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ADULT, FOETAL AND TRANSFORMED FIBROBLASTS DISPLAY DIFFERENT MIGRATORY PHENOTYPES ON COLLAGEN GELS: EVIDENCE FOR AN ISOFORMIC TRANSITION DURING FOETAL DEVELOPMENT

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SUMMARY

Data are presented indicating that the migration of fibroblasts into three-dimensional collagen gels is affected by cell density. We have defined a 'cell density migration index' (CDMI) to express this behavioural response in quantitative terms. The results of a survey of 77 different cell types indicate that the CDMI values expressed by normal adult skin fibroblasts and transformed cell lines fall into two distinct, non-overlapping groups. Measurement of the CDMI therefore provides an additional means of distinguishing between normal and transformed cells and may be used in conjunction with other commonly recognized criteria (e.g. anchorage-independent growth) to assess expression of a transformed phenotype *in vitro*. It is of interest to note that the CDMI values expressed by foetal cells define a group lying intermediate between normal and transformed cells. Both uncloned and cloned foetal cells have been observed to undergo a stable transition to expression of CDMI values characteristic of adult cells when followed throughout the duration of their *in vitro* lifespan. In addition to providing a novel means of distinguishing between normal adult and foetal cells, our results suggest that foetal fibroblasts undergo an 'isoformic' transition at some point in their developmental history, which is manifest *in vitro* by the expression of an adult CDMI.

INTRODUCTION

Normal fibroblasts have been cultured in the laboratory for many years. When grown under conventional tissue-culture conditions, such cells display a common set of behavioural characteristics, which have come to define the normal phenotype *in vitro*. When diploid fibroblasts or so-called 'normal' fibroblastoid cell lines are exposed to oncogenic viruses or other carcinogenic agents, a proportion of the cells subsequently display altered behavioural characteristics (e.g. anchorage-independent growth) and are now said to be transformed. Many such markers of the transformed phenotype have been identified over the past few years (Cameron & Pool, 1981). It is now clear that not one of these aberrant behavioural characteristics is consistently expressed by all transformed cells. Indeed, clones of cells isolated from a given population of transformed cells may display only one, or various combinations, of these transformation markers (Risser & Pollack, 1974). A detailed

Key words: fibroblasts, cell migration, collagen, isoformic transition.

account of the transformed phenotype and the potential difficulties that may be encountered in assaying for transformation are presented in the excellent review by Cameron & Pool (1981).

Much of the previous work in our laboratory has been concerned with examining cell migration on three-dimensional collagen gels. When plated on the surface of collagen gels, both normal and transformed fibroblasts rapidly begin to migrate down into the three-dimensional collagen matrix (Schor, 1980). Cells within the collagen matrix are clearly distinguishable from those confined to the gel surface and are easily visualized by focussing down through the gel. We have previously described techniques for expressing this movement of cells into the gel matrix in quantitative terms (Schor, 1980).

In this paper we describe a new assay system that makes it possible to distinguish between normal adult fibroblasts, foetal fibroblasts and transformed fibroblasts on the basis of their migratory behaviour on such gels.

MATERIALS AND METHODS

Cells and culture conditions

Explant cultures were established from 14 samples of foreskin (male children, age range 2–8 years), 24 samples of human foetal skin, and 24 samples of normal adult skin (Ham, 1980). Samples of foetal skin were obtained from induced abortions (estimated 10–16 weeks) kindly supplied by T. Carr (Clinical Research, Paterson Laboratories). The adult fibroblasts were mainly derived from skin specimens obtained from patients undergoing surgery for conditions unrelated to cancer (e.g. hernia) or from biopsy samples taken from normal individuals under local anaesthesia; skin samples from the following sites were used: 10× females, upper chest (age range 21–76 years); 2× female, abdomen (34, 74 years); 1× female, postauricular skin; 1× female, shoulder (52 years); 2× female, forearm (18, 22 years); 1× male, chest; 4× male, abdomen (31–61 years); 1× male, face (42 years); 1× male, back (67 years) and 1× male, lung. The following transformed cell lines were used: HT1080 (human fibrosarcoma), CC22 (human colon), CC21 (human colon), EJ (human bladder), CHED6T (spontaneously transformed Chinese hamster embryo), SV3T3 (SV40 virus transformed 3T3), PyBHK (polyoma virus transformed BHK), JRS (Jensen rat sarcoma), NCTC2472 (mouse tumorigenic fibroblasts), C6 (rat glial tumour), MG63 (osteogenic sarcoma), Py3T3 (polyoma virus transformed 3T3 cells), RSV (Rous sarcoma virus transformed Syrian hamster fibroblasts), SV-WI38 (SV40 virus transformed WI38 cells) and CCL8 (mouse sarcoma). The CC22 and CC21 cells were a gift from Dr A. Kinsella (Paterson Laboratories), the CHED6T cells were a gift from Dr C. Ockey (Paterson Laboratories), the EJ cells were a gift from Dr R. Hastings and all other cell lines were obtained from Flow Laboratories, Irvine, Scotland. The transformed cell lines all displayed anchorage-independent growth in agar or Methocel; certain of these lines were also tumorigenic.

