Differences between adult and foetal fibroblasts in the regulation of hyaluronate synthesis: correlation with migratory activity

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Summary

We have previously reported that confluent foetal fibroblasts migrate into three-dimensional collagen gel matrices to a significantly greater extent than do adult cells. Hyaluronic acid (HA) is a major constituent of the extracellular matrix deposited by fibroblasts and has been demonstrated to stimulate the migration of a number of different cell types. Previous studies have indicated that the synthesis of HA by normal adult skin fibroblasts declines significantly when the cells achieve confluence. Data presented in this paper indicate that foetal fibroblasts differ from adult cells in this respect, in that they do not show an inverse relationship between cell density and HA synthesis, i.e. confluent foetal fibroblasts continue to produce approximately the same amount of HA as do subconfluent cells. These data suggest that the synthesis of relatively high levels of HA by foetal fibroblasts at confluence may be causally related to the elevated migration displayed by these cells. In this context, a close correlation was observed between the level of HA synthesized by confluent foetal and adult fibroblasts and the differential migratory activity displayed by these cells. Such differences in HA synthesis and migratory behaviour were only apparent at cell confluence, with subconfluent foetal and adult fibroblasts being indistinguishable in terms of these two criteria. Our data further reveal that: (1) cell density affects the size class of HA synthesized by both foetal and adult cells; and that (2) there is a considerable degree of heterogeneity amongst the nine different fibroblast lines examined in this study in terms of the size class of HA that they produce.

Key words: fibroblasts, hyaluronate, heterogeneity, cell density.

Introduction

Foetal fibroblasts may be distinguished from their normal adult counterparts by a number of behavioural and biochemical criteria, including cloning efficiency in semi-solid medium (Nakano and T'so, 1981), the production of peptide growth factors (Clemmons, 1983; Lawrence *et al.* 1984), proliferative response to TGF-β (Hill *et al.* 1986), the synthesis of particular isoforms of matrix macromolecules (Matsuura and Hakomori, 1985; Castellani *et al.* 1986) and the presence of foetal-specific antigenic determinants at the cell surface (Azzarone *et al.* 1985; Bartal *et al.* 1986). We have previously reported that the migration of foetal and adult fibroblasts into three-dimensional gels of type I collagen fibres is differentially affected by cell density (Schor *et al.* 1985). In our migration assay, fibroblasts are plated on the surface of collagen gels at defined subconfluent (10^3 cells cm^{-2}) and confluent (2×10^4 cells cm^{-2}) cell densities; these cultures are then incubated for 4 days, at which time the percentage of cells that have migrated down into the three-dimensional collagen matrix is determined by microscopic observation (Schor, 1980). Using this experimental approach, we have shown that the migration of adult fibroblasts is inversely proportional to cell density, with mean values of 20.6% cells within the gel matrix in subconfluent cultures compared to 5.9% in confluent cultures (Schor *et al.* 1985, 1988). In contrast, the migration of foetal fibroblasts appears to be unaffected by cell density with corresponding mean values of 16.5% and 15.8% cells within the gel matrix in subconfluent and confluent cultures, respectively. These observations have provided the impetus for our current work concerned with understanding the biochemical basis of the differential migratory behaviour of foetal and adult fibroblasts.

Hyaluronic acid (HA) is the major class of glycos-

aminoglycan (GAG) synthesized by fibroblasts *in vitro* (Welch and Roberts, 1975; Hopwood and Dorfman, 1977). HA deposited in the extracellular matrix (ECM) has been shown to stimulate the migration of a number of cell types during embryonic development, including neural crest cells (Pratt *et al.* 1975; Pintar, 1978; Turley and Torrance, 1984), corneal mesenchymal cells (Toole and Trelstad, 1971) and cardiac cushion cells (Markwald *et al.* 1978; Orkin and Toole, 1978). In view of this potentiating effect of HA on cell migration, it is of interest to note that synthesis of HA by adult fibroblasts *in vitro* has been reported to be inversely related to cell density, with significantly less HA produced on a per cell basis by confluent adult fibroblasts compared to subconfluent cells (Tomida *et al.* 1974; Hronowski and Anastassiades, 1980; Matuoka *et al.* 1985, 1987; Mian, 1986). Such a density-dependent inhibition of HA synthesis is not seen in virally transformed fibroblasts and has been discussed in terms of the continued mitotic activity of these cells at confluence (Hopwood and Dorfman, 1977; Matuoka *et al.* 1987). Similar data regarding the density dependence of HA synthesis by foetal fibroblasts have not been reported.

