

## Immunochemical demonstration of the clonal organization of chimaeric mouse epidermis

GÜNTER H. SCHMIDT\*, MARGARET A. BLOUNT and BRUCE A. J. PONDER

*Institute of Cancer Research, Haddow Laboratories, Clifton Avenue, Sutton, Surrey SM2 5PX, UK*

\* Present address for correspondence: Department of Cell Biology, Fraunhofer-Institute, Nikolai-Fuchs-Str. 1, 3000 Hannover 61, FRG

### Summary

The clonal organization of chimaeric mouse epidermis was demonstrated by *in situ* staining of whole-mount preparations using monoclonal antibodies directed against H-2<sup>k</sup> and H-2<sup>b</sup> antigens. A striking pattern of transversely oriented stripes or patches was found which extended from mid-dorsum to the flank region. The orientation of these patches indicates a preferred directional expansion of clones during the development of dorsal/lateral epidermis. The clonal pattern of the belly region differed in that stripes were not found, but a marked ventral midline boundary was observed. This demarcation line may be due to a physical effect, i.e. isolation of the left and right ventral halves of the epidermis during early embryogenesis with relatively little cell mingling following

closure of the abdominal wall. The obvious nonhomogenous distribution of chimaeric components in dorsal/lateral and ventral epidermis contradicts assumptions of homogenous, fine-grained patchiness derived from electrophoretic analysis of tissue samples and used in studies of skin carcinogenesis.

The observation that hair follicles may contain cells of both parental genotypes implies a polyclonal origin. Epidermal proliferative units as described by Potten (1974) were not revealed by the pattern of mosaicism at the cellular level in these chimaeric tissue sheets. This indicates that the proliferative compartment of each putative epidermal unit is polyclonal.

Key words: mouse, chimaera, epidermis, H-2 antigens, immunohistochemistry, clonal development.

### Introduction

Monoclonal antibodies directed against H-2 antigens have been used as strain-specific markers for cellular genotype in various sectioned tissues of adult chimaeric mice (Ponder, Wilkinson & Wood, 1983a; Ponder *et al.* 1985). Here we report on the use of the marker system to visualize the chimaeric components in whole-mount preparations of mouse epidermis.

Chimaeric mice have been used to address a variety of questions about clonal histories in development and in adult animals (e.g. McLaren, 1976; Le Douarin & McLaren, 1984 for reviews; Schmidt, Garbutt, Wilkinson & Ponder, 1985a). Whole-mount preparations are better suited than tissue sections for this purpose because it is difficult to infer clone sizes in the intact tissue from the patterns of mosaic patches in tissue sections (Schmidt, Wilkinson & Ponder, 1985b; Gardner, 1984a).

The examination of mosaic patterns in sheets of chimaeric epidermis provides information about the normal pattern of development of this tissue and the clonal organization of epidermis in the adult mouse. Green, Durham, Mayer & Hoppe (1977) proposed, from observations of the pattern of mutant hair types in chimaeric coat, that the epidermis showed similar directional clonal expansion and coherent proliferation during development as had previously been suggested for melanocytes by Mintz (1967). In the adult, the epidermis of ear and body skin is thought to have a specific clonal architecture based on the 'epidermal proliferative unit' (Potten, 1974): the visible element of structure is found by precisely aligned columns of flattened cells in the spinous, granular and squamous layers. Beneath each column lie groups of approximately ten basal cells, 2-7% of which are clonogenic (i.e. capable of generation as determined by radiobiological experiments). There is

evidence that the single basal cell lying beneath the centre of the column is more slowly cycling than the cells lying beneath the edges. It is proposed that the central cell is the clonogenic stem cell, whose daughters move in the column, and, hence, emigrate in coordination with the interdigitating cells of adjacent columns to form the flattened cells of the column itself. As with other tissues (see above), such a highly geometrically clonal organization of the basal layer of the epidermis should be revealed in a chimaeric system.

Binomial mathematics were employed in a study of chimaeric epidermis (Iannaccone, Gardner & Harris, 1978; Iannaccone, 1980) which asked from how many cells experimentally induced skin tumours arise. In that analysis, mosaic patterns were not visualized by staining; instead, the composition of small tissue fragments was examined using electrophoretic polymorphisms. The variances of the proportions of the chimaeric components obtained were used to calculate an average clone size; a mean patch size was then estimated from the theoretical relationship between clone and patch size (West, 1975). Further calculations, also based on binomial sampling and incorporating the observed frequency of tumours with mixed genotypes, yielded estimates for a hypothetical target size (about eight cells), the number of cells that would give rise to a single tumour. The inherent difficulties with this type of statistical approach have been discussed in detail elsewhere (Mead, Schmidt & Ponder, 1987; Schmidt & Ponder, 1987; Wilkins, 1986). In order to exploit the potential of the chimaeric system for answering questions regarding tumorigenesis, clearly it will be crucial to know the **actual** distribution and sizes of patches rather than to rely on untested assumptions about them. The *in situ* staining method of whole-mount preparations of epidermis reported in the present paper will be of value to these applied studies.

