

Differential growth of facial primordia in chick embryos: responses of facial mesenchyme to basic fibroblast growth factor (bFGF) and serum in micromass culture

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Summary

Differential growth of the three major facial primordia, the frontonasal mass, maxilla and mandible, results in a characteristic face shape. Abnormal growth of any of the primordia can lead to facial defects. In order to dissect out the factors that control growth, we developed a functional assay for cell proliferation using micromass culture and defined medium. Cell number was determined over a 4 day period and BrdU incorporation was used to determine the percentage of cells in S-phase. In defined medium, cell number progressively decreases and proliferation is very reduced in cultures of cells from all three primordia. When foetal calf serum was added, frontonasal mass cell number triples, mandible doubles and maxilla increases by half. The number of cells in S-phase increased in every case but the final cell number reflects a balance between proliferation and cell loss from the culture. The addition of basic fibroblast growth factor (bFGF) to defined medium leads to an increase in cell number in the frontonasal mass, while the cell number of mandibular and maxillary cultures is rela-

tively unaffected. The percentage of cells in S-phase is highest in frontonasal mass cultures. Serum and bFGF both increase chondrogenesis in frontonasal mass cultures when compared to defined medium. In contrast in mandibular cultures, serum does not change the amount of cartilage and with bFGF chondrogenesis is reduced. The coordination of the changes in proliferation and differentiation in frontonasal mass cultures suggest that either these two processes are independently stimulated to the same extent or a single subpopulation of cells is stimulated to divide and differentiate into chondrocytes. The different responses of the populations of individual facial primordia to growth factors may contribute to differential growth *in vivo* and also be linked to the generation of facial defects.

Key words: bFGF, frontonasal mass, mandible, maxilla, micromass culture, defined-medium, facial primordium, BrdU, chondrocytes.

Introduction

Differential growth is a major factor in determining face shape. At early stages in embryogenesis, the chick face consists of primordia, buds of mesenchyme encased in epithelium, which are of more-or-less equal size. As development proceeds, the primordia enlarge differentially and fuse to give rise to the bill. The frontonasal mass, the primordium that lies between the nasal pits, grows out to form most of the upper beak; the maxillary primordia, lateral to the presumptive oral cavity, contribute to only the corners of the upper beak; and the paired mandibular primordia, inferior to the presumptive oral cavity, grow out to form the entire lower beak.

Failure of expansion of the primordia can lead to facial defects. When chick embryos are treated with retinoic acid, the development of the frontonasal mass is specifically affected and this results in absence of the

upper beak and clefting of the primary palate (Tamarin *et al.* 1984). Therefore an understanding of how the growth of each primordium is controlled may give insights into the basis of specific facial defects such as cleft lip.

Facial growth has been difficult to study due to the complex morphology and inaccessibility of the embryonic face. One approach to the problem is to measure growth using morphometric analysis of embryonic material. However, in order to discover the factors controlling growth, it is necessary to use both descriptive and functional approaches. The former technique would involve mapping the distribution of known growth factors and their receptors as well as relevant extracellular matrix molecules. We have chosen to develop a functional assay so that we can identify potentially interesting molecules whose role we can then pursue in the intact face. This approach has also

been used in the developing frog embryo to screen for candidate molecules involved in mesoderm induction (Slack *et al.* 1987; Gillespie *et al.* 1989).

The growth of each primordium is a property of the mesenchyme since the epithelia overlying facial primordia are interchangeable (Richman and Tickle, 1989). Therefore, our strategy was to place facial mesenchyme cells in high density (micromass) culture and define conditions where proliferation did not occur. We then screened substances for their effects on proliferation including foetal calf serum and basic fibroblast growth factor (bFGF) (Gospodarowicz *et al.* 1984).

We show that when facial mesenchyme cells are cultured in micromass with medium containing foetal calf serum, proliferation occurs. Cell number increases in all three primordia but to differing extents. In contrast, bFGF, a well-known mitogen for many mesenchymal cell types (Gospodarowicz *et al.* 1986), preferentially stimulates an increase in cell number in frontonasal mass mesenchyme.

Since each facial primordium contains different proportions of potentially chondrogenic and myogenic cells (see Wedden *et al.* 1986; Ralphs *et al.* 1989), we explored whether the basis of the differential response is related to cellular heterogeneity. The results show that the proportion of chondrogenic cells in each primordium cannot wholly account for the differential response to bFGF.