With this information in mind, the specific objective of the present study has been to compare the effect of cell density on the synthesis of HA by foetal and adult fibroblasts as assessed by the incorporation of radiolabelled precursors into matrix macromolecules, as well as by direct biochemical analysis. The HA synthesized has been further analysed in terms of molecular size distribution and compartmentalization (i.e. HA recovered in the culture medium *versus* cell-associated material). Our results indicate that: (1) in contrast to adult fibroblasts, the synthesis of HA by foetal fibroblasts is not diminished when cell confluence is achieved; (2) both foetal and adult fibroblasts cease to proliferate in confluent culture, suggesting that the relatively elevated production of HA by foetal fibroblasts at confluence is not related to proliferative activity; and (3) there is a considerable degree of heterogeneity amongst fibroblasts (foetal and adult) in terms of the size class, distribution and quantity of cell-produced HA.

Materials and methods

Materials

D-[6-^{3}H]glucosamine hydrochloride (22 Ci mmol^{-1}) and $Na^{35}SO_4$ (aqueous solution, 25–40 Ci mg^{-1}) were obtained from Amersham International plc, Amersham, Bucks., UK. Hyaluronate (type I, sodium salt), chondroitin-6-sulphate, dermatan sulphate, *Streptomyces* hyaluronidase and papain (type III, 2×crystallized) were obtained from Sigma Chemical Co., Poole, Dorset, UK. Sepharose CL-2B was obtained from Pharmacia Fine Chemicals, Milton Keynes, UK. Titan III Zip Zone cellulose acetate electrophoresis plates were purchased from Helena Laboratories, Tyne & Wear, UK. Optiphase Safe scintillation fluid was obtained from LKB. Eagle's minimal essential medium, donor calf serum, sodium pyruvate, glutamine, non-essential amino acids, antibiotics and tissue-culture plastic dishes (Nunclon) were obtained from Gibco Bio-Cult, Paisley, Scotland, UK. All other chemicals were obtained from BDH Chemicals, Poole, Dorset, UK or Sigma Chemical Co., Poole, Dorset, UK.

Table 1. *Confluence densities of skin fibroblast cell lines from adult and foetal origins*

Cell line	Origin	Confluence density (cells cm^{-2}, $\times 10^{-4}$)
SK131	Adult (female, age 21)	1.80±0.28 (2)
SK158b	Adult (male, age 24)	1.65±0.07 (2)
SK172b	Adult (male, age 12)	1.95±0.07 (2)
SK173b	Adult (female, age 13)	1.80±0.28 (2)
FS6	Foetal (12–16 weeks)	3.56±0.09 (5)
FS10	Foetal (12–16 weeks)	3.65±0.07 (2)
F105d	Foetal (7–8 weeks)	3.40±0.14 (2)
F107g	Foetal (7–8 weeks)	3.75±0.35 (2)
FSF37	Foreskin (male, age 6)	1.38±0.10 (5)

Cell numbers at confluence were determined at two or more different passages and expressed as mean±S.D. (number of experiments indicated in parenthesis).

Cell culture

Human fibroblast lines were established from skin biopsies by explant culture as described by Schor *et al.* (1985). Four lines of foetal skin fibroblasts (FS6, FS10, F105d, F107g), four lines of normal adult skin fibroblasts (SK131, SK158b, SK172b, SK173b) and one normal foreskin fibroblast line (FSF37) were used in this study. Samples of adult skin were obtained from the forearm (by pinch biopsy) and foetal skin was obtained from either the abdomen (12–16 weeks) or a limb (7–8 weeks) (see Table 1 for further details). All cultures were maintained in Eagle's minimal essential medium (MEM) supplemented with 15% (v/v) donor calf serum, 1 mM-sodium pyruvate, 2 mM-glutamine, non-essential amino acids, penicillin (100 units ml^{-1}) and streptomycin (0.1 mg ml^{-1}) at 37 °C in a moist atmosphere of CO_2/air (1:19). Stock cultures were maintained in 90 mm plastic tissue-culture Petri dishes and passaged at a split ratio of 1:5 when the cultures reached confluence after 7–10 days of growth. Experiments to determine the accumulation of glycosaminoglycans (GAGs) were carried out with cells cultured in 35 mm plastic tissue-culture Petri dishes containing 1 ml of culture medium as above and supplemented with the appropriate radiolabelled precursors. Cell counts were performed with a Coulter electronic particle counter or with a haemocytometer.