## Materials and methods

### Mice

CBA/CaLac (CBA; H-2<sup>k</sup>) mice were bred in the animal house of the Institute of Cancer Research, C57BL/6JLac (B6; H-2<sup>b</sup>) and AKR (H-2<sup>k</sup>) mice were obtained from Olac Ltd (Bicester, UK). Eight chimaeras were obtained by aggregation of 4- to 8-cell embryos according to methods described by Mintz (1971): five B6↔CBA, two B6↔AKR and one B6↔RIII. The aggregated embryos were brought to term and reared by B6×DBA/2Lac F<sub>1</sub>-hybrid foster mothers.

### Preparation of epidermal sheets

Chimaeras were aged 6 weeks to 2 years when killed by cervical dislocation. The hair of the body (trunk: dorsal/

lateral/ventral) was removed using an electric shaver (Small Animal Clipper, A5-00, Oster Corp. Milwaukee, Wisconsin, USA) followed by a 20 min application of Imaac Cream (Anne French, London) and a final rinse in phosphate-buffered saline (PBS), pH 7.3. In order to loosen the epidermal and dermal layers, the sheets were transferred to 20 mM-disodium ethylene (diamine) tetra acetate (EDTA) in Hanks buffered-salt solution (HBSS) adjusted to pH 7.2, and left with gentle stirring for 19–22 h at 37°C. Following the EDTA treatment, the skin sheets were pinned out (stretched) in a dissecting dish, lined with black paraffin wax and containing PBS, and the remaining preparation was carried out under a dissecting microscope (SD2, Kyowa Optical) using a cold light source (KL, 1500, Schott, Wiesbaden). The epidermis (uppermost) was carefully separated from the dermis by holding down the relatively thick dermis with forceps while gently pushing back – and thus separating – the epidermis with the closed end of a second pair of forceps. The isolated epidermal sheets were transferred (basal surface uppermost) to a Petri dish lined with white paraffin wax, pinned down with fine entomological pins and fixed in 10 mM-periodate–0.15 M-lysine–1 % paraformaldehyde (PLP) pH 7.4 for 2 min.

### Staining

The staining method was adapted from that developed for tissue sections (Ponder *et al.* 1983a). The monoclonal antibodies clone 11.4-1 (anti-H-2<sup>k</sup>) and FT 6 α 9 (anti-H-2<sup>b</sup>) were purified from ascites and conjugated directly to alkaline phosphatase and peroxidase as previously described (Ponder, Wilkinson, Westwood & Wood, 1983b). After fixation, the epidermal preparations were washed with PBS and immediately immersed in 0.1 % phenylhydrazine hydrochloride in PBS for 30 min to block endogenous peroxidase. After several washes in PBS and a final wash in PBS containing 0.5 % bovine serum albumin (PBS–BSA), the preparations were incubated in 10 % mouse serum (from CBA mice) for 30 min before application of the first antibody. Best results were obtained (Ponder *et al.* 1983a) if the anti-H-2<sup>k</sup> monoclonal antibody (11.4-1; conjugated directly with alkaline phosphatase) was applied before the H-2<sup>b</sup> monoclonal antibody (FT 6 α 9; conjugated directly with horseradish peroxidase). The epidermal sheets were incubated for 90 min at room temperature with each of the antibodies (diluted 1:50 to 1:200 in 10 % mouse serum, pH 7.6); preparations were washed in PBS–BSA between and after incubation. Peroxidase was demonstrated first, using 3'3' diaminobenzidine (DAB; Sigma) in 50 mM-Tris pH 7.2 as substrate, yielding a brown reaction product. The alkaline phosphatase substrate was naphthol AS-BI sodium salt (Sigma) coupled to Brentamine fast red TR, in veronal acetate buffer pH 9.2, yielding a red reaction product. Endogenous alkaline phosphatase was inhibited by addition of 1 mM-levamisole (Sigma) to the substrate mixture (Ponder & Wilkinson, 1981). Stained preparations were kept in 5 % formol saline, but the staining intensity became weaker after several weeks of storage.

### Controls

Nonchimaeric B6 and CBA skin sheets were stained with both antibody conjugates simultaneously with a chimaeric sheet as controls.

### Photography

Fig. 2A–G were taken with a Polaroid MP.4 Land Camera System holding a Contax (RTS) camera, using Kodak Ektachrome 50 ASA.

