Decreased expression of fibronectin and the $\alpha_5\beta_1$ integrin during terminal differentiation of human keratinocytes

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Summary

We have examined the expression of fibronectin and the $\alpha_5\beta_1$ fibronectin receptor during terminal differentiation of human epidermal keratinocytes, using involucrin as a terminal differentiation marker. The levels of mRNAs encoding fibronectin and the $\alpha_5$ and $\beta_1$ integrin subunits were measured in keratinocyte populations that had been enriched for involucrin-negative or -positive cells by unit gravity sedimentation or suspension-induced terminal differentiation. All three mRNAs decreased in abundance during terminal differentiation, and the corresponding proteins were localised by immunofluorescence to the basal layer in stratified colonies. We also examined expression in ndk, a strain of epidermal cells with a complete block in terminal differentiation, which, as a result, do not express involucrin. Messenger RNA levels for fibronectin and the $\alpha_5$ and $\beta_1$ subunits were higher in ndk, than in unfrac tionated keratinocytes and the corresponding proteins were expressed by all ndk, consistent with a basal keratinocyte phenotype. We conclude that expression of fibronectin and the $\alpha_5\beta_1$ fibronectin receptor decreases during terminal differentiation and that such changes are likely to play a role in the selective migration of terminally differentiating cells from the basal epidermal layer.

Key words: fibronectin, integrin, keratinocytes.

Introduction

Human epidermal keratinocytes can be grown in culture under conditions in which they retain the basic organisation of the tissue from which they are derived (Green, 1980; Watt, 1988). The cells stratify, proliferation is confined to cells in the basal layer, and cells undergo terminal differentiation as they move upwards through the suprabasal layers. Involucrin, the major precursor of the cornified envelope, is expressed by all cells that have left the basal layer, and as such is a useful marker of terminal differentiation (Rice and Green, 1979; Simon and Green, 1984). The cultures provide a good experimental model with which to analyse the factors that control proliferation and terminal differentiation (Watt, 1988).

In skin, the basal layer of keratinocytes adheres to a basement membrane that contains a range of extracellular matrix molecules, including type IV collagen, laminin, fibronectin and heparan sulphate proteoglycan (reviewed by Briggaman, 1982). Keratinocytes in culture synthesize and deposit the major basement membrane components, although the proteins are not organised into a recognisable basement membrane (Alitalo et al. 1982; Kubo et al. 1984; O'Keeffe et al. 1984; Clark et al. 1985; Oguchi et al. 1985; Bohmert et al. 1986).

Studies with cultured keratinocytes have shown that cell–matrix interactions play an important role in terminal differentiation. During terminal differentiation keratinocytes become less adhesive to extracellular matrix proteins (reviewed by Watt, 1987; Adams and Watt, 1990) and this ensures that cells move upwards out of the basal layer as they undergo terminal differentiation (Watt, 1984). Terminal differentiation can be induced by depriving keratinocytes of contact with the culture substratum; however, induction can be blocked by adding fibronectin to the cell suspension and we have suggested that reduced occupancy of extracellular matrix receptors may act as a signal for terminal differentiation (Watt et al. 1988; Adams and Watt, 1989). We have proposed that a positive feedback loop exists, such that reduced contact with the extracellular matrix causes cells to initiate terminal differentiation and simultaneously migrate upwards out of the basal layer (Watt et al. 1988; Watt, 1988).

Although fibronectin is a minor component of the normal epidermal basement membrane it is abundant in the provisional extracellular matrix over which keratinocytes migrate during wound-healing (reviewed by Clark, 1990). In culture, fibronectin receptor function is upregulated (Toda et al. 1987) and in short-term adhesion assays fibronectin is the preferred adhesive substratum for cultured keratinocytes (Adams and Watt, 1990). Keratinocyte adhesion to fibronectin is mediated by the $\alpha_5\beta_1$ integrin (Adams and Watt, 1990).