The initial cellular outgrowths from the tissue explants generally contained epithelial cells as well as fibroblasts; when present, the epithelial cells were lost from the cultures after one or two passages *in vitro*. All fibroblast cultures were maintained in MEM growth medium supplemented with 15% foetal calf serum, glutamine, non-essential amino acids, sodium pyruvate and penicillin/streptomycin (Schor & Court, 1979). Stock cultures were maintained in 90 mm plastic tissue-culture dishes and passaged at a split ratio of 1:5 when the cultures reached confluency after 7–10 days of growth (Schor & Court, 1979). Cells were counted using a Coulter electronic particle counter. Cell numbers (N) in the confluent stock dishes of adult, foreskin and foetal fibroblasts varied between 1×10^6 and 4×10^6. The number of population doublings per passage were estimated by the following equation:

$$x = \frac{\log N - \log N_0}{\log 2},$$

where x = number of population doublings, N_0 = number of cells initially plated and N = number of cells present in confluent stock cultures before passage. Under our standard conditions using a split ratio of 1:5 we obtained a mean population doubling of 2·3 per passage.

Cloned populations of fibroblasts were obtained from a particular line of foetal skin fibroblasts. This was accomplished by plating 100 cells derived from a confluent stock culture at passage 9 onto 35 mm plastic tissue-culture dishes. Such sparsely plated cultures were incubated in growth medium in the presence of a gas mixture containing 3 % O_2 and 5 % CO_2 in nitrogen. The use of reduced oxygen tension was found to improve plating efficiencies to a significant degree, these varying between 12 and 14 %. Individual colonies containing between 100 and 200 cells were then trypsinized using cloning rings with an internal diameter of 4 mm, the resultant cell suspensions plated onto 35 mm plastic tissue-culture dishes and these again incubated under reduced oxygen tension until a confluent culture was obtained. Cultures of cloned cells could then be maintained and passaged in 90 mm dishes as described above for uncloned cells. Since stock dishes contained $1{\cdot}6\times10^4$ to $6{\cdot}4\times10^4$ cells cm^{-2} at confluence, we estimate that 21–22 cell doublings were required to achieve this final cell density from the original progenitor cell in the two *in vitro* passages used for cloning. This additional number of estimated cell doublings must be taken into account when comparing the passage number of uncloned and cloned cell cultures.

Measurement of cell migration

Type I collagen was extracted from rat tail tendons and used to make three-dimensional collagen gels as previously described (Schor & Court, 1979). In our standard experimental protocol, cells are plated onto the surface of replicate collagen gel substrata at a low initial density (i.e. 10^3 cells cm^{-2}) and a high initial density (i.e. 10^4 cells cm^{-2}). After 4 days of incubation at 37 °C, the percentage of cells present within the three-dimensional collagen matrix was determined by the 'microscopic method' described previously (Schor, 1980). Accordingly, cultures are examined in a Leitz Diavert microscope fitted with a photographic graticule defining a rectangular area of known dimensions. The cells on the gel surface and within the collagen matrix that fall within this rectangular area are counted in 10–15 randomly selected fields for each of the replicate cultures. The number of cells found within the collagen matrix may then be expressed as a percentage of the total number of cells present.

RESULTS

Definition of cell density migration index

Cell migration into the gel matrix is influenced by a number of experimental parameters (Schor, Schor, Winn & Rushton, 1982). The effects of one such parameter, cell density, on the migration of normal human lung fibroblasts (WI38) and their SV40 virus transformed counterparts (SV-WI38) are shown in Fig. 1. Cells were plated at densities between 10^3 and 10^4 cm^{-2} and the percentage of cells within the collagen matrix was measured after 4 days of incubation. As can be seen in Fig. 1, the migration of the WI38 cells is inversely proportional to cell density, whereas the migration of SV-WI38 cells increases in a proportional manner.

In order to express the effect of cell density on migration by a single numerical value, we have defined a cell density migration index (CDMI) as follows:

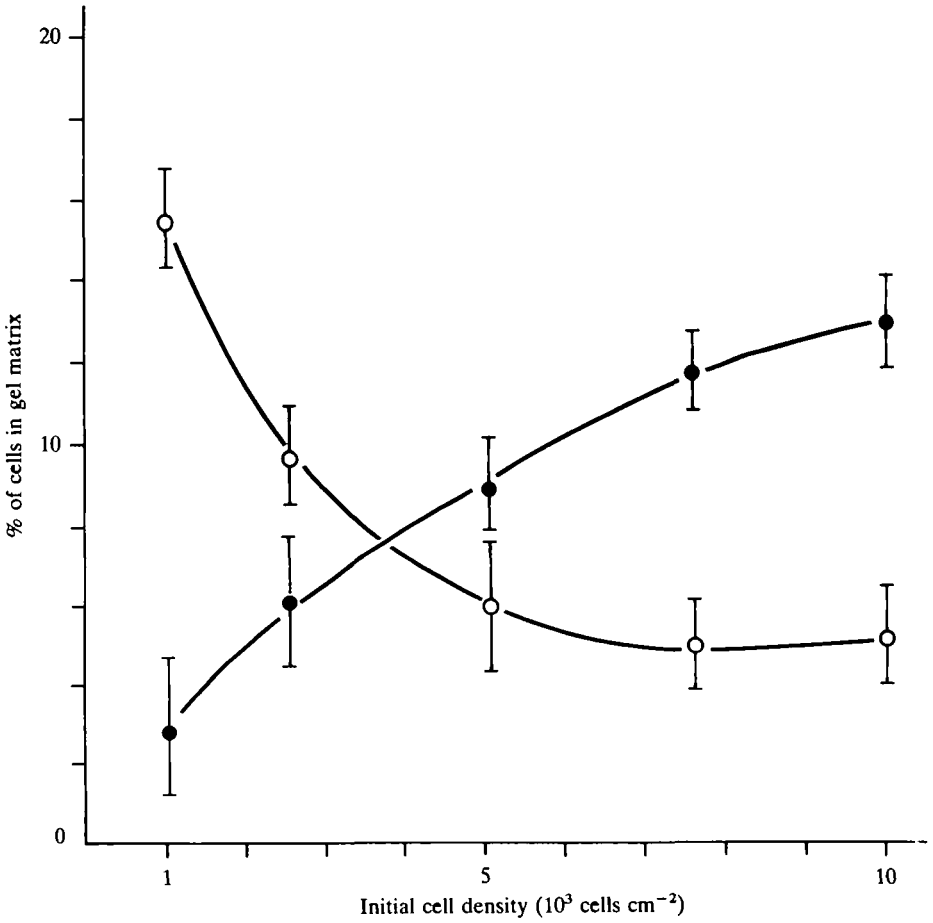

Fig. 1. The effects of different plating densities on the migration of WI38 (○——○) and SV40 virus transformed WI38 (●——●) fibroblasts into three-dimensional collagen gels. Cells were plated on the gel surface at densities ranging between 10^3 and 10^4 cells cm^{-2}. The percentage of cells found within the collagen matrix after 4 days of incubation was determined as previously described (Schor, 1980).

$$\text{CDMI} = \log [\%\ \text{(low density)}/\%\ \text{(high density)}],$$

where '% (low density)' is the percentage of cells found within the gel matrix in cultures plated at 10^3 cells cm^{-2} and '% (high density)' is the corresponding value in cultures plated at 10^4 cells cm^{-2}. According to this formulation, cells whose migration is inversely proportional to cell density will have positive CDMI values, whereas cells whose migration increases proportionally with cell density will have negative CDMI values. Cells whose migration is unaffected by cell density will have CDMI values approximating zero. From the data shown in Fig. 1, the WI38 cells

have a CDMI = +0·48, and the SV-WI38 cells have a CDMI = −0·65. Expressing the cell density dependence of migration in terms of the CDMI greatly facilitates the comparative analysis of different cell types and allows the resultant data to be expressed in quantitative terms. The determinations used to measure the percentage of cells within the gel matrix (and hence calculate the CDMI) may be made with a considerable degree of reproducibility (Schor, 1980); within a given experiment, the standard deviation of such migration measurements rarely exceeds 10 % of the mean value.

Differences in fibroblast migratory behaviour

The data presented in Fig. 1 for WI38 cells and their virally transformed counterparts suggest that it might be possible to distinguish between normal and transformed fibroblasts by the CDMI values they express *in vitro*. In order to test this possibility, we examined the behaviour of 14 strains of normal human foreskin fibroblasts, 24 strains of normal human adult fibroblasts (male and female, obtained from different anatomical sites; see Materials and Methods), 24 strains of human foetal fibroblasts and 15 different transformed fibroblast cell lines. Cells were plated on replicate gels at 10^3 and 10^4 cells cm^{-2} and the percentage of cells found within the collagen matrix was measured after 4 days of incubation. The results of over 500 individual experiments are presented in Fig. 2. No difference was apparent between the CDMI values of normal foreskin and adult fibroblasts; in both cases over 90 % of the cells examined had CDMI values greater than +0·4. The CDMI values of the transformed cells produced a completely non-overlapping distribution profile; in this group greater than 90 % of the cells examined had negative CMDI values. The response of the foetal cells was especially interesting. These cells behaved as a clearly defined group, with CDMI values falling between those of the normal (foreskin and adult) and transformed cells.

The data presented in Fig. 2 indicate that it is possible to define empirically certain ranges of CDMI values, which include the majority of specimens of a given cell type (i.e. normal, foetal or transformed). Such divisions are indicated in Fig. 2 and are defined as follows: the transformed range (T) with CDMI values $<-0{\cdot}4$, the transformed/foetal range (T/F) with CDMI values between −0·4 and 0, the foetal/normal range (F/N) with CDMI values between 0 and +0·4 and the normal range (N) with CDMI values $>+0{\cdot}4$.