Materials and methods

Preparation of cell suspensions

Facial primordia of stage 24 chick embryos were dissected according to the dimensions illustrated in Fig. 1. The entire frontonasal mass, the entire maxillae and the distal half of the mandibular primordia were used. Epithelia were removed using 2% trypsin (Gibco, 1:250) in calcium- and magnesium-free saline (Hanks Buffered Salt Solution, Gibco), pH 7.4 at

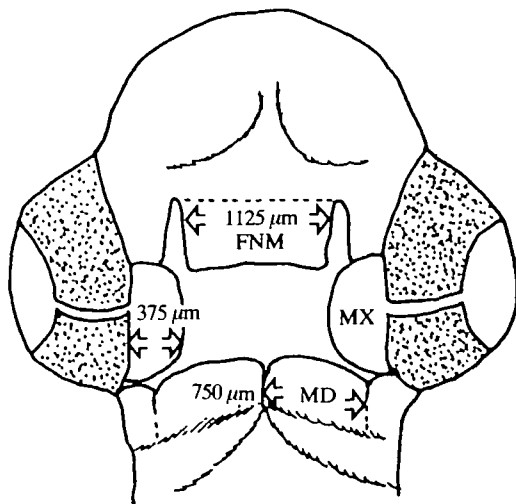


Fig. 1. A diagram of a stage 24 chick face; the dashed lines indicate how facial primordia were dissected. The entire 375 µm width of the maxillary primordium was dissected. KEY: FNM=frontonasal mass, Mx=maxilla, Md=mandible.

4°C for 45 min. The mesenchyme of the facial primordia was dissociated by pipetting, cell number estimated by hemocytometer and the final concentration adjusted to 2×10^7 cells ml^{-1} .

Composition of media

Two types of media were used in these experiments. The serum-containing media consisted of 2 mM L-glutamine, 100 units ml^{-1} penicillin, and 100 $\mu\text{g ml}^{-1}$ streptomycin + 0.25 $\mu\text{g ml}^{-1}$ fungizone (antibiotic/antimycotic, Gibco Biocult) 50:50 ratio of F12:DMEM (Gibco Biocult), and 10% foetal calf serum. The defined media contained a 60:40 ratio of F12:DMEM, 2 mM L-glutamine, antibiotics as described for serum-containing medium, 5 $\mu\text{g ml}^{-1}$ transferrin (bovine, Sigma), 100 nM hydrocortisone (Sigma), 5 $\mu\text{g ml}^{-1}$ porcine insulin (Sigma) and 50 $\mu\text{g ml}^{-1}$ ascorbate. This formula was based on the medium used by Paulsen and Solursh (1988) and Kujawa *et al.* (1989). Where indicated, bFGF (Gift from M. Noble, purchased from British Biotechnology Ltd, Oxford) was added to the defined media on a daily basis. Lyophilized bFGF was rehydrated with defined medium to which Bovine Serum Albumin (BSA) (Miles Scientific, USA) was added. The BSA concentrate was first diluted to a concentration of 2.86% with defined medium. This stock BSA was added to the defined medium used to rehydrate the bFGF at a dilution of 1:100. The stock concentration of rehydrated b-FGF was 1 ng μl^{-1} .

Cell plating

Cells were plated either as 2 micromasses (two, 10 μl drops containing 2×10^5 cells) or as a monolayer of 4×10^5 cells in each well of four-well tissue culture dishes (Nunc, average well diameter=16 mm). Serum was not used in the initial plating of micromass cultures in defined medium. In the growth factor experiments, the surface of the dish was precoated with fibronectin. 10 μl drops of fibronectin (10 $\mu\text{g ml}^{-1}$ of PBS, Calbiochem) were placed in the culture wells and left at 37°C for 2–3 h. The fibronectin was aspirated and a 10 μl drop of cell suspension placed on the same spot. The presence of fibronectin did not have any effect on the final cell number in bFGF-supplemented cultures. The experiments were repeated without precoating the culture dishes and similar responses to bFGF were obtained.

In all the experiments, the micromass cultures were allowed to adhere for 1 hour before flooding with media to give a final volume of 500 μl . Medium was replaced daily with 500 μl of fresh medium. The insulin present in defined medium was essential for cell spreading and flattening during the first few hours of the culture.

Assaying cell number

The cell number was estimated at 4 to 96 h after plating by replacing the medium with 200 μl trypsin/EDTA solution (0.1% Trypsin and 0.001 M EDTA in calcium-/magnesium-free saline, pH 7.2) incubating for 2 to 10 min at 20°C and then gently agitating the dishes. When the cells began to detach, 700 μl serum-containing medium was added to the well to stop the action of the trypsin, and the entire contents of the well aspirated and placed into a centrifuge tube. The cell suspension was spun to form a pellet, resuspended in a known volume of fresh medium, and cell number was estimated with a hemocytometer. To obtain cell suspensions from 4 day cultures of frontonasal mass, the EDTA/trypsin treatment was used to lift the micromasses from the substratum, 700 μl of medium containing serum was added to the well and the contents aspirated into a centrifuge tube. The tube was spun to pellet the cultures, the medium removed and replaced with

a 0.2% solution of crude collagenase (Type IA, Sigma) and the cultures were incubated at 37°C until the cells could be dissociated. The suspension was spun into a pellet and resuspended in a known volume of medium and cell number estimated.