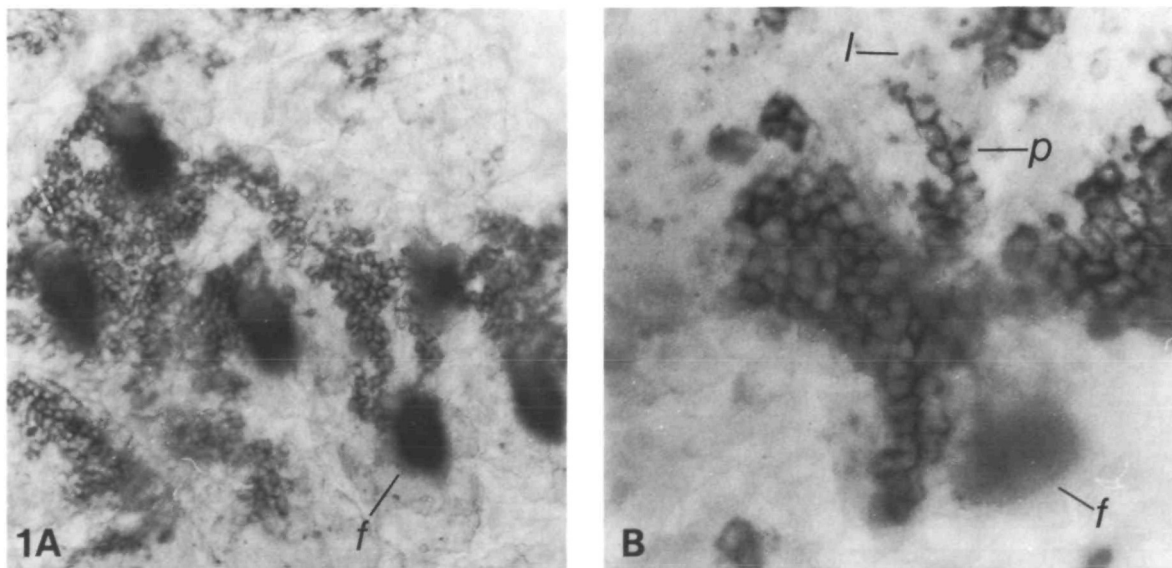
### Results

The resolution of the immunohistochemical staining was sufficient to allow us to identify unequivocally the chimaeric components at the level of a single epithelial cell (Fig. 1). Nonchimaeric, control sheets of epidermis stained homogeneously for the respective H-2 haplotypes (Fig. 2G).

The relative proportions of the mosaic H-2 haplotypes were visually estimated to have varied between 0.3 and 0.7 (Fig. 2A,C,E). Thus, we did not obtain chimaeras in which the components were overall highly unbalanced (e.g. one component less than 0.05); such unbalanced animals are particularly suitable for *quantitative* clonal analysis of tissues in which the arrangements of clones show little obvious spatial pattern (Schmidt, Wilkinson & Ponder, 1985c, 1986b). In the epidermis, however, the clonal patterns observed were striking: transverse clonal stripes were observed in all specimens, although more pronounced in some than in others (Fig 2A,C,E). They

extended from approximately mid-dorsal, laterally down both flanks; a dorsal midline was, however, not sharply defined (Fig. 2A). In epidermal sheets in which large dorsal/lateral areas were dominated by only one component, small transversely oriented, longitudinal patches of the other genotype were clearly delineated, probably representing single coherent clones (Fig. 2D). These patches were always smaller, often considerably so, than the obvious transverse bands in areas of epidermis with relatively more equal contributions of the two component genotypes (compare Fig. 2A,E). The edges of both stripes and coherent clones showed fine-grained mosaicism (Fig. 2B).

A very different clonal pattern was found for the belly region, the most conspicuous feature being a relatively marked patch boundary along the ventral midline (Fig. 2C,E). The length over which this demarcation line was visible varied depending on whether the adjoining regions were of dissimilar genotypes. In some animals it extended to 3 cm, whereas no line at all was visible in one specimen in which the entire belly region was dominated by the B6 component (Fig. 2A). The definition of the boundary was less pronounced when viewed at higher magnification (Fig. 2F), the irregularity extending over some 20 cell diameters. Other large areas of the belly were often dominated by either one of the two chimaeric components (Fig. 2A,C) and the striking patterns of clonal stripes observed in the dorsal region and flanks were absent.



**Fig. 1.** All preparations (Figs 1, 2) are whole-mount sheets and viewed from the dermal side. Patches of CBA (H-2<sup>k</sup>) keratinocytes in CBA↔B6 (H-2<sup>k</sup>↔H-2<sup>b</sup>) chimaeric epidermis. (A) Individual CBA keratinocytes are outlined by dark staining. The pattern of squames can be indistinctly seen at top right of the field. f, hair follicle (out of plane of focus). (B) Irregular-shaped projection (p) from patch of CBA keratinocytes. Smaller, less strongly stained cells are seen scattered in the epidermal sheet away from the CBA patch (e.g. l). These are probably Langerhans cells which have bound the anti-H-2<sup>k</sup>-antibody through F<sub>c</sub> receptors. ×100.