The majority of our experiments were conducted with fibroblasts originally established from explant cultures using small pieces of tissue; such cultures are consequently derived from the relatively small number of cells capable of active migration out from the tissue fragment. In order to determine whether a particular subset of cells (possibly exhibiting a 'migratory phenotype') might be selected preferentially in such explant cultures, a number of experiments were performed in which replicate samples of skin were digested with collagenase overnight and the resultant freed cells and tissue debris used to establish parallel fibroblast cultures. Fibroblasts derived from cultures established by either technique (i.e. explant or

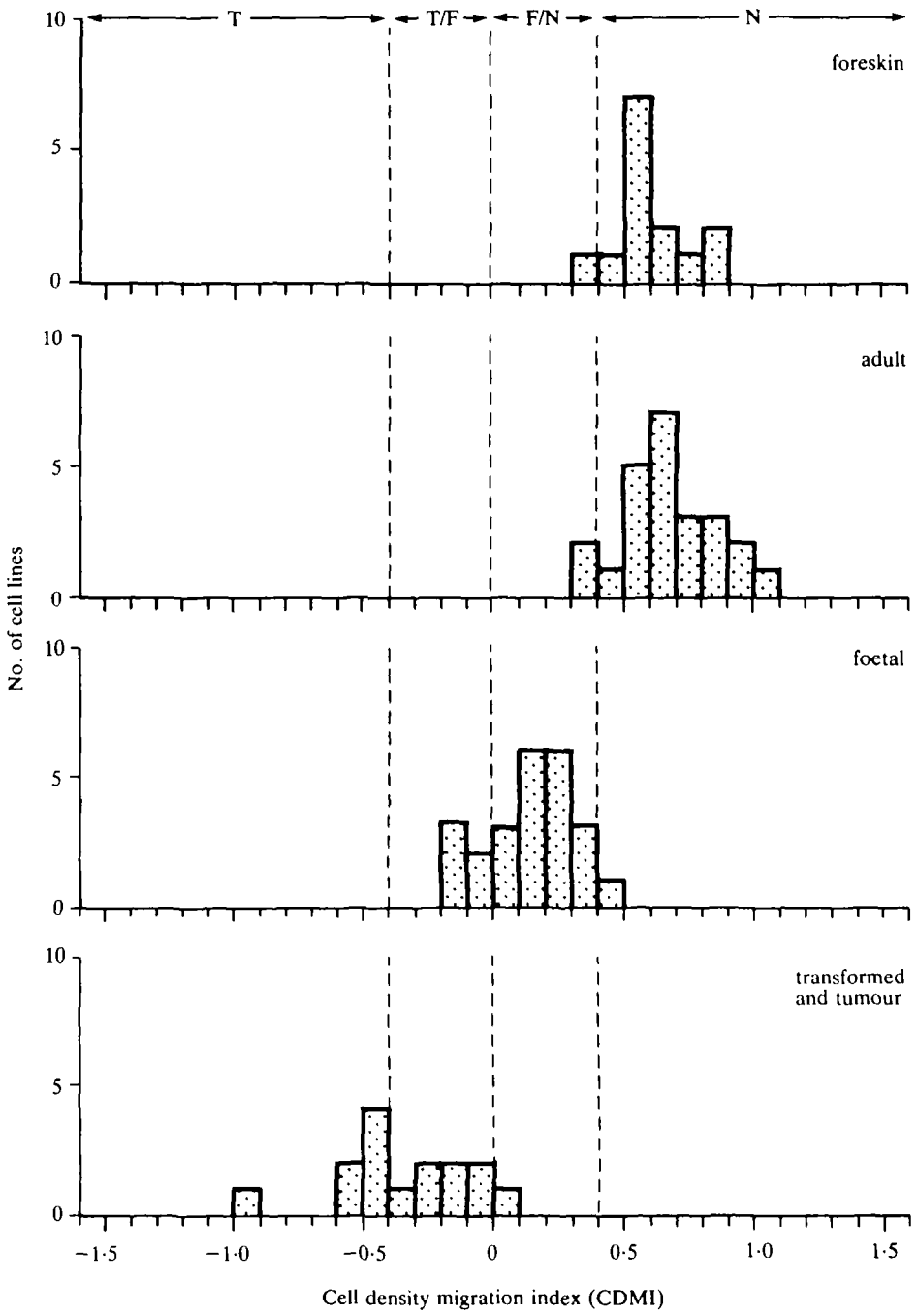
T
T/F
F/N
N
foreskin
adult
foetal
transformed
and tumour
No. of cell lines
10
5
0
−1·5
−1·0
−0·5
0
0·5
1·0
1·5
Cell density migration index (CDMI)