Use of BrdU and its antibody to detect cells in S-phase

One hour prior to counting the cell number, cultures were fed with 25 μ M 5-bromodeoxyuridine (BrdU) in culture medium. Cells were removed from the wells as previously described and counted. The suspension was spun a second time to form a pellet and 40 μ l of fresh medium used to resuspend the cells. 20 μ l samples of concentrated suspension were spread on two gelatin-subbed slides. The cell smears were allowed to dry and then slides were dipped in 70% industrial methylated spirits at room temperature for 10–15 min to fix and permeabilise the cells. Slides were stored for up to 2 weeks at -20°C before continuing with antibody staining. Smears were hydrolysed for 10 min at 45°C using 1.5 M HCl. A mouse monoclonal antibody to BrdU (Becton-Dickinson, California), diluted 1:10, was applied for 1 h at 37°C and binding was routinely detected with fluorescently labelled rabbit anti-mouse antibody diluted 1:50 (Dakopatts, Denmark). A random sample of 1000 cells was chosen and the number of labelled cells was counted.

Detection of myoblasts

Based on the work of Ralphs *et al.* (1989), myogenic cells were counted after 48 h of culture since this is the time point at which they are most numerous and the myoblasts have not yet fused into myotubes. Cultures grown for 48 h, were rinsed in phosphate-buffered saline (PBS) and then fixed and permeabilized with 70% industrial methylated spirits at 4°C for 3 to 5 min. The cultures were gradually rehydrated with PBS and a monoclonal antibody to myosin heavy chains of striated muscle, 83B6 (gift of G.K.C. Dhoot), diluted 1:200, was applied to the cultures for 2 h at room temperature. The cultures were then rinsed and incubated overnight at 4°C in the secondary antibody linked to 5 nm gold particles, diluted 1:40. The following day, labelled cells were visualized with a silver enhancement kit (Jenssen, Belgium). Numbers of muscle cells per culture could then be counted under bright-field illumination.

Staining for cartilage in micromass culture

4-day micromass cultures were rinsed in PBS and then fixed with 1/2-strength Karnovsky's fixative (Karnovsky, 1965) for 2 to 12 h and stained with Alcian blue pH 1.0 for no more than 2 h (Wedden *et al.* 1986). Cartilage area was measured by tracing Alcian-Blue-stained regions in a camera-lucids image which was projected onto a digitizing pad. The digitizing pad was linked to an Archimedes computer and the image was measured using the DIGIT program (B. Hayes).

Results

Growth of facial mesenchyme in serum-free medium

In defined medium alone, cell number progressively decreased in cultures from cells of all three primordia and the percentage of cells incorporating BrdU was low (Fig. 2A, Table 1).

Effect of serum-containing medium on cell number and proliferation in cultures of facial cells