Hair follicles lying within a patch of epithelium of a single parental type would be expected to be homogeneously stained whether they arose from one or from more cells; but at the border between patches of differing parental type, mixed hair follicles would be expected if their origins were polyclonal. We observed that hair follicles at such boundaries were indeed often mixed.

There were irregularities of the patch boundaries and occasionally isolated patches of two or three cells in the skin of dorsal, flank and belly regions (e.g. Fig. 1) whose size and shape did not conform to that expected if the basal layer were composed of monoclonal epidermal proliferative units (EPU's) as described by Potten (1974).

## Discussion

The marker system employed in the present study is suitable for positive identification of both parental components in whole-mount preparations of chimaeric mouse epidermis. Patches of dorsal/lateral but not ventral epidermis showed a directional preference of clonal expansion consistent in different animals. Hence, our study supports the suggestion made by Green *et al.* (1977) regarding the dorsal growth of epidermis (see Introduction), but it revealed a different clonal pattern for ventral epidermis. The clonal sizes observed in the epidermal sheet with relatively unbalanced components (Fig. 2D) suggest that most of the large conspicuous stripes in some other specimens (e.g. Fig. 2A) are due to aggregation of adjacent coherent clones of the same genotype into larger transverse patches (i.e. stripes) in a manner analogous to that described for melanocyte patterns (Silvers, 1979). Since the epidermis grows as a cell sheet, the stripy patterns may be interpreted as showing preferred orientations of mitoses, while in the coat, the migratory behaviour of the melanocyte population greatly contributes to the final patterns (Mintz, 1967).