Fig. 2

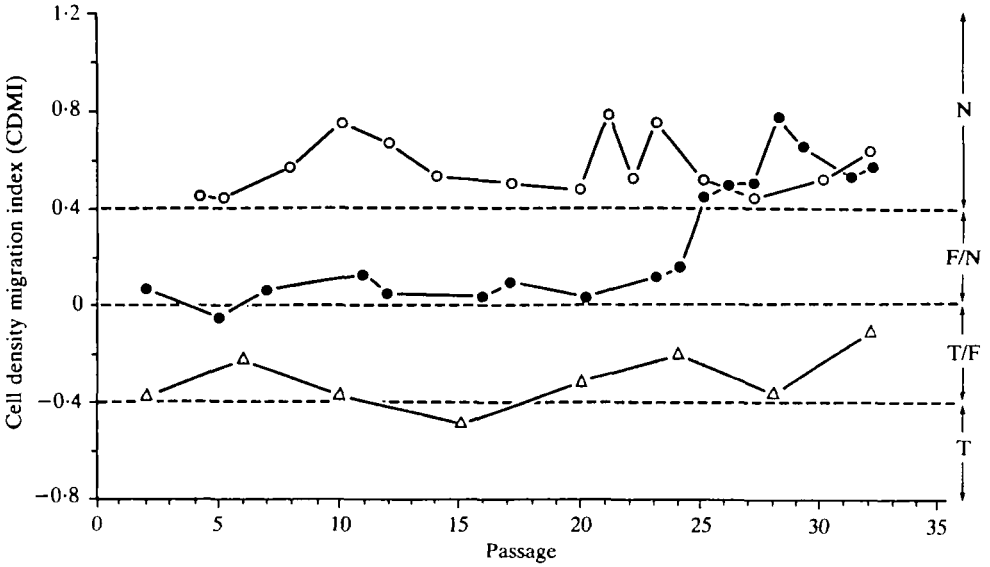

Fig. 3. The CDMI values expressed by a normal foreskin fibroblast, FSF36 (○——○); a human foetal fibroblast, FS8 (●——●); and a human fibrosarcoma cell line, HT1080 (△——△), as a function of passage number *in vitro*. Stock cultures were passaged at a split ratio of 1:5 every 7–10 days. The data presented here for the foreskin and foetal fibroblasts cover their entire *in vitro* lifespan. Each passage represents an estimated 2·3 population doublings (Materials and Methods).

collagenase digestion) were morphologically indistinguishable, displayed the same kinetics of growth and saturation cell densities and had identical CDMI values (results not shown).

Transition to adult CDMI occurs in foetal fibroblasts cultured in vitro

The stability of the CDMI as a function of *in vitro* passage number is shown in Fig. 3. Data are presented for a normal adult fibroblast, a foetal fibroblast and a human fibrosarcoma cell line (HT1080). Cells were passaged every 7–10 days at a split ratio of 1:5 and the period of study shown in Fig. 3 covers the entire *in vitro* lifespan of the normal adult and foetal cells. The HT1080 cells are a 'permanent cell line' and hence do not exhibit a finite lifespan *in vitro*; these cells were, however, studied over a similar number of passages in order to compare their CDMI values directly with those obtained with the diploid cells. The CDMI values of the adult

Fig. 2. The CDMI values expressed by 14 strains of human foreskin fibroblasts, 24 strains of human adult fibroblasts, 24 strains of human foetal fibroblasts and 15 transformed cell lines (see Materials and Methods for details concerning cell types). Cells were plated onto the surface of replicate collagen gels at both 10^3 and 10^4 cells cm^{-2} and incubated at 37 °C. The percentage of cells present within the three-dimensional collagen matrix was determined after 4 days of incubation (Schor, 1980) and the resultant data were used to calculate the respective CDMI values as described in Results. Data are expressed as the mean CDMI value obtained for each strain of cell examined (4–20 individual experiments done with each strain).

fibroblast strain remained confined to the N range over its entire *in vitro* lifespan (estimated 74 population doublings), giving a mean value of $+0{\cdot}57 \pm 0{\cdot}13$ during this time. The CDMI values obtained with the HT1080 cells were similarly consistent, with the majority of determinations falling within the T/F range (mean CDMI $= -0{\cdot}32 \pm 0{\cdot}12$).

The results presented in Fig. 2 suggest that foetal cells undergo a transition at some point in their developmental history, which results in the expression of elevated CDMI values falling in the adult range. That such a transition may also occur *in vitro* is shown in Fig. 3. Before passage 25 (estimated 58 population doublings), this strain of foetal cells had CDMI values that tended to fall within the F/N range (mean value $= +0{\cdot}05 \pm 0{\cdot}04$). We observed an abrupt transition occurring at passage 25, with the cells now expressing CDMI values in the N range. This transition appeared to be a stable attribute of the cell phenotype and continued to be expressed for the remainder of the fibroblast's *in vitro* lifespan (mean value $= +0{\cdot}57 \pm 0{\cdot}13$). A similar transition of CDMI values from the F/N range to the N range was observed to occur in another population of FS8 cells at passage 24, as well as in another uncloned foetal cell line (results not shown).

Since an uncloned population of foetal cells was used to obtain the data shown in Fig. 3, the observed transition in CDMI values may have resulted from either the preferential growth (or survival) of cells expressing CDMI values in the N range, which existed in the population before passage 25, or from an actual phenotypic transition in the progeny of cells previously expressing CDMI values in the F/N range. In order to examine these possibilities further, 110 clones of cells were obtained from the uncloned strain of foetal cells used to obtain the data presented in Fig. 3 (i.e. strain FS8); clones were isolated from the FS8 strain at passage 9 of its *in vitro* lifespan. A total of 42 clones were subsequently subcultured and CDMI values obtained at passage 14 (i.e. estimated 53 population doublings; N.B. estimated population doubling of cloned cells calculated by adding 21 to estimated population doubling of uncloned cells; see Materials and Methods). As can be seen in Fig. 4A, there is indeed a considerable degree of clonal heterogeneity in CDMI values expressed at this time, with 33·3% of the clones falling within the T/F range, 45·2% within the F/N range and 21·5% within the N range. The range of CDMI values obtained in 36 replicate experiments using one particular clone of cells (FS8/102) is shown in Fig. 4B. A tight modal distribution was obtained in this series of experiments with a mean value of $+0{\cdot}05 \pm 0{\cdot}07$. These results suggest that the rather broad distribution profile shown in Fig. 4A represents a true clonal heterogeneity in the population and is not due to experimental error inherent in determining the CDMI value. Since clones of cells were isolated from the uncloned population at one particular *in vitro* passage number, no information is consequently available regarding the precise time at which cells displaying 'adult-like' CDMI values first appeared in the uncloned population.