In medium containing foetal calf serum, the number of

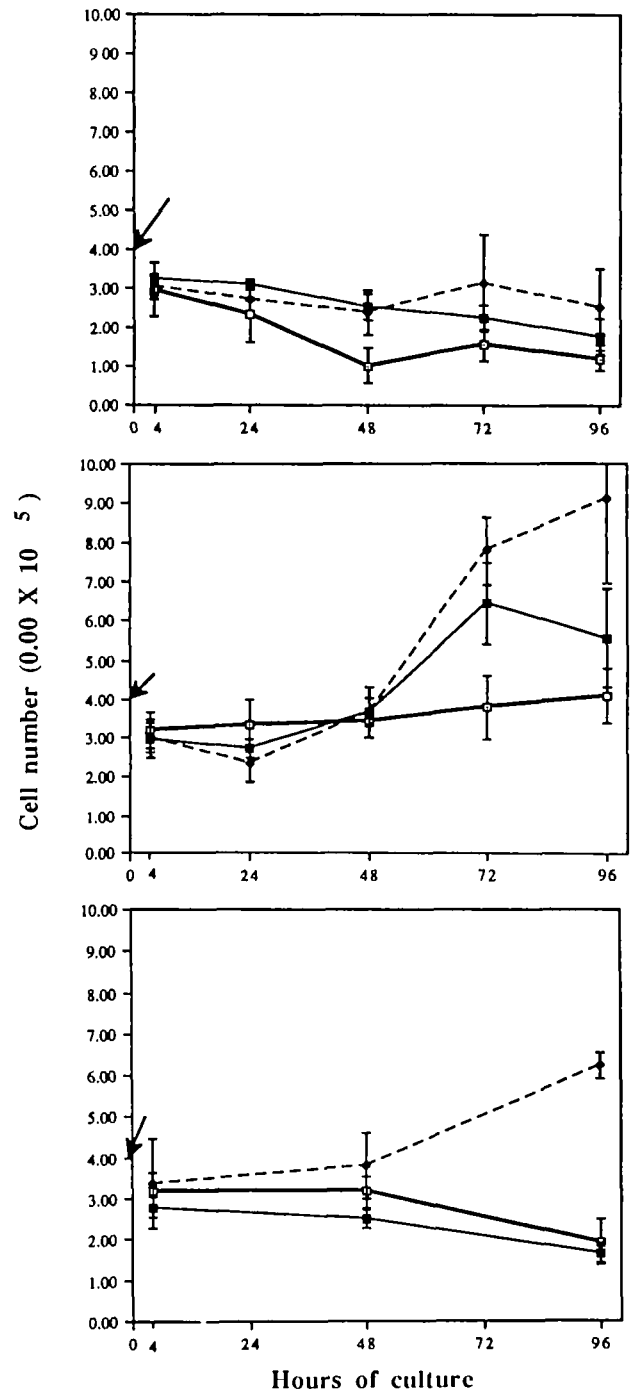


Fig. 2. (A) Cell number plotted as a function of duration of culture in defined medium. Each value represents the mean of 3 to 11 separate experiments. (B) Cell number plotted as a function of duration of culture in medium containing foetal calf serum. Each value represents the mean of 3 to 8 separate experiments. (C) Cell number plotted as a function of duration of culture in defined medium containing 1 ng ml⁻¹ of bFGF. Each value represents the mean of 4 to 6 separate experiments. These cultures were plated on fibronectin spots but this did not affect the number of cells adhering at 4 h (compare with 3B). Key: Arrow=number of cells plated, 4 h time point illustrates the number of cells that adhered from the suspension. --- frontonasal mass — mandible — maxilla cultures. Error bars represent 1 standard deviation on either side of mean.

Table 1. Percentage of BrdU-labelled cells at 48 h in different culture media

Type of Medium	FNM	Mx	Md
Defined	6.2 (0.4) n=4	3.7 (0.8) n=4	2.9 (1.4) n=5
Serum (10%)	18.6 (3.5) n=6	19.7 (2.1) n=3	10.7 (4.0) n=3
bFGF (1 ng ml ⁻¹)	10.7 (1.2) n=3	5.5 (3.8) n=4	4.6 (1.5) n=5

FNM=Frontonasal mass
Mx=Maxilla
Md=Mandible
()=1 standard deviation

cells in micromass cultures of all three facial primordia had increased after 4 days but the cells from each primordia behaved differently; the number of frontonasal mass cells tripled, the number in mandible cultures doubled and the number in maxillary cultures increased by 50% (Fig. 2B). Equal numbers of cells adhered to the dish (as seen at 4 h on Fig. 2B), and the cell numbers began to increase after 48 h and appeared to be reaching a plateau by 72 h.

The percentage of cells in S-phase in each type of culture at 48 h is shown in Table 1. The growth curves in Fig. 2B begin to diverge between 48 and 72 h. Therefore the percentage of cells labelled at 48 h should relate closely to subsequent differences in cell number. At 48 h in serum-containing medium, the frontonasal mass and maxilla have 18.6% and 19.7% of cells labelled, respectively, whereas the labelled cells in the mandible cultures is only 10.7% (Table 1). The maxilla has therefore an unexpectedly high percentage of cells undergoing DNA synthesis given the very small increase in cell number (Fig. 2B).

Effect of bFGF on cell number and DNA synthesis in serum-free medium

bFGF preferentially stimulated an increase in cell number in frontonasal mass cultures. When 1 ng ml⁻¹ bFGF is added to defined medium, the number of cells in frontonasal mass cultures at 96 h was double that at 4 h, whereas, in the mandible and maxilla cultures, cell number still decreased (Fig. 2C). Increasing the concentration of bFGF to 10 and 100 ng ml⁻¹ led to a further stimulation of cell number in the frontonasal mass (Fig. 3), whereas in mandible and maxilla cultures the number of cells was still below the number of cells plated. A concentration of 100 ng ml⁻¹ bFGF gives the same cell number in frontonasal mass cultures as that seen in foetal calf serum (compare Fig. 3 to 96 h data in Fig 2B). Preliminary studies using a different batch of bFGF but a dose equivalent to 100 ng ml⁻¹ showed that mandible and maxillary mesenchyme do not increase in cell number significantly compared to the number plated (mean for mandible=4.45×10⁵, s.d.=0.93; mean for maxilla=3.32×10⁵, s.d.=0.60). The addition of BSA, used as a carrier protein when diluting bFGF,