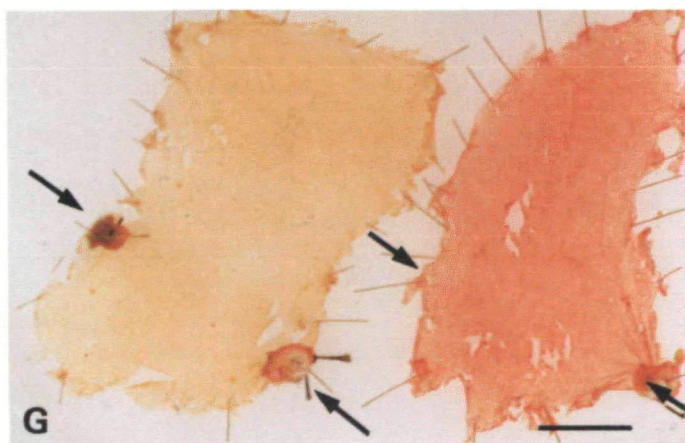
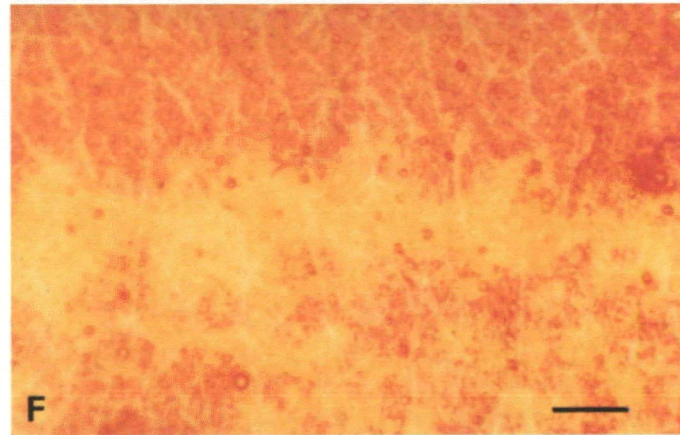
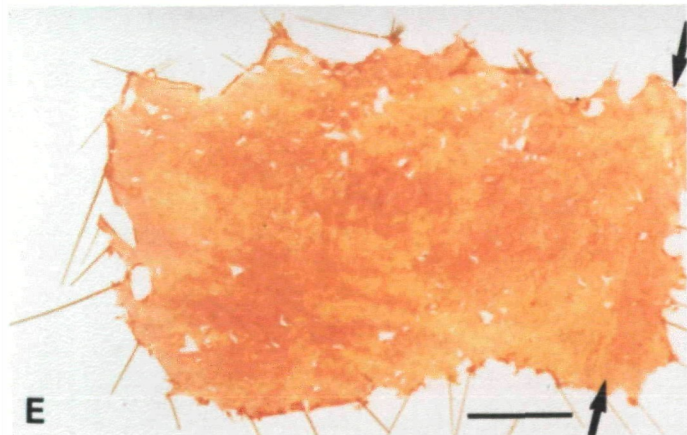
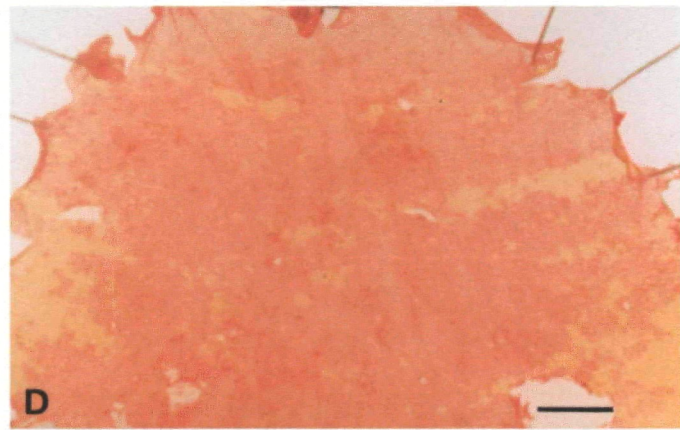
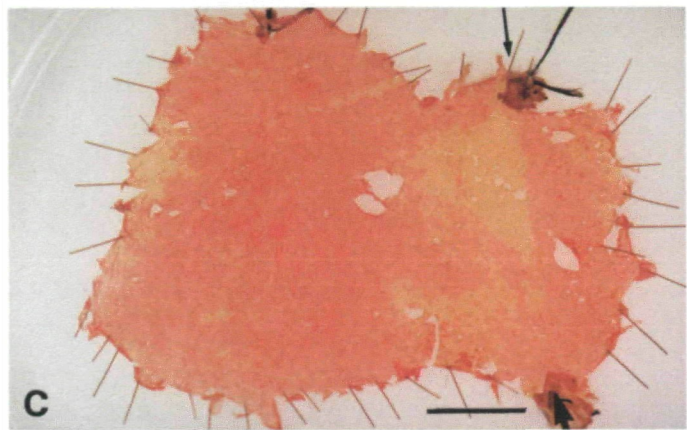
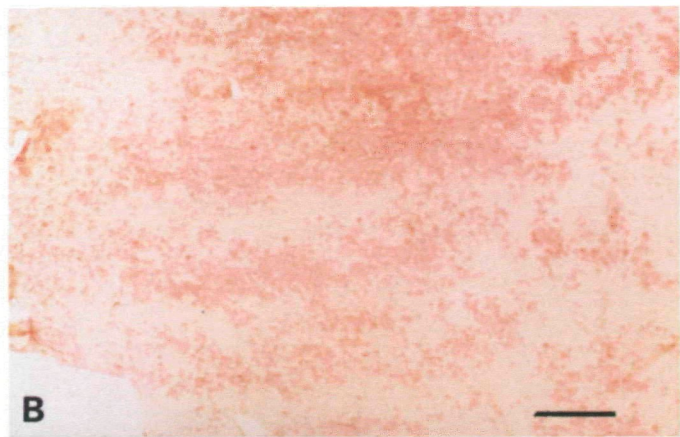
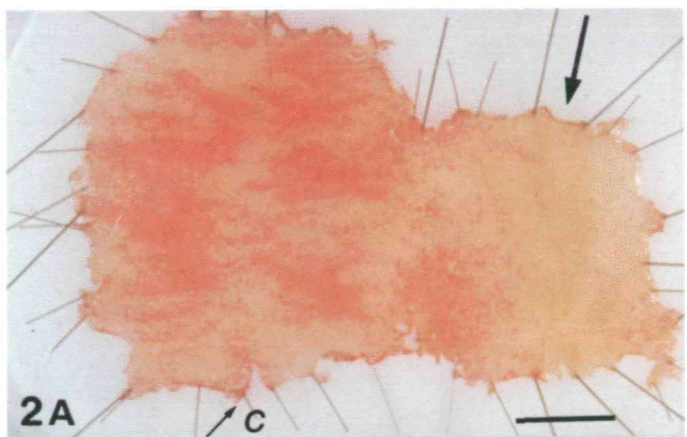
The clonal pattern in the ventral epidermis was markedly different. There were no stripes anywhere from the midthoracic region to the groin, but a straight midventral clonal boundary was observed. These findings were consistent in chimaeras of each of the three strain combinations studied. The lack of obvious ventral striping in epidermis suggests that the growth of the epidermis is less-strongly directional here than it is dorsolaterally, perhaps because of the growth associated with the closure of the anterior abdominal wall. In contrast, striping in melanocyte chimaeras may extend to the midline in the upper abdominal region or over the belly (Fig. 3), implying

that by the time of melanocyte migration, the direction of epidermal growth no longer leads to disruption of the dorsal/lateral striping pattern.