The CDMI values expressed by 14 randomly selected clones were followed throughout the duration of their *in vitro* lifespan. Three general patterns of behaviour were observed (Fig. 5). Clones that initially displayed CDMI values

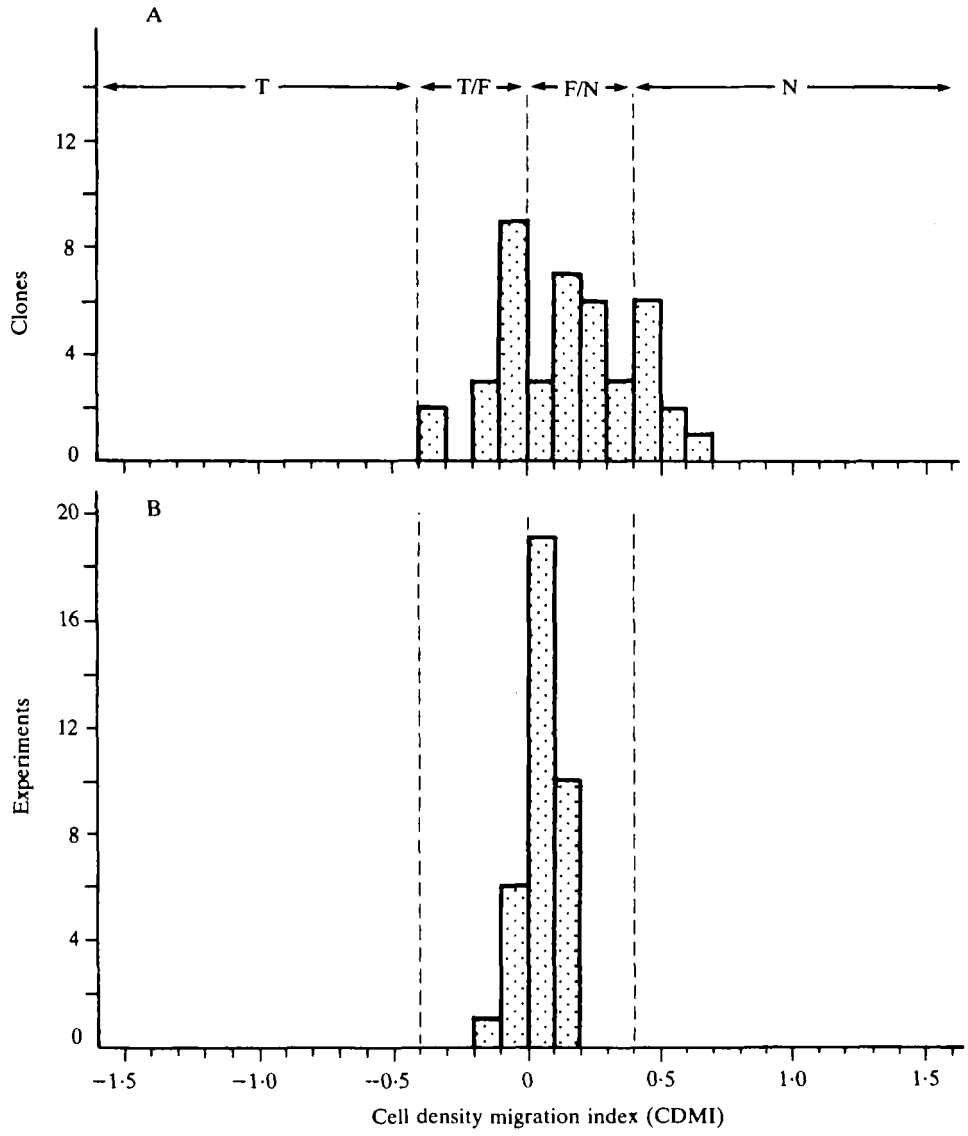

Fig. 4. A. The CDMI values expressed by 42 different clones of foetal skin fibroblasts isolated from the FS8 uncloned strain. Data are expressed as the mean CDMI value obtained for each clone in three separate experiments. B. The CDMI values expressed by one particular clone of foetal cells (FS8/102) in 36 separate experiments. All the clones were at passage 14 (i.e. estimated 53 population doublings *in vitro*).