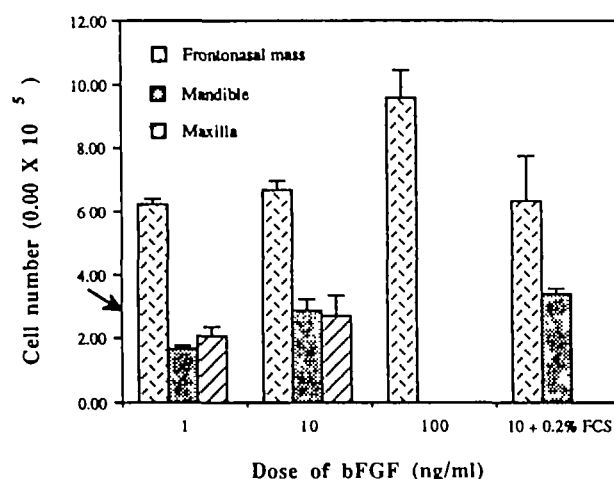


Fig. 3. Histogram showing cell number in micromass cultures after 96 h after addition of 1, 10, 100 ng ml⁻¹ bFGF and 10 ng ml⁻¹+0.2% FCS to defined medium. Each bar for the 1 and 10 ng ml⁻¹ data represents the mean of four to six separate experiments. The 100 ng ml⁻¹ data is the mean of two experiments. The data for serum+bFGF was the mean of two to three experiments. Error bars represent 1 standard deviation. KEY: Arrow=mean number of cells adhering 4 h after plating.

did not alter the cell number (data not shown) from the values obtained in defined medium (Fig. 2A).

Determination of the percentage of cells in S-phase showed that 1 ng ml⁻¹ bFGF stimulated DNA synthesis in frontonasal mass cells. The labelling index at 48 h (10.7%, Table 1), was greater than that seen in defined medium (significant at $P \leq 0.1$). In mandibular and maxillary cultures, the percentage of labelled cells at 48 h also increased compared with defined medium but these differences were not found to be significant.

The plating efficiency of facial cells (the number of cells present at 4 h) was identical in all cultures whether serum or bFGF was added and therefore the starting cell populations in all the experiments appear to be equivalent.

The addition of 1–10 ng ml⁻¹ of bFGF led to a significant increase in cell number; however, this was still less than that obtained with serum. In order to see whether serum contains factors that interact with b-FGF in controlling proliferation of facial cells, 0.2% FCS was added to defined medium containing 10 ng ml⁻¹ b-FGF. This small quantity of serum had no effect on the number of cells in frontonasal mass and mandibular cultures (Fig. 3) (*t*-test showed no significant difference between the number of cells/well in cultures treated with 1–10 ng ml⁻¹ b-FGF versus cultures treated with 10 ng ml⁻¹ b-FGF+0.2% FCS).

Cell differentiation

Myogenesis

In defined medium, the number of myogenic cells that differentiated in mandibular cultures was much higher than that in frontonasal mass or maxillary cultures (data not shown). The number of myoblasts is very small in relation to the total number of cells (1% of the culture).

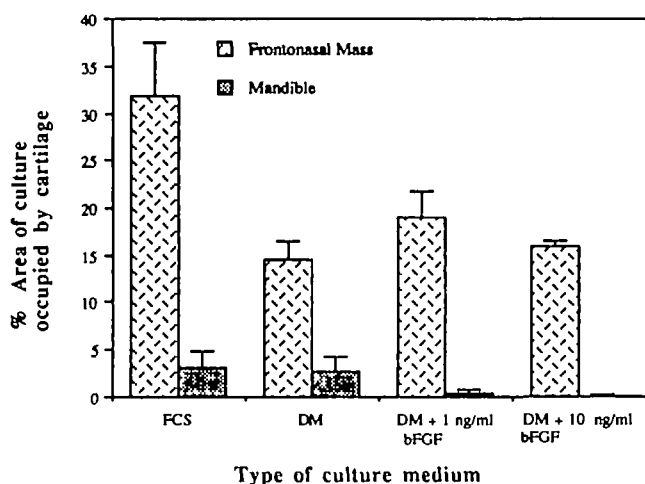


Fig. 4. Histogram showing the percentage area occupied by cartilage in micromass cultures in different culture media. Each bar represents the mean of 4 to 9 cultures for frontonasal mass cells and the mean of 6 to 16 separate cultures for mandibular cells. The error bars represent 1 standard deviation. KEY: FCS= Foetal calf serum-containing medium, DM=defined medium.

The number of myoblasts was reduced when serum was added to mandibular cultures (DM, mean=5348; 1 s.d.=660; FCS, mean=998, 1 s.d.=292). The addition of 1 or 10 ng ml⁻¹ bFGF to defined medium had no effect (1 ng ml⁻¹ bFGF, mean=5731, 1 s.d.=1361; 10 ng ml⁻¹ bFGF, mean=5285, 1 s.d.=553). In frontonasal mass and maxillary cultures, the number of myogenic cells that differentiated was not changed in different culture media (data not shown).

Chondrogenesis

In defined medium, a lacy pattern of cartilage formed in the frontonasal mass (Fig. 5A); small nodules of cartilage developed in the mandible cultures that are distributed over the whole culture (Fig. 5B), and in maxillary cultures tiny, very faintly stained nodules could be detected (Fig. 5C).

