EVIDENCE FOR A REACTION/DIFFUSION SYSTEM IN THE CONTROL OF CELL PROLIFERATION AND DIFFERENTIATION IN THE WOOL FOLLICLE

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SUMMARY

Specific mechanisms responsible for the control of cell proliferation and differentiation in growing hair fibres are still uncharacterized. However, the Reaction-Diffusion (RD) system of morphogens advanced by Nagorcka and Mooney (1982) makes specific quantitative predictions about wavelike patterns of putative morphogen distributions as functions of wool follicle bulb size and shape and dermal papilla volume. These theoretical morphogen distributions can then be used to predict patterns of cell proliferation and differentiation in the growing wool fibre. In order to test these predictions, we used the BrdU/anti-BrdU technique in conjunction with a monoclonal antibody (Hewish and French 1986) specific for fibre orthocortex cells to visualize patterns of cell proliferation and differentiation in the wool follicle bulb. Sheep were injected with BrdU and skin samples embedded in paraffin and serially sectioned before immunohistochemical staining. Individual follicles were followed from their base to the level at which cortical differentiation was obvious. For every follicle examined to date (n=15) from one sheep whose fibres exhibit bilateral cortical segmentation, there is good agreement between the observed asymmetric pattern of cell proliferation and the predicted morphogen gradient.

INTRODUCTION

The quantity and morphology of wool fibres in the fleece are functions of the follicle population in the skin. In general, there is significant variation in follicle density, follicle size distribution, follicle type and follicle grouping within an *animal,* and much greater variation between animals. This variation in follicle characteristics is reflected in the fleece because of the relationship between the fibre length growth rate and fibre diameter and the follicle bulb diameter and/or the size of the dermal papilla (Rudall 1956; Henderson 1965). Furthermore, the efficiency of the follicle, i.e. the proportion of cells produced in the follicle bulb that enter the fibre, has been observed to vary substantially between animals and between breeds (Black and Reis 1979; Hynd 1989). However, because of the difficulty of these measurements, no relationship between follicle efficiency and follicle size within an animal has, as yet, been reported. Nevertheless, it is known that fibre structure is dependent on fibre diameter, and hence follicle bulb diameter, since the proportion and distribution of ortho- and paracortical cells within the fibre are observed to *vary* with fibre diameter (Ahmad and Lang 1957; Orwin *et al.* 1984). It follows that, in general, the skin follicle population must be regarded as a heterogeneous population and the variation in the efficiency and quality of the fibres produced in individual follicles needs to be understood,

A prepattern mechanism was proposed recently that controls not only follicle initiation and development but also cellular proliferation and differentiation in the follicle bulb leading to the formation of the fibre (Nagorcka and Mooney 1989). The prepattern is based on a reaction diffusion (RD) system (Turing 1952) which, in its simplest form, consists of two chemicals (X and Y) reacting with each other as they diffuse through a tissue of specific shape and

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boundaries. In the case of the mature wool follicle, X and Y are considered to be confined to the follicle bulb (Nagorcka and Mooney 1982). If the reaction and diffusion rates of X and Y obey certain broad constraints it can be shown that stable wavelike patterns in the distribution of X and Y will arise spontaneously.

A range of different prepatterns in X have been predicted to arise depending on the size and shape of the follicle bulb. These predictions have been found to be in qualitative agreement with the observed fibre cross-sectional shapes and patterns observed in the spatial ditribution of ortho- and paracortical cells. The prepattern predicted to arise in the smallest follicle bulbs is the familiar bilateral pattern in the distribution of ortho- and paracortex. It follows from the proposed mechanism that the mitotic activity associated with this pattern should also be bilateral or asymmetrical in the bulb. There have been conflicting reports concerning the presence of asymmetrical mitotic activity (Fraser 1964; Williams and Winston 1987; Hynd 1989), and in this paper we examine in more detail the predicted bilateral prepattern and the observed patterns of cortical cell distribution and mitotic activity.