Metabolic labelling

Glycosaminoglycans synthesized by the fibroblasts were metabolically labelled by incubating cultures for 48 h with growth medium supplemented with 2.5 μCi ml^{-1} of [^{3}H]glucosamine and/or 20 μCi ml^{-1} of $Na^{35}SO_4$. Labelling was commenced when the cells were either at 40–60% confluence (i.e. exponential phase of growth) or at 100% confluence (i.e. stationary growth phase).

Metabolically labelled cell cultures were separated into medium and pericellular fractions. To obtain the former, the incubation medium was transferred into another vessel, the cell layer was washed twice with 1 ml samples of phosphate-buffered saline, and these were pooled to form the medium fraction. The pericellular fraction was obtained by extracting the cell layer with 1 ml 50 mM-Tris–HCl, 4 M-guanidinium chloride, pH 7.5, for 24 h at 4 °C. The insoluble material was removed by centrifugation at 12 000 *g* for 2 min in a microcentrifuge and the supernatant was collected as the pericellular fraction.

Quantification of total HA

Isotope incorporation into total HA. The medium and pericellular fractions were dialysed exhaustively against 50 mM-sodium phosphate, 5 mM-EDTA, pH 6.5. Protein was removed by subjecting the samples to papain digestion (1 mg ml^{-1}) in the presence of 15 mM-β-mercaptoethanol at 65°C for 24 h. Papain was then inactivated by heating the samples to 90°C for 1 min. Each sample was then divided into four 200-μl aliquots. Hyaluronate was removed from two aliquots by digestion with *Streptomyces* hyaluronidase (0.5 units, dissolved in 20 μl distilled water) for 17 h at 37°C. The remaining aliquots were similarly incubated after addition of 20 μl water without hyaluronidase as control. Carrier HA (50 μg, in 200 μl sodium phosphate/EDTA buffer) was added to all the aliquots. GAGs were precipitated with cetyl-pyridinium chloride (1 %, w/v, final concentration) for a minimum of 2 h at room temperature. The precipitate was sedimented by centrifugation at 12 000 ***g*** for 10 min, washed once with 1 ml of 60 mM-NaCl and redissolved in 100 μl of 66 % propan-1-ol (v/v). Scintillation fluid (1 ml) was then added and radioactivity was determined in a Beckman 9800 scintillation counter. The amount of radioactivity removed by hyaluronidase digestion was taken as the radioactivity associated with HA.

Biochemical determination of total HA. The media from [^{3}H]glucosamine-labelled cultures were dialysed against 50 mM-sodium phosphate, 5 mM-EDTA and subjected to papain digestion as above. GAGs were precipitated with cetyl-pyridinium chloride without addition of carrier GAGs, washed with 60 mM-NaCl and redissolved in 100 μl 66 % propan-1-ol (v/v). The GAGs were then further purified by addition of 1 ml of ethanol saturated with sodium acetate to reprecipitate the GAGs, which were sedimented by centrifugation at 12 000 ***g*** for 10 min and washed once with absolute ethanol. After air-drying, the GAGs was redissolved in 20–50 μl of 5 mM-EDTA, 10 mM-dithioerythritol. Classes of GAGs were separated by cellulose acetate electrophoresis as described by Cappelletti *et al.* (1979). The GAGs were visualised by staining with 1 % Alcian Blue 8GX in 50 mM-sodium acetate, 50 mM-$MgCl_2$, pH 5.8, and quantified by densitometry using an LKB 2202 laser densitometer. GAG standards (chondroitin-6-sulphate (CS), hyaluronate (HA) and dermatan sulphate (DS)) were prepared by further purification of commercially obtained material (Sigma) by two cycles of papain digestion and cetyl-pyridinium chloride/ethanol precipitation and quantified gravimetrically. Amounts of HA ranging from 20 ng to 100 ng can be reliably quantified by this method.