Both melanocytes and epidermal cells observe a well-defined midventral boundary. The straightness of the ventral midline in the epidermis appeared to be intermediate between the borders reflecting compartment boundaries in *Xenopus* (Jacobson & Klein, 1985) or *Drosophila* (Postlethwait, 1978), and the (presumably noncompartment) edges of clones in dorsal epidermis (Fig. 2B). The most probable explanation of the ventral midline boundary in epidermis is that it results from the physical isolation of the left and right halves of the ventral body wall during early embryogenesis before the ventral closure of the body wall at 9 days *post coitum*. The relative straightness of the boundary in the adult indicates that cell mingling

**Fig. 2.** (A) B6↔CBA chimaera. Whole-mount preparation of dorsal-ventral epidermis. *c* marks the cranial end of the dorsal midline. Marked clonal stripes of CBA type (H-2<sup>k</sup>; red) and B6 (H-2<sup>b</sup>; yellow-brown) cells can be seen running dorsoventrally in the dorsal and flank regions. The broad bands are probably aggregates of smaller bands such as those seen in the lower left region (which is shown at higher magnification in B). Note that the ventral region (arrow) is almost completely dominated by the B6 component. Bar, 1.5 cm. (B) Higher magnification of part of A (lower left region) reveals a considerable degree of cell mingling at the edges of stripes. Bar, 0.2 cm. (C) RIII↔B6 chimaera. The dorsal and flank regions in this specimen are dominated by the RIII component. Obvious, striped, patterns are not found, but several small transverse patches (probably representing single coherent clones) are apparent (see D). In contrast, the parental components in the belly region occur in more or less equal proportions; however, the RIII component is almost confined to the one ventral half and the B6 component to the other, thus creating a well-defined clonal midline, arrows). Orientation of the sheet as in A and C. Bar, 1.5 cm. (D) Higher magnification of dorsal part and flanks of sheet shown in C. Some oblong clonal patches are apparent. They may be regarded as single coherent clones. Bar, 0.5 cm. (E) Specimen with more or less equal proportions of CBA and B6 components. Orientation as in A and C: caudal ventral midline arrowed at bottom right. The clonal pattern gives the impression of a relatively homogeneous chimaeric mosaicism; nevertheless, some transverse stripes are visible. The ventral midline is shown at high magnification in F. Bar, 1.5 cm. (F) Close up of midventral clonal boundary line of preparation shown in Fig. 2E. The midventral line is often relatively irregular at the cellular level. Small, round circular structures are broken hair follicles. Bar, 0.25 mm. (G) Control sheets of part of CBA and B6 epidermis, double stained in the same Petri dish. The staining is homogeneous. Arrows denote fragments of dermis left attached to the epidermal sheets to mark the midventral region. Bar, 1.5 cm.