METHODS

One Border Leicester x Merino ewe was labelled by injection into the jugular vein of 450 mg of 5-Bromo-2'-Deoxyuridine (Sigma B-5002) and 50 mg 5-Fluoro-2'-Deoxyuridine (Sigma F-0503) in 20 ml of phosphate buffered saline. After thirty minutes skin samples from the mid-flank were taken under local anaesthesia (Xylocaine 2% w/Adrenaline) with a 10 mm diameter trephine. Biopsies were fixed in Bouin's fixative for four hours and stored in 70% ethanol for no longer than four days prior to dehydration and embedding in paraffin. Samples were embedded and serial 8 micron transverse sections were cut from the dermal side of the biopsy. Sections were rehydrated, treated for half an hour with 0.3% H202 in methanol to inactivate endogenous peroxidase activity, washed in 50 mM Tris pH 7.5, 150 mM NaCl (TBS) covered in 10% sheep serum diluted in TBS. BrdU and orthocortex antigen were detected simultaneously with an Amersham Cell Proliferation Kit used according to the manufacturer's instructions in combination with the monoclonal antibody Hit 96 (Hewish and French 1986). Anti-BrdU and Hit 96 were mixed in a 1:1 ratio and 0.1-0.2 ml of this solution used on each slide. Slides were counterstained with 3% acid eosin in 70% alcohol.

RESULTS

Since the two morphogens are spatially equivalent, only one (X) is considered to be involved in regulating cellular proliferation and differentiation during fibre formation. An additional morphogen Z, which diffuses radially outwards from the dermal papilla, is also required. Z is necessary in order to satisfy a range of experimental observations (Nagorcka and Mooney 1982, Nagorcka 1984) as well as Oliver's (1980) finding that the dermal papilla provides a diffusible factor required for hair and follicle development. Thus the spatial pattern of wool follicle bulb cellular proliferation and differentiation is determined by the two superimposed patterns of X and Z.

Previously, the spatial patterns of X in the follicle bulb were calculated on the basis of approximations of the follicle bulb shape and the RD system equations (Nagorcka and Mooney 1982). We have now solved the coupled nonlinear partial differential equations defining the diffusion and reactions of the RD system components in a realistic representation of the three dimensional follicle bulb using the finite element method. We have also solved the partial differential equations describing the diffusion of Z away from the "tear-drop" shaped dermal papilla within the follicle bulb using a boundary integral method. Details of these calculations are available from the authors. Using the results of these calculations we have produced a contour plot of the

spatial distribution of the quantity [X][Z], which is assumed to determine the activity in the follicle bulb. The contour plots at three different levels in the follicle bulb are shown in Fig. 1.