The aim of the present experiments was to obtain more information about keratinocyte–fibronectin interactions by comparing expression of fibronectin and the $\alpha_5\beta_1$ integrin in proliferating and terminally differentiating cells. Three different experimental approaches were chosen. The first was to harvest stratified cultures of keratinocytes and separate the cells on the basis of size by unit gravity sedimentation (Pavlovitch et al. 1989), since
keratinocytes enlarge during terminal differentiation (Sun and Green, 1976; Watt and Green, 1981). The second approach was to induce the majority of cells to undergo near-synchronous terminal differentiation by disaggregation and resuspension in methyl-cellulose (Green, 1977; Rice and Green, 1978; Adams and Watt, 1989).

Rice and Green, 1978; Adams and Watt, 1989). Finally, expression of fibronectin and the a5β1 fibronectin receptor in normal keratinocytes was compared with expression in nondifferentiating keratinocytes (ndk), a strain of epidermal cells that fail to stratify and have a complete block in terminal differentiation (Adams and Watt, 1988). Our findings provide more information about the mechanism by which terminally differentiating keratinocytes are selectively expelled from the basal layer during stratification.

Materials and methods

Cell culture

Human keratinocytes (strain a, fifth to twelfth passage) were isolated from newborn foreskin and grown in the presence of a feeder layer of 3T3 cells (clone J2) pretreated with 4 μg/ml 1 cholera toxin (ICN Biomedicals, High Wycombe, UK), 10 ng/ml 1 epidermal growth factor (prepared by C. George-Nascimento and generously donated by Chiron Corp., Emeryville, USA) and 10% foetal calf serum (Imperial Laboratories (Europe) Ltd, Andover, UK) (Allen-Hoffmann and Rheinwald, 1984). Cultures were seeded at 2 x 10^6 keratinocytes per 75 cm^2 flask (Falcon, Becton Dickinson UK Ltd, Oxford, UK), 10^5 keratinocytes per 35 mm coverslips. ndk cells were grown in culture medium without feeder support (Adams and Watt, 1988). Cultures were incubated at 37°C in a fully humidified, 5% CO_2 atmosphere.

Cell separation by unit gravity sedimentation

Cultured human keratinocytes were separated by sedimentation under unit gravity through a 880 ml gradient of 3% to 5% Ficoll 400 in PBS (Pharmacia Ltd, Central Milton Keynes, UK) using a CelSep (trade mark), according to the manufacturers' instructions (Diagen Control Co., Wilmington, USA). 3.8 Ficoll has a density of 1.010 g/ml^-1 and 5% Ficoll has a density of 1.015 g/ml^-1. J2 3T3 feeder cells were removed from subconfluent keratinocyte cultures by treatment with EDTA, and then keratinocytes were removed using trypsin–EDTA. A single cell suspension of keratinocytes, previously sieved through a 60 μm Nitex nylon monofilament cloth (Cadiach and Sons, London, UK), was prepared at 1 x 10^6 cells/ml^-1 in PBS containing 1% Ficoll. A total of 3 x 10^6 keratinocytes were routinely loaded onto the top of the gradient and after a separation time of 80 min at room temperature, 50 ml fractions were collected and the cells recovered by centrifugation. Eighteen fractions of cells were collected; the first fraction contained cells of the largest size and subsequent fractions contained cells of progressively smaller size. A sample of cells from each fraction was stained with anti-involucrin antibody, and RNA extracted from the remaining cells.

Induction of terminal differentiation

Cultured human keratinocytes were induced to terminally differentiate by suspension in growth medium made viscous by the addition of methyl-cellulose (4000 centipoises; Aldrich Chemical Co., Milwaukee, USA) to 1% (Green, 1977). Keratinocytes were seeded at 10^6 cells per ml methyl-cellulose medium into bacteriological Petri dishes coated with polyHEDMA (Hydrion Labordstories, New Brunswick, USA) to prevent cell adhesion (Folkman and Moscona, 1978), and incubated at 37°C in a fully humidified 5% CO_2 atmosphere. At various times, cells were harvested by diluting the methyl-cellulose with PBS and recovering the cells by centrifugation. Samples of cells were stained with antibodies to involucrin or the a5β1 fibronectin receptor, and RNA was extracted from the remaining cells.