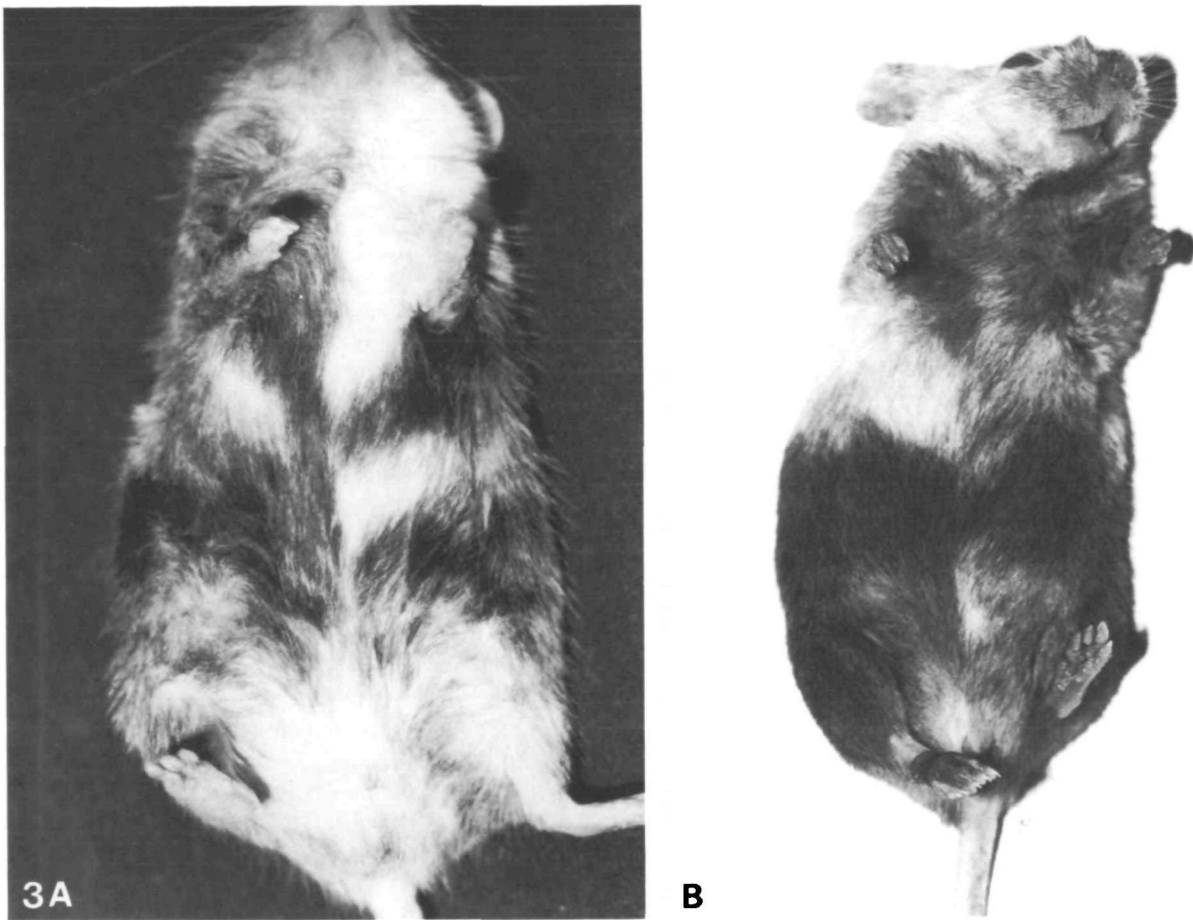


Fig. 3. (A) AKR ↔ B6 chimaera. (B) RIII ↔ B6 chimaera, the epidermis from which is shown in Fig. 2C. Note in each case melanocyte striping patterns extending to the ventral midline in the belly region.

in this region does subsequently occur, but only over a region extending to about 20 cell diameters. The absence of a clear dorsal midline boundary in epidermis suggests that there is a greater degree of mingling in this region, following closure of the neural folds.

The present study is a further example of the very informative approach of *in situ* staining of whole-mount preparations using chimaeras. So far, tissues that have been studied by this method have revealed a considerable spectrum of clonal organization, with different characteristics for particular tissues (Gardner, 1984*b*, 1985; Ponder *et al.* 1985; Ponder, Schmidt & Wilkinson, 1986; Schmidt, Wilkinson & Ponder, 1984; Schmidt *et al.* 1985*a,b,c*, 1986*a,b*; Schmidt & Ponder, 1987). The demonstration of pattern in chimaeric epidermis, i.e. clonal stripes, midventral line, large areas of predominantly one phenotype in tissues with otherwise relatively balanced components, is consistent with a non-random distribution of the two cellular genotypes (in the *statistical* sense, pattern is defined as a departure from a random (Poisson) distribution of the entities

under study (see Schmidt *et al.* 1985*c*)). The nonrandom mosaicism found is in contrast to assumptions made and results obtained by Iannaccone *et al.* (1978; see Introduction). In that study epidermal fragments (0.5–1 mm<sup>2</sup>) were analysed electrophoretically and 92% of a total sample of 495 contained contributions of both chimaeric components, albeit in varying proportions. This would indeed imply a fine-grained mosaicism and a possible random distribution of small patches as was suggested by Iannaccone *et al.* (1978). Although in some regions of the epidermis of our chimaeras (e.g. Fig. 2B,F to left of midline) a fine mosaicism was present, in others (Fig. 2C) large areas were uniformly of one parental type or the other. The discrepancy between Iannaccone *et al.*'s results and ours remains unexplained, although one possibility is the different strain combinations used to make the chimaeras (McLaren, 1976).

The chimaeric system has considerable potential for testing the clonality of anatomical structures. For example, whereas, in the adult, epithelial cells of individual intestinal crypts are always of the same parental type (Ponder *et al.* 1985), hair follicles are



mixed. We can conclude that the epithelium of the former, but not the latter, is derived from a single progenitor cell. The chimaeric system can also demonstrate the migratory pathway taken by cells from proliferative through functional anatomical zones (Schmidt *et al.* 1985*b*). Cells originating in the epidermal proliferative, basal layer are believed to migrate vertically in precisely defined columns of cells, several columns giving rise to well-determined geometric patterns (EPU; see Introduction). If EPUs were monoclonal the mosaicism of chimaeric epidermis would be expected to reveal also a well-determined geometric pattern at patch boundaries and in areas where one of the parental components is in the minority. We did not, however, observe such regular patterns and conclude that our data indicate a polyclonal composition for EPUs. A polyclonal composition would not be consistent with the view of the centrally located basal cell being the sole progenitor of all cells in a single EPU.