Molecular size distribution of isotopically labelled HA

Samples of medium and pericellular fractions from metabolic labelling experiments were exhaustively dialysed against 50 mM-sodium acetate, 10 mM-NaCl, pH 5.5. To determine the molecular size distribution of labelled material, aliquots were analysed by Sepharose CL-2B gel filtration chromatography without being subjected to papain digestion. Sepharose CL-2B columns containing 15 ml of gel were prepared in glass columns with an internal diameter of 8 mm and equilibrated with 50 mM-Tris–HCl, 4 M-guanidinium chloride, pH 7.4. Aliquots of the samples (0.4 ml) were chromatographed. Each sample was analysed with and without prior digestion with 2 units ml^{-1} hyaluronidase for 17 h to determine the susceptibility of macromolecules to hyaluronidase digestion and identify the radioactivity associated with HA. The columns were eluted with the Tris/guanidinium chloride buffer at 12 ml h^{-1}. Fraction (0.5 ml) were collected and radioactivity determined after addition of 3.5 ml scintillation fluid. V_o and V_t of the column were determined by the elution volumes of Blue Dextran and Phenol Red, respectively.

Cell migration assay

Fibroblasts were plated at confluent density on collagen gels and the percentage of cells found within the three-dimensional gel matrix was determined after 4 days as previously described (Schor *et al.* 1985).

Statistical analyses

Variation of total HA and [^{3}H]glucosamine incorporation into HA in subconfluent and confluent cultures of the different cell lines were analysed using analysis of variance (one-way classification) (ANOVA); comparisons between sample means of individual cell lines were made using multiple range tests as described by Parker (1979).

Results

Cell growth

The fibroblasts were routinely subcultured at a split ratio of 1:5, and all took 7–10 days to reach confluence. Saturation cell densities were found to vary between foetal and foreskin/adult lines (Table 1); the foreskin and adult skin fibroblasts achieved saturation densities of 1.3×10^4 to 2.0×10^4 cells cm^{-2}, whereas the foetal cells achieved significantly higher densities of 3.4×10^4 to 3.8×10^4 cells cm^{-2}.

Metabolic labelling of HA in the medium fraction

Accumulation of total labelled HA in media of subconfluent and confluent fibroblasts was studied by isolating the GAGs by cetyl-pyridinium chloride/ethanol precipitation and determination of the activity susceptible to hyaluronidase digestion. The data indicated as 'label' in Table 2, which represent independent experiments on each cell line at two different passages, indicate that while there are significant differences in the amount of [^{3}H]glucosamine incorporated into the secreted HA, adult fibroblasts generally incorporate less radiolabel into the secreted HA on a per cell basis in confluent culture than in subconfluent culture; the ratio of incorporated [^{3}H]glucosamine in subconfluent cultures varied between 2.86 and 7.36. This phenomenon was not observed in foetal fibroblasts or in the FSF37 foreskin line, with subconfluent to confluent ratios varying between 0.73 and 1.36.

The heterogeneity of ^{3}H incorporation into secreted HA was analysed by ANOVA without taking into account their foreskin, adult or foetal origins. Differences between individual cell lines were further analysed by multiple range tests at the $P<0.05$ significance level. The results (Fig. 1) indicate that adult fibroblasts at confluence generally incorporate less [^{3}H]glucosamine into secreted HA than their foetal counterparts. The FSF37 cell line more closely resembles foetal fibroblasts by this criterion.

Data presented in Fig. 2 compare the level of migration displayed by the four foetal and adult fibroblast lines used in this study with the total amount of ^{3}H-labelled HA accumulated in the medium fraction

Table 2. *Accumulation of ^{3}H-labelled and total hyaluronate in the culture supernatant at 40–60% and 100% cell confluence*

Cell line		Hyaluronate accumulated during 48 h: Subconfluent	Hyaluronate accumulated during 48 h: Confluent	Mean ratio subconf.: conf.
Adult				
SK131	Labelled	30.75±2.55	10.91±1.91	2.87±0.38
	Total		(not determined)	
SK158b	Labelled	26.73±4.02	9.56±2.32	2.86±0.39
	Total	53.37±12.46	12.49±1.61	4.24±0.45
SK172b	Labelled	45.98±4.65	7.47±2.73	7.36±0 09
	Total	43.02±3.80	8.89±1.09	4.84±0.17
SK173b	Labelled	17.43±2.57	4.06±0.54	4.28±0.09
	Total	40.02±6.31	8.85±0.68	4.56±1.06
Foetal				
FS6	Labelled	12.75±0.87	17.52±1.52	0.73±0.02
	Total	15.86±3.10	18.06±3.21	0.91±0.33
FS10	Labelled	15.39±1.89	17.89±1.08	0.87±0.22
	Total		(not determined)	
F105d	Labelled	29.65±3.53	22.10±1.74	1.36±0.38
	Total	22.95±4.17	26.80±2.31	0.85±0.08
F107g	Labelled	24.59±0.64	20.87±1.53	1.18±0.08
	Total	16.02±4.29	16.99±5.00	1.02±0.55
Foreskin				
FSF37	Labelled	16.44±0.94	19.61±6.39	0.92±0.35
	Total	34.41±2.86	24.68±6.15	1.45±0.48

* Values are mean±S.D. of two independent experiments. Labelled HA, disints min^{-1} per 10^6 cells, $\times 10^{-5}$; total HA, μg HA per 10^6 cells.