Probes

A human involucrin cDNA clone (pl-2; Eckert and Green, 1986) was generously provided by H. Green, Harvard Medical School. A mouse 18S rRNA probe (100 Df; Edwards et al., 1987) was a kind gift from D. Edwards, University of Oxford. A cDNA clone to mouse integrin β1 subunit (pMINTβ; D.W. De Simone, V. Patel, R.O. Hynes, unpublished) was kindly provided by R.O. Hynes, Massachusetts Institute of Technology, and a cDNA clone to the human fibronectin receptor α5 subunit (pGEMI-P7; Argraves et al., 1986, 1987) was a generous gift from E. Ruoslahti, La Jolla Cancer Research Foundation. A human cellular fibronectin cDNA clone (pFH23; Korolghi et al., 1984) was a kind gift from H. Marond, University of Oxford.

RNA extraction and Northern analysis

Total RNA was extracted from cultured human keratinocytes essentially as described by Chirgwin et al. (1979). Briefly, cells were washed three times with PBS, then lysed in a 4 M solution of guanidine thiocyanate (4 M guanidine thiocyanate (Fluka Chemicals Ltd, Glossop, UK), 0.5 M sodium N-lauroylsarcosine, 25 mM sodium citrate, pH 7.0, 0.1% antifoam A emulsion (Sigma Chemical Co.) and 0.1 M 2-mercaptoethanol; the solution was adjusted to pH 7.0 with a small amount of 1 M sodium hydroxide and filtered. An 80-ml sample of lysate was layered on top of 4 ml of 5.7 M caesium chloride containing 25 mM sodium acetate, pH 6.0, and spun at 32,000 revs min^-1 for 21 h at 20°C. The pellet of RNA was washed with 70% ethanol and dissolved in sterile distilled water containing heparin (sodium salt from porcine intestinal mucosa, Grade I, Sigma Chemical Co.) at 200 μg/ml^-1. The concentration and purity of RNA was determined by absorbance at 260 and 280 nm.

Polyadenylated (poly(A))^-1 RNA was isolated from total RNA preparations by chromatography on oligo(dT)-cellulose (Sigma Chemical Co.), essentially as described by Aviv and Leder (1972). Oligo(dT)-cellulose was suspended in sterile loading buffer (20 mM Tris–HCl, pH 7.5, 0.5 M NaCl, 1 mM sodium EDTA, pH 8.0, 0.1% SDS) and poured into a column. The column was washed with three column-volumes of 0.1 M NaOH, three column-volumes of water containing heparin (sodium salt from porcine intestinal mucosa), Grade I, Sigma Chemical Co.) at 200 μg/ml^-1. The pH of the column effluent was less than 8. The solution of total RNA was heated at 65°C for 5 min, then cooled to room temperature. An equal volume of 2× sterile loading buffer was added to the RNA solution, the mixture applied to the column and the eluate collected. One column-volume of sterile loading buffer was added to the column and the eluate collected. The pooled eluate was heated to 65°C for 5 min, cooled to room temperature and reapplied to the column. The column was washed with five column-volumes of sterile loading buffer, then the poly(A)(+) RNA eluted from the oligo(dT)-cellulose with three column-volumes of sterile elution buffer (10 mM Tris–HCl, pH 7.5, 1 mM sodium EDTA, pH 8.0, 0.05% SDS). The poly(A)(+) RNA was heated to 65°C for 5 min, cooled to room temperature, the NaCl concentration adjusted to 0.5 M, and then a second round of chromatography was carried out using the same column. Sodium acetate (3 M, pH 5.2) was added to the eluted poly(A)(+) RNA to a final concentration of 0.3 M and the RNA precipitated with 2.5 volumes of ethanol at −20°C. The RNA was recovered by centrifugation, the pellet rinsed in 70% ethanol and then dissolved in sterile distilled water containing heparin at 200 μg/ml^-1.