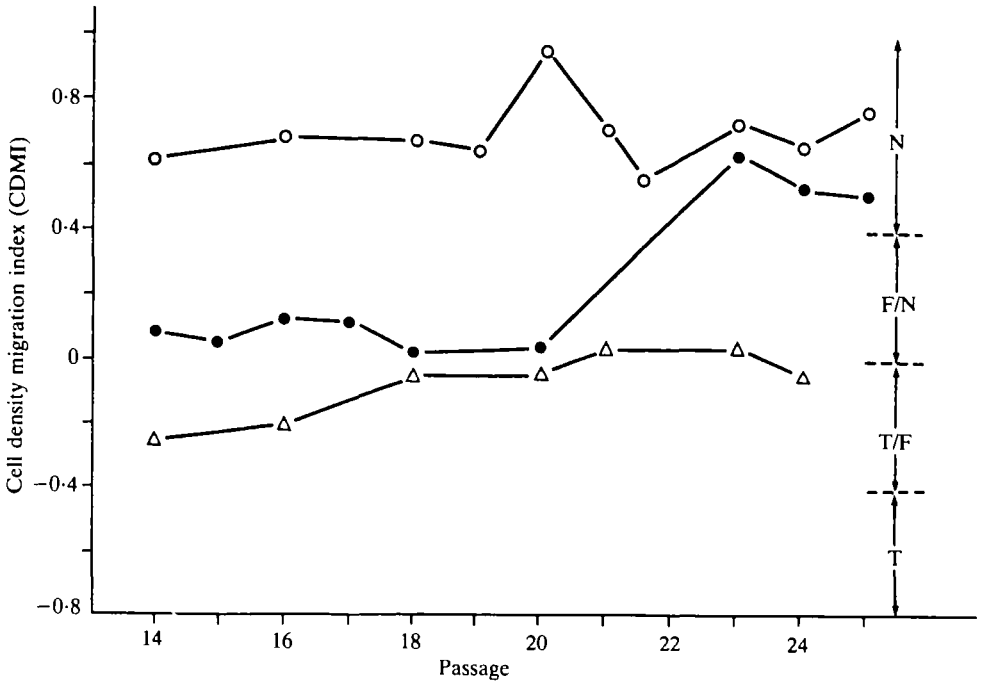

Fig. 5. The CDMI values expressed by three representative clones of foetal fibroblasts from passage 14 (estimated 53 population doublings) until senescence. Three different patterns are shown. Pattern A: clone expressed CDMI value falling within the N range when initially examined at passage 14 and continued to do so until senescence. This pattern shown by clone FS8/51 (○——○). Pattern B: clone expressed CDMI value within foetal range when initially examined and continued to do so until senescence. This pattern shown by clone FS8/101 (△——△). Pattern C: clone initially expressed CDMI value within foetal range and undergoes transition before senescence to ekpression of CDMI values in the N range. This pattern shown by clone FS8/86 (●——●).

falling within the N range invariably continued to do so for the remainder of their *in vitro* lifespan (pattern A). Clones that initially displayed CDMI values falling within the T/F or F/N ranges either continued to do so until senescence (pattern B), or underwent a well-defined transition to a CDMI value falling within the N range (pattern C). The data obtained from all 14 of these clones are summarized in Table 1. The results indicate that the majority (75 %) of clones initially displaying CDMI values in the T/F and F/N ranges underwent a transition to an adult phenotype before becoming senescent. There was no apparent correlation between the initial CDMI expressed by the clones and the estimated number of population doublings at the time of senescence. These data support the view that the transition observed in the uncloned foetal cell population (Fig. 3) is due to a change in phenotype expressed by individual cells and not by the selection of a particular subpopulation.

Table 1. *The CDMI values expressed by 14 clones of foetal fibroblasts as a function of* in vitro *passage*

Pattern	Clone	CDMI Initial	CDMI Final	Passage Transition	Passage Senescence
A	FS8/ 51	+0·62 (N)	+0·70 (N)	–	26
	FS8/ 69	+0·48 (N)	+0·42 (N)	–	25
B	FS8/ 31	−0·05 (T/F)	+0·07 (F/N)	–	22
	FS8/ 35	+0·11 (F/N)	+0·03 (F/N)	–	23
	FS8/101	−0·03 (T/F)	+0·04 (F/N)	–	28
C	FS8/ 19	−0·01 (T/F)	+0·48 (N)	24	25
	FS8/ 61	+0·12 (F/N)	+0·72 (N)	15	25
	FS8/ 63	+0·05 (F/N)	+0·51 (N)	15	23
	FS8/ 68	+0·34 (F/N)	+0·43 (N)	18	26
	FS8/ 70	+0·10 (F/N)	+0·44 (N)	19	22
	FS8/ 71	+0·15 (F/N)	+0·45 (N)	15	23
	FS8/ 75	+0·06 (F/N)	+0·58 (N)	21	24
	FS8/ 86	+0·06 (F/N)	+0·43 (N)	23	25
	FS8/ 88	−0·08 (T/F)	+0·94 (N)	18	24

Clones were initially examined at passage 14. Three different patterns of CDMI expression were observed as the clones were followed throughout the remainder of their *in vitro* lifespans (see Fig. 5). Of the 14 clones examined, 2 initially displayed CDMI values falling within the N range and continued to do so for the duration of their *in vitro* lifespans (pattern A). Of the remaining 12 clones, which initially displayed CDMI values falling in the foetal range, 3 continued to do so for the remainder of their lifespans (pattern B), while 9 underwent a transition to expression of CDMI values falling in the N range (pattern C). The passage numbers at which this transition occurred and the passage numbers at which the cultures became senescent are indicated. The estimated population doublings achieved by the cloned cells are calculated from these passage numbers as described in Materials and Methods. The CDMI values expressed by all clones at passage 14 are presented as 'initial' values in the Table. The final CDMI values are those expressed by the clones in their last passage *in vitro* (i.e. when they became senescent).

