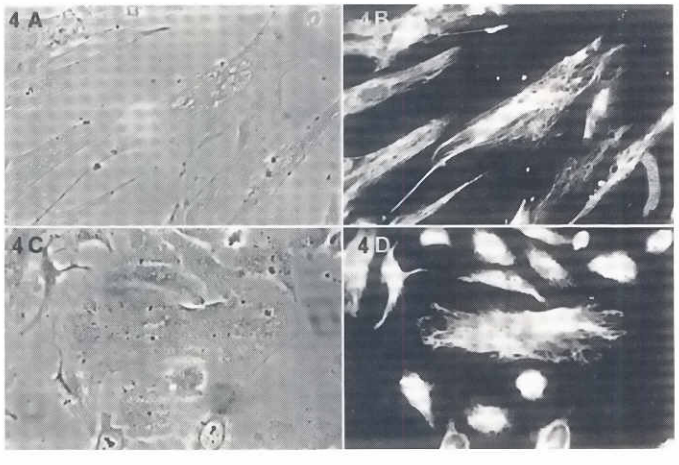

Fig. 4. *Expression of intermediate filaments in enucleated cells. (A) Phase contrast micrograph of enucleated human fibroblasts. (B) A fibrillar network of cytoplasmic vimentin filaments (from Laurila* et al., *1981). (C) Enucleated and two nucleated HeLa cells (bottom). (D) A fibrillar cytoplasmic keratin network can be seen both in the cytoplasts and in the intact cells.*

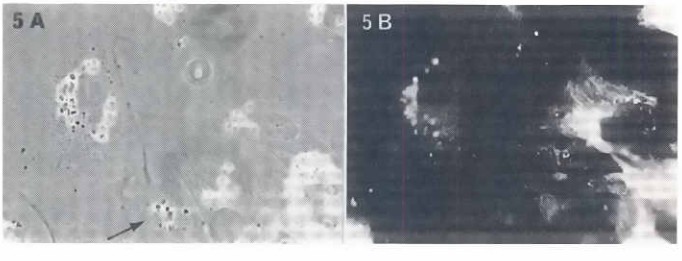

Fig. 5. *Expression of fibronectin in cybrids of fibroblasts and epithelial cells. (A) A cybrid (left) with one nucleus and both large and small polystyrene particles. One enucleated epithelial, MDCK cell (bottom, arrow) and several fibroblasts (large particles). (B) A moderate intracellular fibronectin fluorescence in the cybrid and strong fluorescence in the fibroblasts (from Laurila and Stenman, 1982).*

most extensively studied model in this respect is, however, the expression of the malignant phenotype in cybrids and reconstituted cells (reviews, Shay, 1983; Sager, 1986). Tumorigenicity in nude mice was suppressed in cytoplasmic hybrids of malignant cells and cytoplasts from normal cells (Shay, 1983; Israel and Schaeffer, 1987; Shay and Werbin, 1988). The suppression was, however, often only partial. The results have been used to support the concept of cytoplasmic factors, present in non-tumorigenic cells, which can modify the expression of the tumorigenic phenotype (review, Sager, 1986). In contrast, malignant cytoplasts were recently fused with normal cells, and the tumorigenic phenotype was found to be mediated by the cytoplasts to the cybrids (Israel and Schaeffer, 1988). Thus, a malignant cytoplasm alone would be able to induce the malignant phenotype on normal cells. This is in line with our results showing at least temporary expression of the transformed, non-fibroblastic matrix phenotype in cybrids (Laurila and Stenman, 1982). As a mechanism the authors suggested modulatory effects of mitochondrial genes operating in the cytoplasmic hybrids (Israel and Schaeffer, 1988). This hypothesis is interesting, especially since different types of mitochondrial genomes have recently been observed to have varying abilities of propagation within a common cytoplasm (Hayashi *et al.*, 1986).