RNA samples were prepared for electrophoresis in the following way. RNA was mixed with 4 volumes of sample buffer (2 parts 37% formaldehyde (AlaraK, BDH Ltd, Poole, UK), 5 parts deionised formamide, 1 part 10× Hepes buffer (0.5 M Hepes, 10 mM sodium EDTA, pH 7.8), incubated at 70°C for 10 min, then allowed to cool to room temperature. Loading buffer (50% glycerol, 226 L. J. Nicholson and F. M. Watt
The binding of specific antisera to human keratinocytes grown on glass coverslips or to suspensions of keratinocytes dried onto coverslips (Read and Watt, 1988) was visualised by indirect immunofluorescence. The first antibody was incubated with the cells for 1h at room temperature, washed extensively in PBS, then incubated with the second antibody (GAR-FITC or RAR-FITC diluted 1:32 in PBS) for 1h at room temperature. The cells were washed in PBS, mounted in Gelvatol (Monsanto Co., St Louis, USA) and examined using a Zeiss Axiophot microscope.

Antibodies

The preparation of rabbit antiserum to human involucrin has been described previously (Dover and Watt, 1987). Rabbit antiserum to human fibronectin (Ruusalhi et al. 1982) was obtained from Novabiochem (UK) Ltd, Nottingham, UK. Rabbit antiserum against the human fibronectin receptor was supplied by Novabiochem (UK) Ltd, and recognises epitopes on both $\alpha_6$ and $\beta_1$ subunits; it therefore cross-reacts with members of the $\beta_1$ integrin family in addition to $\alpha_6\beta_1$ (Argraves et al. 1986, 1987; Kramer et al. 1989). Rat monoclonal antibodies 13 and 16 recognise the $\beta$ and $\alpha$ subunits, respectively, of the $\alpha_6\beta_1$ fibronectin receptor, and were a generous gift from K. Yamada, National Institutes of Health (Akiyama et al. 1989; Matsuyama et al. 1989). Fluorescein-conjugated goat anti-rabbit IgG (GAR-FITC) and rabbit anti-rat IgG (RAR-FITC) were supplied by ICN Biomedicals Ltd, High Wycombe, UK.

Indirect immunofluorescence

The binding of specific antisera to human keratinocytes grown on glass coverslips or to suspensions of keratinocytes dried onto coverslips (Read and Watt, 1988) was visualised by indirect immunofluorescence. Cells were fixed with 3.7 % formaldehyde in PBS for 10 min at room temperature. Cells to be stained with anti-involucrin antibody were subsequently permeabilised with absolute methanol for 5 min on ice.

Cells were incubated with the first antibody for 1h at room temperature, washed extensively in PBS, then incubated with the second antibody (GAR-FITC or RAR-FITC diluted 1:32 in PBS) for 1h at room temperature. The cells were washed in PBS, mounted in Gelvatol (Monsanto Co., St Louis, USA) and examined using a Zeiss Axiophot microscope.

Results

Physical separation of basal and terminally differentiating keratinocytes

Keratinocytes were loaded onto a 3 % to 5 % Ficoll gradient in a CellSep apparatus and allowed to sediment under unit gravity. After 80 min the gradient was fractionated and cells were harvested from each fraction. Samples were stained for involucrin (Fig. 1A) and total RNA was extracted from the remaining cells for Northern analysis (Fig. 1B). As shown in Fig. 1A, the first two, most dense, fractions were enriched in large, involucrin-positive cells and there was a progressive increase in the proportion of small, involucrin-negative cells in the later fractions. The immunofluorescence results were in good agreement with the levels of involucrin mRNA in the different fractions, as shown in Fig. 1B, which includes tracks of total RNA extracted from unfractionated keratinocytes harvested just prior to confluence, and from 3T3 clone J2 feeder cells that do not express involucrin. The results presented in

![Fig. 1. Immunofluorescence and Northern analysis of cell fractions obtained by unit gravity sedimentation.](image-url)
Fig. 1B are from a single experiment; similar results were obtained with RNA from three other cell separation experiments. The relative enrichment for involucrin mRNA was 10-fold between fractions 1 and 11; note, however, that no fraction consisted entirely of involucrin-negative cells (or indeed, involucrin-positive cells, as judged by immunofluorescence). An 18S rRNA probe was used as a control to show the amount of RNA loaded per track.