With the addition of serum continuous sheets of cartilage formed in the frontonasal mass (Fig. 5D), nodules formed in the mandible (Fig. 5E) but no cartilage was seen in the maxilla (Fig. 5F) (see also Wedden *et al.* 1986). The area of cartilage in the frontonasal mass cultures in serum-containing medium was significantly larger than in defined medium (Fig. 4, $P \leq 0.05$). In mandibular cultures, although the pattern of chondrogenesis was different in serum-containing and defined medium, the quantity of cartilage was the same (Fig. 4).

bFGF has primordium-specific effects on both the pattern and quantity of cartilage matrix. In the frontonasal mass cultures supplemented with 1 ng ml⁻¹ bFGF, a nodular pattern of cartilage developed (Fig. 6A) and with 10 ng ml⁻¹ the pattern was sheet-like (Fig. 6B) resembling that obtained with serum-containing medium. With even higher doses of bFGF, a larger, denser sheet of cartilage was formed (data not shown). The cartilage area in frontonasal mass cultures treated with

1 and 10 ng ml⁻¹ bFGF was 24% larger than in defined medium (Fig. 4) (*t*-test, $P \leq 0.05$). In contrast, when bFGF is added to mandibular cultures, the extent of cartilage differentiation is significantly reduced compared to defined medium (eg. 1 ng ml⁻¹ bFGF versus defined medium, $P \leq 0.02$). When 10 ng ml⁻¹ bFGF was added, cartilage was scarcely detectable (Fig. 4, Fig. 6D).

Discussion

The aim is to discover the factors that control proliferation of facial mesenchyme and ultimately understand their role in differential growth *in vivo*. We established a defined culture system in which to rapidly screen growth factors for their effects on proliferation and, as a first step, we tested the effects of serum and bFGF.

Foetal calf serum stimulates an increase in cell number and labelling index in cultures of cells from all three facial primordia. In contrast, bFGF leads to an increase in cell number in only frontonasal mass mesenchyme and labelling index was highest in these cultures. The increase in proliferation of frontonasal mass cultures when either serum or bFGF was added was accompanied by an increase in chondrogenesis. In mandibular cultures, the addition of serum did not change the extent of cartilage differentiation while with bFGF the amount of cartilage that differentiated was reduced.

Role of proliferation and cell loss in determining cell number

The labelling indices under the three culture conditions were determined to find out whether the cell number is an accurate reflection of cell proliferation. However, with serum, the maxilla had an unexpectedly high labelling index in light of the small increase in cell number. Therefore the number of cells in a culture is a balance between proliferation and cell loss.

The maxillary cultures in serum make very little cartilage matrix and this could allow round, mitotic cells to float away. However, in mandible cultures the extent of cartilage differentiation is unaffected by the addition of serum and yet cell number and labelling index increase. This suggests that other factors in addition to chondrogenic differentiation may act to control cell loss.

Serum-derived and tissue-derived growth factors affect proliferation of facial mesenchyme

Serum generally stimulates cell proliferation whereas bFGF has a preferential effect on frontonasal mass cultures. Foetal calf serum contains growth factors such as platelet-derived growth factor (PDGF) (Ross *et al.* 1986). Further analysis is required to determine which factors in serum are involved in mediating proliferation of facial cells and whether these are present in the developing face.

bFGF is a tissue-derived factor that is not present in serum (Gospodarowicz and Moran, 1976; Thomas,