(Table 2). The data indicate a close correlation between the levels of migration displayed by these cells and HA production.

Elution profiles of dialysed metabolically labelled medium fractions from three representative fibroblast lines are shown in Fig. 3. The results indicate that there is considerable variation in molecular size distribution of ^{3}H-labelled material between different cell lines and also within a given cell line at exponential and stationary growth phases. In addition to the material eluted at V_o, a proportion of material eluted between V_o and V_t was also susceptible to hyaluronidase digestion; this observation indicates that HA synthesized by some of the cell lines is polydisperse and can be classified into high M_r ($M_r > 2\times10^6$) and low M_r ($M_r < 2\times10^6$) classes. The specificity of the hyaluronidase for HA has previously

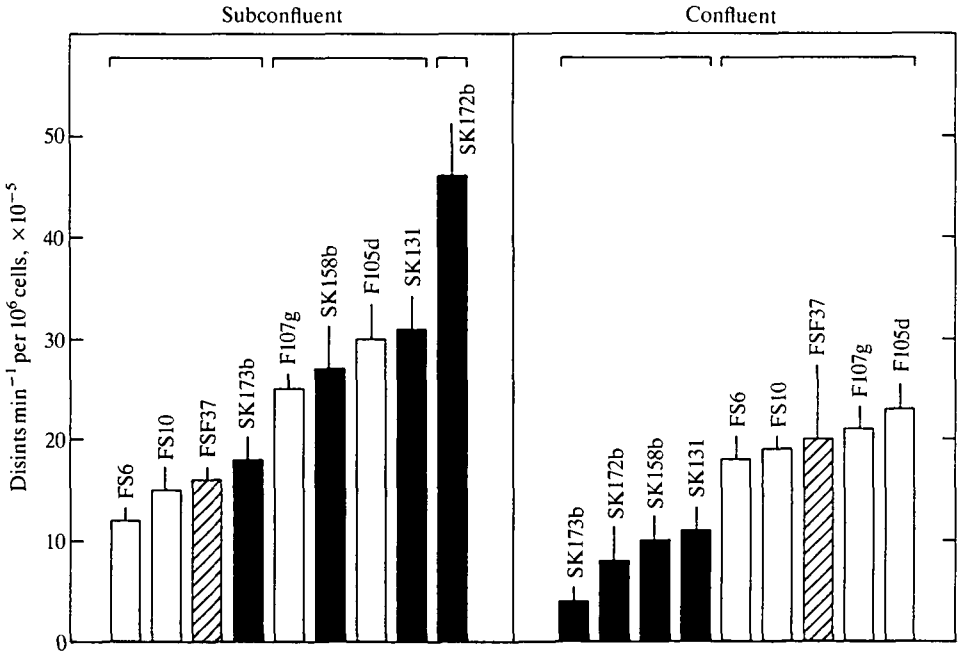

Fig. 1. Accumulation of [^{3}H]glucosamine-labelled hyaluronate in medium fractions of cultured fibroblasts metabolically labelled for 48 h with 2.5 μCi ml^{-1} [^{3}H]glucosamine as described in Materials and methods. Data were analysed by one-way analysis of variance and ranked by multiple-range tests. Bars within brackets are not significantly different from each other ($P>0.05$). Data represent mean±S.D. of two independent experiments. Filled bars, adult fibroblasts; open bars, foetal fibroblasts; stippled bar, foreskin fibroblasts.