Messenger RNA levels for fibronectin and the α5 and β1 integrin subunits were examined. As shown in Fig. 1B, fibronectin mRNA was detected throughout the gradient, but was slightly more abundant in fractions 1–9 than in 10 and 11. Fibronectin mRNA levels in J2 cells were higher than in keratinocytes. There was an inverse relationship between β1 mRNA levels and involucrin expression, with more β1 expressed in the fractions enriched for small, involucrin-negative cells (fractions 8–11). There was a similar enrichment for α5 mRNA in these fractions. J2 cells expressed more β1 mRNA than keratinocytes, but less α6.

Induction of terminal differentiation by suspension
Keratinocytes were disaggregated and resuspended in medium containing methyl-cellulose, then poly(A)+ RNA was isolated for Northern analysis after 0, 4 and 24 h (Fig. 2). β Actin and glyceraldehyde phosphate dehydrogenase mRNAs are commonly used as loading controls, but the levels of both decreased with time when keratinocytes were placed in suspension (results not shown). In Northern blots of total RNA, in which 18S rRNA served as a control, the level of involucrin mRNA increased fourfold between 0 and 24 h in suspension and there was a slight increase detectable by 4 h (results not shown). We therefore used involucrin mRNA levels as a guide to relative loading on poly(A)+ RNA blots. As shown in Fig. 2, there was a decrease in involucrin mRNA between 0 and 4 h and a fourfold increase between 0 and 24 h, indicating that equivalent amounts of RNA had been loaded in the 0 and 24 h tracks, while a lower amount was loaded in the 4 h track. The involucrin mRNA results are in general agreement with published data on the number of cells expressing involucrin and cornified envelopes with time in suspension (Adams and Watt, 1989; Rice and Green, 1978).

Messenger RNA levels for fibronectin and the α5 and β1 integrin subunits were examined. As shown in Fig. 2, mRNA levels of fibronectin and both integrin subunits had decreased by 24 h in suspension. Given the down-loading of the 4 h track, message levels for fibronectin and the β1 subunit were unchanged or slightly decreased between 0 and 4 h in suspension, whereas there was an increase in abundance of the α5 subunit mRNA.

ndk
Fig. 3 shows the results of Northern analysis of total RNA from normal keratinocytes, J2 feeder cells and ndk. The data in Fig. 3 are from a single experiment; similar results were obtained in three further experiments. No involucrin mRNA was detected in ndk; this is consistent with the absence of detectable involucrin protein (Adams and Watt, 1988). The level of fibronectin mRNA was 15-fold higher in ndk than in normal keratinocytes and the levels of α5 and β1 were both approximately threefold higher.

Expression of fibronectin and the β1 subunit by J2 cells was intermediate between keratinocytes and ndk, but there was less α6 mRNA in J2 than in keratinocytes and ndk (see also Fig. 1).

Immunofluorescence studies
To complement the Northern analysis, the distribution of fibronectin and the α5β1 fibronectin receptor on the surface of keratinocytes was examined. As shown in Fig. 3, fibronectin was distributed throughout the epithelium and the β subunit was expressed in the basal region of the spinous layer. In normal keratinocytes, fibronectin and the β1 subunit were expressed in the lower spinous cells, but in ndk, fibronectin was expressed in the lower spinous and granular layers and the β1 subunit was expressed in the granular layer. In J2 cells, fibronectin was expressed in the lower spinous and granular layers and the β1 subunit was expressed in the granular layer.

Fig. 2. Northern analysis of keratinocytes induced to undergo terminal differentiation in suspension. RNA was extracted from keratinocytes after 0, 4 and 24 h in suspension. A 4 μg sample of poly(A)+ RNA was loaded per track and hybridised to various cDNA probes: involucrin (INV), fibronectin (FN), fibronectin receptor α5 subunit (FNRα5), β1 subunit (FNRβ1). 