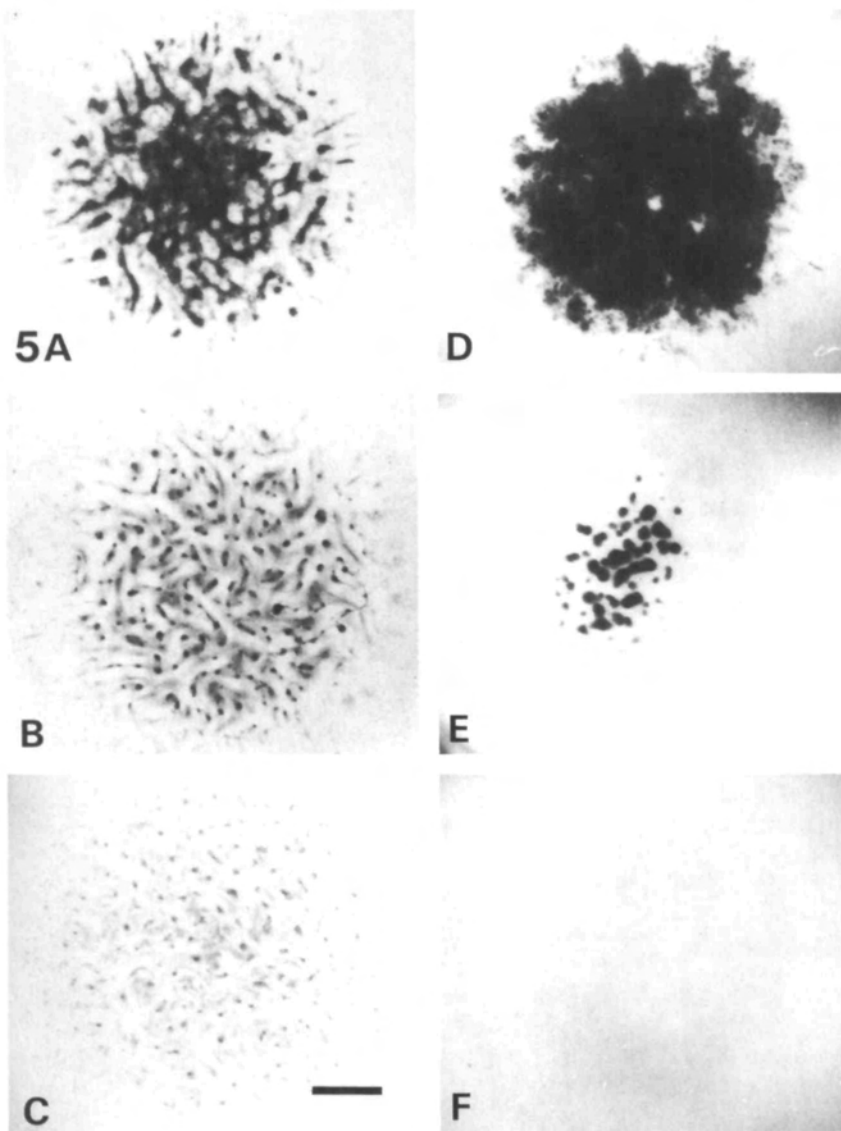


Fig. 5. Micromass cultures stained with Alcian blue at 96 h in defined or serum-containing medium. (A) Frontonasal mass cells in defined medium. The Alcian-blue stained matrix has a lacy appearance (compare with solid sheet in 5D). (B) Mandible cells in defined medium. An array of tiny nodules extends out to the edges of the culture (compare to 5E). (C) Maxilla cells in defined medium. Faintly staining nodules can be detected. (D) Frontonasal mass cells in serum-containing medium. A central sheet of cartilage is present. (E) Mandible cells in serum-containing medium. The cartilage matrix is arranged in nodules confined to the centre of the culture. (F) Maxilla cells in serum-containing medium. There is no visible cartilage matrix. Scale bar for all figures=1.0 mm.

1987; Rifkin and Moscatelli, 1989). Nevertheless, the effects of serum and bFGF on frontonasal mass cells are very similar. b-FGF can, on its own, stimulate an increase in cell number in frontonasal mass cultures equal to that seen in serum. Moreover, when both bFGF and serum are added simultaneously, there is no synergistic effect on cell number. Therefore, it appears that the same cell population in the frontonasal mass is affected by bFGF and serum-derived growth factors. However, we cannot exclude indirect actions of growth factors. It is possible that bFGF acts by stimulating target cells to produce a second factor that affects cell proliferation. For example, bFGF has been shown to stimulate transforming growth factor beta 1 (TGF beta) mRNA synthesis in osteoblasts (Noda and Vogel, 1989).

There are many other tissue-derived growth factors that are absent in serum such as nerve growth factor (Korsching *et al.* 1985), epidermal growth factor (Carpenter and Cohen, 1979) and beta TGFs (Sporn *et al.*

1986). Some of these could act as mitogens for maxillary and mandibular cells.

The role of cellular heterogeneity in the response to serum and bFGF

The cell population constituting the facial primordia is heterogeneous and can differentiate into myogenic and chondrogenic cells in high density culture (Wedden *et al.* 1986; Langille *et al.* 1989). Myogenic cells constitute a very small proportion of the total cells in the culture (see also Ralphs *et al.* 1989), whereas large numbers of chondrogenic cells differentiate in frontonasal mass and mandible cultures in defined medium. Could the relative number of chondrogenic cells in each type of facial mesenchyme account for the differences in cell number produced by serum and bFGF? In serum-containing medium, the increase in cell number is correlated with the number of chondrogenic cells. Similarly bFGF stimulates frontonasal mass cells to proliferate. However, bFGF does not lead to an increase in cell number