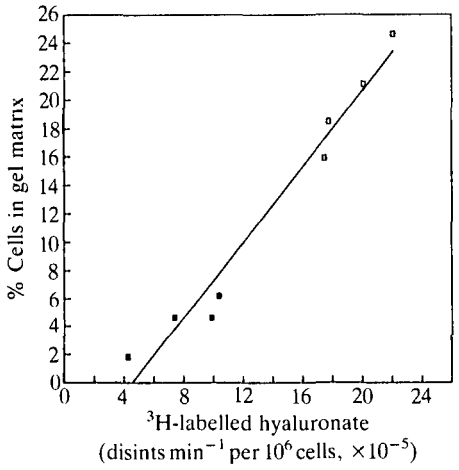

Fig. 2. Correlation between HA synthesis and migratory activity in confluent foetal and adult fibroblasts. The synthesis of HA in confluent cultures as measured by radioisotope incorporation (see Table 2) are here compared with the migration of these cells into collagen gels. Cells were plated at confluent cell density and the percentage of cells within the gel matrix was measured after 4 days of incubation. The migration data represent the mean results obtained in two replicate experiments. (■) Adult fibroblasts; (□) foetal fibroblasts.

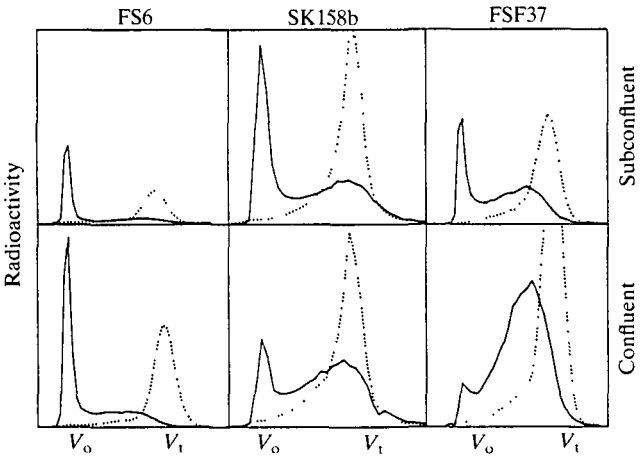

Fig. 3. Elution profile of medium fractions from foetal (FS6), adult (SK158b) and foreskin (FSF37) fibroblasts at 40–60% and 100% confluence metabolically labelled with 2.5 μCi ml^{-1} [^{3}H]glucosamine for 48 h. Dialysed medium fractions were chromatographed on a Sepharose CL-2B column without (——) or with *Streptomyces* hyaluronidase treatment (·····) as described in Materials and methods.

been confirmed by its absence of degradative activity on biological molecules biosynthetically labelled with $^{35}SO_4$ (Schor *et al.* 1989).

The accumulation of [^{3}H]glucosamine specifically into the high M_r class of HA in medium was studied by gel-filtration chromatography and determination of ^{3}H activity eluted at V_o, which was susceptible to hyaluronidase digestion. The results (Table 3) indicate that the accumulation of ^{3}H in high M_r secreted HA is dependent on cell density in adult fibroblasts but not in foetal

Table 3. *Accumulation of ^{3}H label in high M_r hyaluronate in the medium at 40–60% and 100% cell confluency*

Cell line	^{3}H-labelled high M_r hyaluronate accumulated during 48 h (disints min^{-1} per 10^6 cells, ×10^{-5})		Mean ratio subconf.:conf.
	Subconfluent	Confluent	
Adult			
SK131	25.33±2.5	9.20±1.80	2.81±0.39
SK158b	24.84±3.54	7.09±2.15	3.69±0.88
SK172b	26.89±5.11	3.63±1.47	9.54±7.56
SK173b	16.89±2.89	2.90±0.40	5.80±0.28
Foetal			
FS6	9.71±1.12	12.82±2.04	0.79±0.16
FS10	10.42±0.87	7.08±0.44	1.49±0.30
F105d	20.61±0.61	20.96±2.38	1.00±0.20
F107g	15.68±0.32	15.45±0.09	1.01±0.02
Foreskin			
FSF37	8.23±1.09	3.11±0.75	3.05±1.15

* Values are mean±S.D. of two independent experiments.

fibroblasts. It should be noted that in this particular situation (where only high M_r HA is considered), the FSF37 foreskin fibroblasts more closely resemble adult cells.

Biochemical measurement of HA in the medium fraction

In order to determine whether the different levels of ^{3}H incorporation into secreted HA represent real differences in the level of HA accumulated in the medium, GAGs were isolated from the culture media used in the above experiments and analysed by cellulose acetate electrophoresis. The main class of GAGs present was HA, with smaller amounts of DS and CS also present. The HA was quantified by laser densitometry. The results, indicated as 'total' in Table 2, reveal that over a 48-h period, less HA on a per cell basis is accumulated in the culture media of confluent foreskin and adult fibroblasts than those of subconfluent cells; this phenomenon was not observed with foetal fibroblasts, and is in agreement with the [^{3}H]glucosamine incorporation data also presented in Table 2.