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## References

- GARDNER, R. L. (1984*a*). Mammalian chimaeras – future perspectives. In *Chimaeras in Developmental Biology* (ed. N. M. Le Douarin & A. McLaren), pp. 431–443. London: Academic Press.
- GARDNER, R. L. (1984*b*). An *in situ* cell marker for clonal analysis of development of the extraembryonic endoderm in the mouse. *J. Embryol. exp. Morph.* **80**, 251–288.
- GARDNER, R. L. (1985). Clonal analysis of early mammalian development. *Phil. Trans. R. Soc. Lond. B* **312**, 163–178.
- GREEN, M. C., DURHAM, D., MAYER, T. C. & HOPPE, P. C. (1977). Evidence from chimaeras for the pattern of proliferation of epidermis in the mouse. *Genet. Res. Camb.* **29**, 279–284.
- IANNACCONE, P. M., GARDNER, R. L. & HARRIS, H. (1978). The cellular origin of chemically induced tumours. *J. Cell Sci.* **29**, 249–269.
- IANNACCONE, P. M. (1980). Clone and patch size in chimaeric mouse skin. *Math. Biosci.* **51**, 117–123.
- JACOBSON, M. & KLEIN, S. L. (1985). Analysis of clonal restriction of cell mingling in *Xenopus*. *Phil. Trans. R. Soc. Lond. B* **312**, 57–65.
- LE DOUARIN, N. & McLAREN, A. (1984). *Chimaeras in Developmental Biology*. London: Academic Press.
- McLAREN, A. (1976). *Mammalian Chimaeras*. Cambridge: University Press.
- MEAD, R., SCHMIDT, G. H. & PONDER, B. A. J. (1987). Calculating numbers of tissue progenitor cells using chimeric animals. *Devl Biol.* (in press).
- MINTZ, B. (1967). Gene control of mammalian pigmentary differentiation. I. Clonal origin of melanocytes. *Proc. natn. Acad. Sci. U.S.A.* **58**, 344–351.
- MINTZ, B. (1971). Allophenic mice of multiembryo origin. In *Methods in Mammalian Embryology* (ed. J. Daniels, Jr.), pp. 186. San Francisco: Freeman.
- PONDER, B. A. J. & WILKINSON, M. M. (1981). Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. *J. Histochem. Cytochem.* **29**, 981–985.
- PONDER, B. A. J., WILKINSON, M. M. & WOOD, M. (1983*a*). H2 antigens as markers of cellular genotype in chimaeric mice. *J. Embryol. exp. Morph.* **76**, 83–93.
- PONDER, B. A. J., WILKINSON, M. M., WESTWOOD, J. & WOOD, M. (1983*b*). Immunohistochemical demonstration of H2 antigens in mouse tissue sections. *J. Histochem. Cytochem.* **31**, 911–919.
- PONDER, B. A. J., SCHMIDT, G. H., WILKINSON, M. M., WOOD, M. J., MONK, M. & REID, A. (1985). Derivation of mouse intestinal crypts from single progenitor cells. *Nature, Lond.* **313**, 689–691.
- PONDER, B. A. J., SCHMIDT, G. H. & WILKINSON, M. M. (1986). Immunohistochemistry in the analysis of mouse aggregation chimaeras. *Histochem. J.* **18**, 217–227.
- POSTLETHWAIT, J. H. (1978). Clonal analysis of *Drosophila* cuticular patterns. In *The Genetics and Biology of Drosophila*, vol. 2C (ed. M. Ashburner & T. R. F. Wright), pp. 359–441. London: Academic Press.
- POTTEN, C. S. (1974). The epidermal proliferative unit: the possible role of the central basal cell. *Cell Tissue Kinet.* **7**, 77–88.
- SCHMIDT, G. H., GARBUIT, D. J., WILKINSON, M. M. & PONDER, B. A. J. (1985*a*). Clonal analysis of intestinal crypt populations in mouse aggregation chimaeras. *J. Embryol. exp. Morph.* **85**, 121–130.
- SCHMIDT, G. H. & PONDER, B. A. J. (1987). From patterns to clones in chimaeric tissues. *BioEssays* **6**, 104–108.
- SCHMIDT, G. H., WILKINSON, M. M. & PONDER, B. A. J. (1984). A method for the preparation of large intact sheets of intestinal mucosa: application to the study of mouse aggregation chimaeras. *Anat. Rec.* **210**, 407–411.
- SCHMIDT, G. H., WILKINSON, M. M. & PONDER, B. A. J. (1985*b*). Cell migration pathway in the intestinal epithelium – an *in situ* marker system using mouse aggregation chimaeras. *Cell* **40**, 425–429.
- SCHMIDT, G. H., WILKINSON, M. M. & PONDER, B. A. J. (1985*c*). Detection and characterization of spatial

- pattern in chimaeric tissue. *J. Embryol. exp. Morph.* **88**, 219–230.
- SCHMIDT, G. H., WILKINSON, M. M. & PONDER, B. A. J. (1986a). Clonal analysis of chimaeric patterns in aortic endothelium. *J. Embryol. exp. Morph.* **91**, 197–207.
- SCHMIDT, G. H., WILKINSON, M. M. & PONDER, B. A. J. (1986b). Non-random spatial arrangement of clone size in chimaeric retinal pigment epithelium. *J. Embryol. exp. Morph.* **93**, 267–280.
- SILVERS, W. K. (1979). *The Coat Colors of Mice*. New York: Springer-Verlag.
- WEST, J. D. (1975). A theoretical approach to the relation between patch size and clone size in chimaeric mice. *J. theor. Biol.* **50**, 153–160.
- WILKINS, A. S. (1986). *Genetic analysis of animal development*. New York: John Wiley & Sons.

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