Expression of intermediate filaments in heterokaryons

The expression of fibronectin matrix and the fibroblastic phenotype was suppressed in our heterokaryons of fibroblasts and epithelial cells (Laurila, 1981). To further characterize the expression of the fibroblastic and epithelial cell phenotypes, intermediate filaments were selected as phenotypic markers. Cytoplasmic keratin filaments have been regarded as reliable markers for the epithelial cell phenotype both in cultured cells and in normal as well as in pathological tissues *in vivo*. The vimentin type of intermediate filaments, on the other hand, occur primarily in mesenchymal cells, although most cells in culture appear to express vimentin filaments in addition to their tissue-specific filament proteins (Franke *et al.*, 1979; Lazarides, 1982; Steinert and Roop, 1988). However, the amnion epithelial cells used in the fusion studies showed vimentin filaments only in a few cells as typical perinuclear aggregates (Virtanen *et al.*, 1981; Laurila *et al.*, 1982) and most of the cells distinctly lacked vimentin under our culture conditions. The expression of fibrillar arrays of vimentin filaments was, therefore, regarded as a phenotypic marker for the mesenchymal or undifferentiated phenotype (Laurila *et al.*, 1982).

Both the vimentin and keratin filaments were intermixed within the cytoplasm of the heterokaryon. No cytoplasmic organizing centers could be found (Fig. 6). Our results are in accordance with those of Eckert *et al.*, (1982), suggesting that nuclei or sites close to the nuclei have an organizing role for intermediate filaments. This is supported by the observation that shortly after fusion the different parental cell nuclei were surrounded by the respective intermediate filaments, and complete intermixing of the filaments was seen only after the nuclei had aggregated in the center of the heterokaryon (Fig. 7). Finally, the fibroblast and the epithelial cell nuclei became surrounded by both types of filaments to similar extents, and no separate compartments were formed within the cytoplasm of the heterokaryon (Laurila *et al.*, 1982).

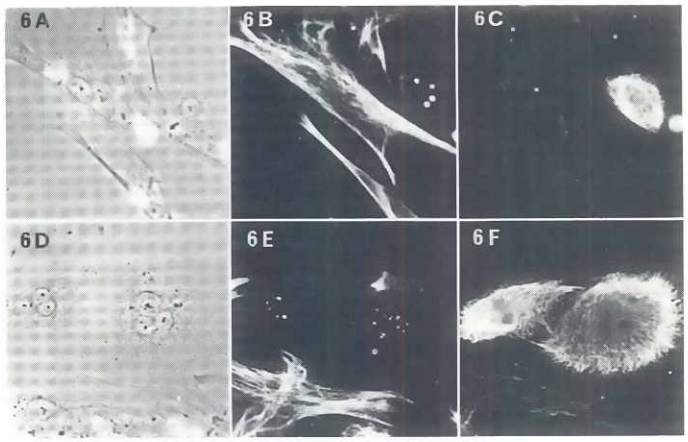

Fig. 6. *Fused cultures of human fibroblasts and amnion epithelial cells after double staining for vimentin (B, E) and keratin (C, F). (A) Fibroblast homokaryon (center) and one unfused amnion epithelial cell. (B) A dense network of cytoplasmic vimentin filaments can be seen in the fibroblast homokaryon, whereas only spotty fluorescence is visible in the epithelial cell. (C) A dense network of keratin fibrils is seen in the epithelial cell, whereas the fibroblast homokaryon lacks fluorescence. (D) Amnion epithelial cell homokaryons (center) and one heterokaryon (bottom). (E) The epithelial cell homokaryons show a weak spotty vimentin fluorescence. Fibrillar arrays of vimentin filaments are seen in the heterokaryon. (F) The epithelial cell homokaryons show fibrillar keratin-specific staining, with typical cell-to-cell junctions. Keratin filaments are also observed in the heterokaryon (from Laurila* et al., *1982).*