Accumulation of labelled HA in pericellular fraction

The amount of ^{3}H activity in pericellular HA was determined by gel filtration chromatography as for high M_r HA (see above). The amount of low molecular weight activity (M_r<2×10^6) susceptible to hyaluronidase was negligible in all cell lines examined (data not shown), suggesting that most of the pericellular HA is in the high M_r form (M_r>2×10^6). Fig. 4 shows the levels of incorporated radioactivity in pericellular fractions of the cell lines examined. Heterogeneity of pericellular HA accumulation was observed among the cell lines at both confluent and subconfluent states. There was no apparent relationship between cell density and the amount of ^{3}H incorporation in pericellular HA of either adult or foetal fibroblasts.

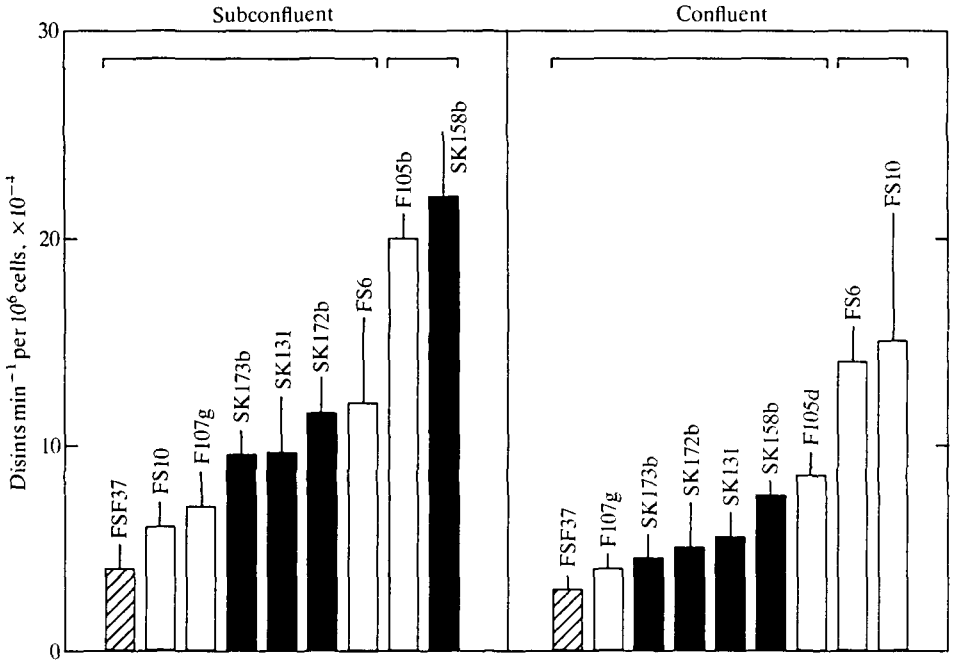

Fig. 4. Accumulation of [^{3}H]glucosamine-labelled hyaluronate in pericellular fractions of cultured fibroblasts. Data were analysed by one-way analysis of variance and ranked by multiple-range tests. Bars in parenthesis are not significantly different from each other ($P>0.05$). Data represent mean±S.D. of two or more independent experiments. Filled bars, adult fibroblasts; open bars, foetal fibroblasts; stippled bar, foreskin fibroblasts.

Discussion

Data presented in this paper indicate that: (1) foetal fibroblasts differ from their normal adult counterparts in terms of the effect of cell density on HA synthesis, i.e. the accumulation of HA by adult fibroblasts declines sharply when the cells reach confluence, whereas foetal fibroblasts do not show such an inverse relationship between cell density and HA synthesis; (2) there is a close correlation between the differential migratory activity displayed by confluent foetal and adult fibroblasts and the quantity of HA synthesized by these cells; and (3) there is a considerable degree of heterogeneity amongst foetal and adult fibroblast lines in terms of the relative proportion of high and low M_r HA synthesized.

Our results are in accord with previous studies (Tomida *et al.* 1974; Hronowski and Anastassiades, 1980; Matuoka *et al.* 1985, 1987; Mian, 1986) indicating that adult fibroblasts display a marked inverse relationship between HA biosynthesis and cell density. Such an inverse relationship was not observed in virally transformed fibroblasts (Hopwood and Dorfman, 1977; Matuoka *et al.* 1987). Our results suggest that transformed fibroblasts resemble foetal cells in this respect.