Fig. 3. Northern analysis of normal keratinocytes (strain a), ndk and J2 3T3 cells. A 30 μg sample of total RNA was loaded per track and hybridised to various cDNA probes: involucrin (INV), fibronectin (FN), fibronectin receptor α5 subunit (FNRα5), β1 subunit (FNRβ1) and 18S rRNA (18S). Each set of tracks is from the same exposure of the same gel.
of keratinocytes and ndk was visualised by immunofluorescence. When keratinocytes were harvested with trypsin/EDTA, fibronectin could not be detected on the cell surface, but positive staining for the α5β1 integrin was observed. Small cells stained brightly with a rabbit antiserum to α5β1, whereas the larger, terminally differentiating cells generally showed only background fluorescence (Fig. 4A). After 24 h in suspension the proportion of positively stained cells had decreased from 65 to 33% (Fig. 4B).

The distribution of fibronectin and the α5β1 fibronectin receptor in adherent keratinocytes and ndk was also examined by indirect immunofluorescence (Figs 5,6). In order to compare staining in basal and terminally differentiating keratinocytes, small stratified colonies were used, in which there is a peripheral zone of basal cells, with terminally differentiating suprabasal cells in the centre. Only the suprabasal cells stained positive with anti-involucrin (Fig. 5A,B) as reported previously (Banks-Schlegel and Green, 1981; Barrandon and Green, 1987).

Fibronectin was found in association with the surface of basal keratinocytes, whereas suprabasal cells were largely unstained (Fig. 5C,D). The J2 feeder layer had a prominent lacy network of fibronectin and in comparison keratinocyte fibronectin was much less abundant, consisting of diffuse patches at the cell surface with some concentrated at cell–cell boundaries (Fig. 5D).

The β1 integrin subunit was present on basal keratinocytes, but suprabasal cells stained weakly or not at all (Fig. 5E,F). β1 subunit was concentrated at cell–cell boundaries and largely absent from the free margins of basal cells (Fig. 5F). The α5 subunit was also found primarily on basal cells; staining was rather diffuse, with some bright patches (Fig. 5G,H). Permeabilisation resulted in loss of staining with antibodies to either subunit, and concentration of the integrin subunits in focal contacts was not observed (Nicholson, unpublished observations; Adams and Watt, unpublished data).

ndk were also stained with antibodies to fibronectin and the α5β1 receptor (Fig. 6). As described previously, ndk have prominent ruffled membranes; they do not grow as compact colonies; and they do not express involucrin (Adams and Watt, 1988; Fig. 6). Staining for fibronectin was more extensive than in normal keratinocytes and revealed a lacy network over and around the cells, similar in appearance to that associated with J2 cells (compare Fig. 6A with Fig. 5D). The β1 subunit was localised to regions of cell–cell contact and in a speckled pattern over the cell surface (Fig. 6B). α5 Subunit staining was diffuse, with some bright spots (Fig. 6C). Again, permeabilisation resulted in loss of staining (Nicholson, unpublished observations; Adams and Watt, unpublished data). All cells within the ndk population stained positive for fibronectin and the α5 and β1 integrin subunits, in contrast to the heterogeneity of staining in colonies of normal keratinocytes.

**Discussion**

The aim of the present experiments was to obtain more information about keratinocyte–extracellular matrix interactions by comparing expression of fibronectin and the α5β1 integrin in proliferating and terminally differentiating cells. Messenger RNA levels were measured in populations that had been enriched for involucrin-negative or -positive cells by unit gravity sedimentation or suspension-induced terminal differentiation and in ndk, a strain of epidermal cells that do not terminally differentiate. The distribution of the proteins was studied by immunofluorescence.