DISCUSSION

Transformed fibroblasts are commonly distinguished from their normal counterparts by the expression of certain phenotypic characteristics *in vitro*, e.g. anchorage-independent growth, focus formation and secretion of elevated quantities of plasminogen activator (Cameron & Pool, 1981). Fibroblasts *in vivo* are generally embedded within a three-dimensional matrix containing type I collagen. Normal fibroblasts embedded within a three-dimensional collagen matrix *in vitro* have been shown to exert tension on the collagen fibres and thereby induce the compaction of the gel when it is detached from the walls of the tissue-culture dish (Bell, Ivarsson & Merrill, 1979; Bellows, Melcher & Aubin, 1981; Allen & Schor, 1983). Transformed fibroblasts were subsequently shown to exhibit a considerably reduced capacity for gel compaction (Steinberg, Smith, Colozzo & Pollack, 1980; Buttle & Ehrlich, 1983). The results presented here indicate that normal and transformed cells may also be distinguished from each other by assessing the effects of cell density on migration down into the three-dimensional collagen gel matrix. These

data are presented in the form of a single numerical value, the cell density migration index (CDMI).

Although there are many criteria that may now be used to distinguish normal and transformed cells from each other, foetal and adult fibroblasts generally behave in a similar fashion when cultured *in vitro* and are therefore difficult to distinguish from each other by a simple assay procedure. The differences that we observe in the CDMI values expressed by foetal and adult cells are of particular interest in this context. Foetal fibroblasts have previously been reported to express certain transformation-associated phenotypic characteristics (e.g. anchorage-independent growth) when assayed under the appropriate conditions (Peehl & Stanbridge, 1981). All of the cloned and uncloned foetal fibroblasts examined in our study were indistinguishable from normal (adult and foreskin) cells in terms of their morphology, serum requirements for growth, ability to compact a floating collagen gel, and inability to form colonies in Methocel when studied in the appropriate assay systems (results not shown).

Previous studies have indicated that certain differences in fibroblast behaviour *in vitro* may be related to the age and sex of the donor, as well as the particular anatomical site of derivation (Conrad, Hart & Chen, 1977; Harper & Grove, 1979; Azzarone & Macieira-Coelho, 1982). Differences in the protein synthetic activity of foreskin fibroblasts and fibroblasts derived from other (non-genital) anatomical sites have recently been demonstrated by Thompson *et al.* (1983). In contrast, our data suggest that the CDMI value expressed by fibroblasts is not a sex- or site-dependent phenotypic characteristic, since no differences were observed between foreskin fibroblasts and adult fibroblasts derived from a variety of anatomical sites, from both male and female donors.

The underlying mechanisms responsible for the observed effects of cell density on fibroblast migration are not known. Several possible levels of control may be involved, including: (a) cell-produced changes in the organization and/or biochemical composition of the collagen matrix (Steinberg *et al.* 1980; Schor, Schor & Bazill, 1981; Erickson & Turley, 1983; Yamada, 1983); (b) the production of soluble chemokinetic or chemotactic factors by the cells (Hayashi, Yoshida, Ozaki & Ushijima, 1970); and (c) an effect of local cell density on the social interactions operative between cells (Abercrombie, 1970). These various possibilities are amenable to experimental investigation and will be discussed in a forthcoming article.

It should be emphasized that the CDMI value determined for a given population of cells does not itself provide information concerning the heterogeneity of migratory phenotypes expressed by individual cells within that population. The CDMI is a single numerical value reflecting the average behaviour of cells within the population examined, irrespective of the degree of heterogeneity present. In this context, note that the histograms shown in Figs 2 and 4A depict the distribution of the mean CDMI values expressed by the *different* uncloned or cloned cell populations examined. The standard deviations of the mean CDMI values obtained for each of the cell populations examined (either cloned or uncloned) were of a similar

magnitude (compare data presented in Figs 3, 4B and 5), generally being less than ±0·1. Information regarding the heterogeneity of CDMI values within a given population of cells can be estimated only by examining the behaviour of individual clones of cells derived from that population.

The transition from a foetal to an adult form of haemoglobin has been recognized to occur for some time. Many similar types of 'isoformic' transitions have subsequently been observed to occur during embryogenesis and are now believed to play an important role in the coordinate control of normal development (Caplan, Fiszman & Eppenberger, 1983). Our data suggest that foetal fibroblasts undergo such an isoformic transition at some point in their developmental history, which is manifest *in vitro* by the expression of elevated CDMI values falling in the N range. A similar transition in the *in vitro* behaviour of foetal fibroblasts during development has been described by Nakano & Ts'o (1981). They reported that clones of fibroblasts that displayed anchorage-independent growth (i.e. a transformation-associated phenotypic characteristic) could be isolated from 10- to 13-day-old Syrian hamster embryos; the number of clones displaying this 'transformed' behavioural characteristic decreased as a function of both gestation period and *in vitro* passage number.

The common occurrence of isoformic transitions allows the interesting hypothesis to be put forward that certain disease states are characterized by the persistent expression of the foetal isoform (of a particular molecule or cell type) in the adult organism (e.g. see Fitzsimmons & Hoh, 1982). Assessment of the CDMI values expressed by fibroblasts in adult patients suffering from different pathological conditions may provide information relevant to this hypothesis.

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