Matuoka *et al.* (1987) have suggested that HA synthetase activity is controlled by the proliferative state of the cells, i.e. elevated in subconfluent cultures in exponential growth and reduced in confluent cultures at stationary phase in which cell proliferation is minimal. Our data suggest that such a tight coupling between HA biosynthesis and cell proliferation may not be a universal phenomenon, since foetal cells at confluence cease to proliferate (as do normal adult cells), but continue to synthesize HA at rates comparable to rapidly proliferating cells in subconfluent culture.

The involvement of HA in mediating cell migration has been well documented in a variety of systems (see Introduction). The data presented in this paper suggest a causal relationship between the continued elevated level of HA production by foetal fibroblasts at cell confluence and their relatively enhanced migration into three-dimensional collagen matrices compared to adult cells (Schor *et al.* 1985, 1988). This conclusion is supported by our recently reported observation that exposure of confluent foetal fibroblasts to *Streptomyces* hyaluronidase results in a significant inhibition of cell migration (Schor *et al.* 1989); interestingly, exposure of either confluent adult fibroblasts or subconfluent fibroblasts (foetal or adult) to hyaluronidase had no effect on cell migration. These data were interpreted as support for the conclusion that HA is specifically required for the elevated migration displayed by confluent foetal fibroblasts.

We have recently reported that foetal fibroblasts secrete a peptide factor, migration-stimulating factor (MSF), which is not produced by normal adult cells (Schor *et al.* 1988, 1989; Grey *et al.* 1989). Exposure of confluent adult fibroblasts to MSF results in a stimulation of cell migration to the elevated levels characteristic of foetal cells and a corresponding increase in HA synthesis (Schor *et al.* 1989). Exposure of adult fibroblasts treated with MSF to hyaluronidase completely blocked this stimulation of cell migration. These data support the view that MSF constitutively produced by confluent foetal fibroblasts induces an autocrine stimulation of HA synthesis, which consequently results in an enhanced level of cell migration. It should be emphasized that the HA produced by foetal fibroblasts (possibly in response to MSF) may affect various fundamental aspects of cell behaviour in addition to migration, such as the mediation of inductive epithelial–mesenchymal interactions (Schor *et al.* 1989).

In this study, HA has been measured both by direct biochemical determination (cellulose acetate electrophoresis) and by the incorporation of [^{3}H]glucosamine into hyaluronidase-sensitive GAGs. Our results (Table 2) indicate that there is not necessarily a good correlation

between the estimated amounts of HA synthesized, as ascertained by these two methods. Such apparent discrepancies most likely result from differences in sugar transport and in metabolic rates between different cell lines, with consequent differences in the specific activity of ^3H in the synthesized HA, and/or differences in the turnover of the secreted HA. These results suggest that the commonly used method of measuring the incorporation of [^3H]glucosamine into GAGs over a 1- to 2-day period may not provide an accurate assessment of the relative amount of HA synthesized by different cell lines; caution should therefore be exercised in interpreting the results of studies in which this method has solely been used. The degree of heterogeneity further indicates that results obtained with one particular fibroblast line (either adult or foetal) with respect to the quantity or size distribution of the synthesized HA cannot be assumed to be representative of other fibroblasts of similar origins.

In spite of their wide-spread tissue distribution and common use in cell culture, fibroblasts remain a rather poorly defined group of cells generally identified *in vitro* by such non-specific attributes as spindle-shaped morphology and the presence of vimentin intermediate filaments. It is, however, now apparent that cells classified as fibroblasts on the basis of these criteria actually represent a highly heterogeneous group displaying both developmental and site-specific differences in such fundamental aspects of cell behaviour as proliferation, migration and matrix biosynthesis (reviewed by Schor and Schor, 1987). The heterogeneity in HA biosynthesis displayed by the different fibroblast lines examined in this study should be viewed in this context.

It is of particular interest that the FSF37 foreskin fibroblasts resembled foetal fibroblasts by certain criteria and adult fibroblasts by others. It is unclear at the moment whether this represents a site-specific attribute of these cells or is due to their intermediate position in terms of donor age.

This work was supported by a project grant from the Medical Research Council. We also thank Dr M. A. Cattell for much help and stimulating discussions.

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(Received 23 May 1989 – Accepted 12 July 1989)