Messenger RNA levels for the α5 and β1 integrin subunits decreased during suspension-induced terminal differentiation and were lower in CelSep fractions enriched for involucrin-positive cells than in those enriched for involucrin-negative cells. The proteins were predominantly present in the basal layer, as observed by Adams and Watt (unpublished data). In fully developed epidermis, β1 integrins are localised in the basal layer although staining for α5 is weak (Peltonen et al. 1989; Staquet et al. 1989; Hertle, Adams and Watt, unpublished data). Thus, both in vivo and in culture, α5β1 receptor expression is reduced when keratinocytes leave the basal layer. The Northern analysis results suggest that this is due either to decreased transcription or to a reduction in mRNA stability; further experiments are required to distinguish between these possibilities.

By immunofluorescence, fibronectin was localised to the basal layer, in agreement with the earlier observations of Alitalo et al. (1982). A decrease in fibronectin mRNA was observed by 24 h in suspension; however, the message was equally abundant in involucrin-positive and -negative fractions of the CelSep. There are a number of possible explanations for this discrepancy. One is that the suspension effect is due to loss of cell–substratum contact, rather than to induction of terminal differentiation; however, at least in 3T3 cells, suspension actually results in an increase in fibronectin mRNA (Dike and Farmer, 1988). Alternatively, the CelSep Northern may simply have been less sensitive than the suspension Northern, because total RNA was used for the former and poly(A)+ RNA for the latter; certainly the reduction in fibronectin mRNA in suspension is less marked than that of the α5.

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Fig. 5. Phase-contrast images and indirect immunofluorescence staining of stratified colonies of keratinocytes. Colonies were stained with (A,B) anti-involucrin, (C,D) anti-fibronectin, (E,F) anti-β1 integrin, (G,H) anti-α5 integrin. Note that the large suprabasal cells in the centres of the colonies are positively stained for involucrin (A,B), but are largely unstained with the other antibodies (C–H). Bars: 15 μm (A,B), 30 μm (C–H).
ndk cells do not undergo terminal differentiation and have some of the properties of basal keratinocytes (Adams and Watt, 1988). Consistent with a basal phenotype, the levels of $\alpha_5$, $\beta_1$ and fibronectin mRNAs were higher in ndk than in unfractionated keratinocytes and all of the cells were positive for each protein by immunofluorescence. One function of the J2 feeder layer is to provide an extracellular matrix to promote attachment of keratinocytes (Alitalo et al. 1982) and the high level of expression of fibronectin by ndk may partly explain why ndk do not require a feeder layer for growth (Adams and Watt, 1988). However, it is unclear why fibronectin mRNA levels should be so much higher in ndk than J2 (Fig. 3).

In conclusion, our findings provide new information about the link between terminal differentiation and migration out of the basal layer (Watt, 1984, 1987). During terminal differentiation keratinocyte adhesiveness to a range of extracellular matrix proteins, including fibronectin, is reduced (Adams and Watt, 1990). Adhesion to fibronectin is mediated by the $\alpha_5\beta_1$ integrin and receptor function is down-regulated in two stages during terminal differentiation: there is an early reduction in the ability of $\alpha_5\beta_1$ to bind fibronectin, followed by loss of the integrin from the cell surface (Adams and Watt, 1990). Our results now show a correlation between the level of $\alpha_5\beta_1$ protein on the cell surface and the abundance of the $\alpha_5$ and $\beta_1$ subunit mRNAs. By 4 h in suspension, adhesion to fibronectin has begun to decrease, but there is no reduction in the amount of receptor on the cell surface (Adams and Watt, 1990) or in the abundance of the subunit mRNAs (Fig. 2). However, by 24 h the level of $\alpha_5\beta_1$ on the cell surface has decreased (Adams and Watt, 1990) and there has been a reduction in the $\alpha_5$ and $\beta_1$ mRNA levels (Fig. 2). Two questions that remain to be answered are whether the mRNA levels reflect transcriptional regulation and, if so, whether decreased transcription is a consequence of the earlier reduction in ligand-binding capacity. It is clear, however, that the integrin-mediated interaction of basal keratinocytes with the extracellular matrix can be controlled at more than one level.

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References


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