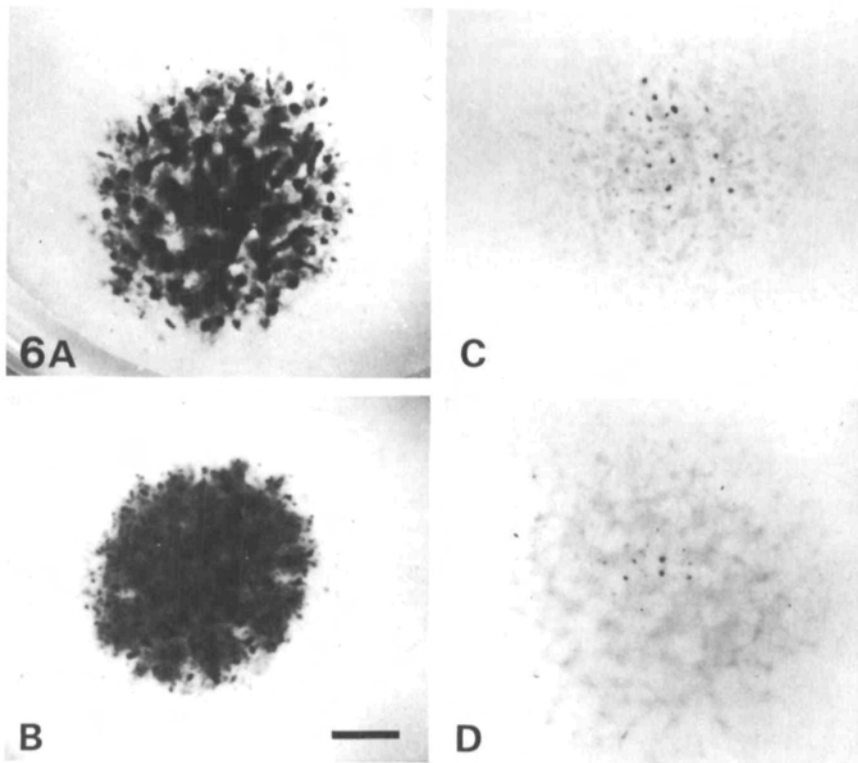


Fig. 6. Micromass cultures stained with Alcian blue after 96 h of culture in defined medium with added bFGF. (A) Frontonasal mass culture in defined medium supplemented with 1 ng ml^{-1} bFGF. Darkly stained chondrogenic area in the centre of the culture contains nodules, some of which have coalesced. (B) Frontonasal mass culture in defined medium supplemented with 10 ng ml^{-1} of bFGF. A dense sheet of cartilage has formed in the centre of the culture similar to that which forms in serum (compare to Fig. 5D). (C) Mandibular culture in defined medium supplemented with 1 ng ml^{-1} bFGF. A few tiny nodules of cartilage in the centre of the culture have developed but far fewer than in media without bFGF (compare with Fig. 6B). (D) Mandibular culture in defined medium supplemented with 10 ng ml^{-1} bFGF. Virtually no cartilage differentiates. Scale bar = 1.0 mm.

in mandibular cultures even though many potentially chondrogenic cells are present.

A separate issue is the purely differentiative effects that serum and bFGF have on chondrocytes. In mandibular cultures, chondrocyte differentiation is inhibited by bFGF and unchanged by serum whereas in frontonasal mass cultures both serum and bFGF increase chondrogenesis over that seen in defined medium. Therefore, the mandible cultures lack the positively responsive chondrogenic cells that are found in frontonasal mass cultures. In limb buds, subpopulations of myogenic cells that differ in their response to bFGF have also been detected (Seed and Hauschka, 1988).

Relevance to normal facial development

Frontonasal mass cultures have a lower percentage of cells in S-phase than frontonasal mass at comparable stages *in vivo* (19% in foetal calf serum cultures compared to 35% at stage 28 *in vivo* [Minkoff and Kuntz, 1977]) while maxillary cultures are closer to the labeling index found in the intact primordium (20% in foetal calf serum cultures compared with 25% in the maxillary primordia [Bailey *et al.* 1988]). Nevertheless, it is striking that maxilla cultures, under all conditions tested, have the smallest change in cell number and that this primordium also makes the smallest contribution to the bill.

The results suggest that bFGF may be involved in differential growth of the facial primordia in the embryo. The growth factor may be unequally distributed, the number of receptors (Neufeld and Gospodarowicz, 1985) may vary, and the efficiency of signal transduction (Gillespie *et al.* 1989) may be different in each facial cell

population. It will be important to explore these possibilities in the intact head. Although bFGF has been purified from brains (Risau *et al.* 1988), bodies (Seed *et al.* 1988; Lee *et al.* 1989) and limb buds (Seed *et al.* 1988; Munaim *et al.* 1988) of chick embryos, it is not known whether bFGF is present in the face.

The development of the frontonasal mass is specifically affected by retinoic acid treatment *in vivo* (Tamarin *et al.* 1984; Wedden, 1987). Since both bFGF and retinoic acid affect the same primordium, it is possible that a single cell population within the frontonasal mass is responsive to both. It will be interesting to find out whether there is a functional interaction between retinoic acid and bFGF and whether this is related to the facial defect.

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