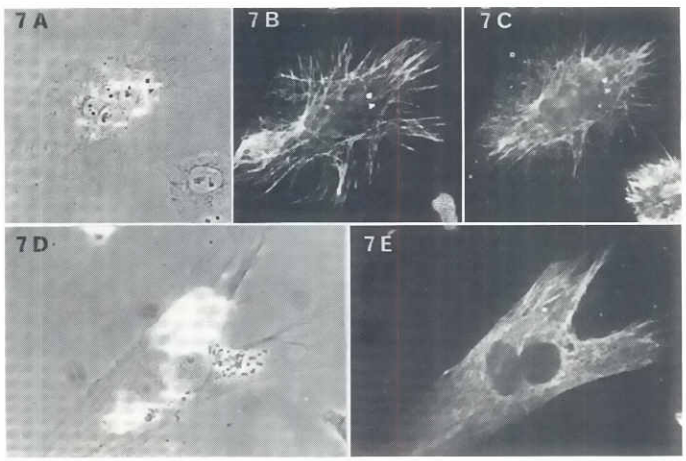

Fig. 7 *Expression of intermediate filaments in heterokaryons. (A) A heterokaryon of fibroblasts and human amnion epithelial cells (center). One unfused amnion epithelial cell (below, right). (B) After staining for vimentin the heterokaryon shows typical fibrillar fluorescence, whereas no fluorescence can be seen in the epithelial cell. (C) After double staining, both cells exhibit fibrillar arrays of keratin filaments (from Laurila* et al., *1982). (D) A heterokaryon formed between normal fibroblasts and HeLa cells. (E) After staining for keratin, a fibrillar cytoplasmic fluorescence can be seen.*

Analogously, in heterokaryons formed between human amnion epithelial cells and glial cells, three types of intermediate filaments, vimentin, keratin and glial filaments, were co-expressed within the same cytoplasm. The filaments were distributed uniformly within the cytoplasm of the heterokaryon, and the redistribution pattern of the filaments was similar to that seen in the parental cells. This indicated that the keratin fila-

ments, on the one hand, and the vimentin and glial filaments, on the other, were structurally independent (Laurila and Virtanen, 1985; Laurila and Virtanen, unpublished results). The rate and sequence of the intermixing process of the filaments suggest that the filaments are mechanically rearranged in the cytoplasm of the heterokaryon, as it is known that cytoskeletal structures and extracellular matrix proteins are associated via specialized intramembranous receptor molecules (review, Buck and Horwitz, 1987). This intermixing of intermediate filaments may be associated with the concomitant redistribution of surface receptors known to occur rapidly after fusion (Edidin *et al.*, 1982; Cheng-Zhi, 1988).

Our results are in line with the idea of autonomous coexpression of the two or even three different types of intermediate filaments within the same cell. In analogous experiments, when HeLa cells were fused with fibroblasts, the resulting continuous hybrid cell lines showed varying degrees of expression of keratin filaments (Stanbridge *et al.*, 1982; Knapp *et al.*, 1985). There was also correlation of keratin expression with unlimited growth potential. The karyotype of the hybrid cells was not, however, determined in detail and thus gene loss as a cause for the observed phenomena could not be ruled out (Knapp and Bunn, 1987).

Concluding remarks

Heterokaryons and fused cells in general have proved valuable in the analysis of the regulation of the differentiated phenotype in cells. Our results are in agreement with the presence of diffusible transacting factors regulating the expression of the extracellular matrix. On the other hand, different types of intermediate filaments were co-expressed in our heterokaryons, which suggests that the cytoskeletal and extracellular matrix structure reflect differing aspects of cellular differentiation and have a varying susceptibility to cytoplasmic influences. In the future, the role of the possible transacting factors should be studied using molecular markers in addition to the immunocytochemical methods used so far. Interesting candidates for the transacting factors are various DNA-binding proteins now being discovered in various laboratories. They open very promising avenues for the study of the regulation of the differentiated phenotype of cells.

Acknowledgements

The helpful assistance of Ms. Marja-Leena Rissanen in the preparation of this manuscript is gratefully acknowledged. This study was supported by grants from the Finnish Cancer Society and the Paulo Foundation.

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