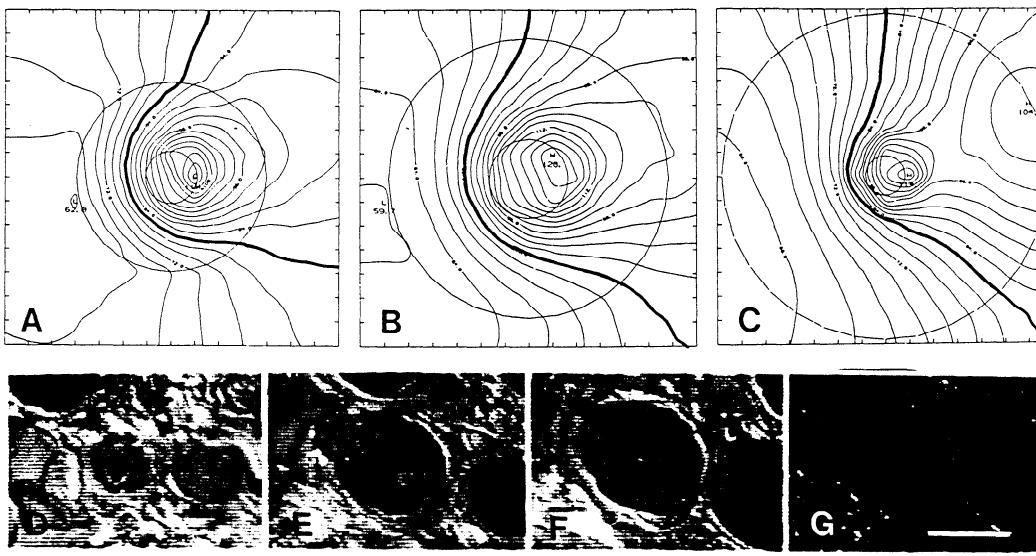

Fig. 1. Comparison of predicted morphogen gradients with observed patterns of mitotic activity and cellular differentiation. Panels A, B and C are contour plots of [X][Z] determined at different heights within the follicle bulb; the outer circle is the outer boundary of the follicle bulb. The inner circle is the bulb/dermal papilla boundary. The contour levels are in arbitrary units, Panel A is a cross section through the base of the bulb, B at midheight of the dermal papilla and C at the top of the dermal papilla. The bold line indicates the chosen threshold concentration for the stimulation of cell proliferation. Panels C, D and E are 8 micron sections from a single bulb taken at the base, mid-dermal papilla height and the top of the dermal papilla. Nuclei of dividing cells are darkly stained with anti-BrdU and are restricted to areas above the predicted [X][Z] threshold. Panel F is a section of the same bulb from the bottom of the keratogenous zone stained with HiT 96. The pattern of orthocortical cell differentiation is complementary to the pattern of cell proliferation. Scale bar = 100 microns.

The bold contour lines seen in panels A, B and C represent the minimum [X][Z] threshold for cell proliferation.

Fifteen follicles were traced and their patterns of cell proliferation and orthocortical cell differentiation analyzed, Follicles were selected for tracing if all contiguous serial sections were available and if the follicle did no bend or twist out of its transverse orientation. The follicles from the Border Leicester x Merino ewe used in this experiment produced almost exclusively fibres of the bilaterally segmented variety. The following pattern of labelled nuclei was observed in every follicle bulb traced. At the base of the follicle, BrdU labelled nuclei are seen to cover most of the area with the exception of a thin crescent of tissue at one margin of the bulb, Moving up the bulb, this pattern becomes more pronounced and approximately mid-way up the dermal papilla, the asymmetric distribution becomes obvious with a large crescent shaped area on one side of the bulb devoid of labelled nuclei. This non-labelled portion of the bulb matrix often includes a number of cells

directly in contact with the dermal papilla. Further up the bulb, the asymmetric distribution of proliferating cells gradually becomes more and more restricted until it dwindles to a few cells clustered to one side of the tip of the dermal papilla. A typical example of this three dimensional pattern can be seen in Fig. 1, panels D,E,F. Panel D shows an 8 micron thick section cut at the very bottom of a typical follicle bulb; the labelled nuclei are confined to one side of the bulb. In panel E, the same bulb is seen at the fifth serial section (40 microns from the base) the labelled nuclei are clearly restricted to one side with a non-proliferating crescent shaped area to the left. Panel F shows the ninth section of the same bulb (72 microns) near the top of the dermal papilla with labelled nuclei confined to a small area neighbouring the papilla. The pattern of cell proliferation in panels D, E and F is in good agreement with that predicted from calculated morphogen ([X][Z])concentration thresholds in panels A, B and C.

Fig. 1, G shows a section from the same follicle at the bottom of the keratogenous zone where cortical cell differentiation first manifests itself. The darkly stained area indicates the location of orthocortex specific antigen bound by HiT 96. The observed pattern of orthocortical cell differentiation is complementary to the pattern of cell proliferation. This pattern also, is consistent with the threshold plot for orthocortical cell differentiation as a function of [Z]/[X] (Nagorcka 1984) (plot not shown), which is essentially the reciprocal pattern to that produced by [X][Z].

DISCUSSION

Our results confirm the observations of Fraser (1964), who noted that bulbs exhibiting asymmetric mitotic activity produced bilaterally segmented fibres, with the paracortex always associated with the mitotically more active side of the bulb. Assumptions of symmetrical mitotic activity are probably not warranted in fine to medium wool animals whose fibres exhibit the typical bilateral segmentation of ortho- and paracortex. The results also confirm the predictions of the prepattern mechanism based on an RD system for small follicle bulbs. More complex prepatterns, and hence, more complex patterns of mitotic activity and differentiation are predicted to arise in larger follicle bulbs. Work is in progress to determine if these additional prepatterns do, in fact, appear and whether they are able to account for differences observed in the efficiency of fibre production.

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