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The different steps of skin formation in vertebrates

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ABSTRACT Skin morphogenesis occurs following a continuous series of cell-cell interactions which can be subdivided into three main stages: 1- the formation of a dense dermis and its overlying epidermis in the future appendage fields (macropattern); 2- the organization of these primary homogeneous fields into heterogeneous ones by the appearance of cutaneous appendage primordia (micropattern) and 3- cutaneous appendage organogenesis itself. In this review, we will first show, by synthesizing novel and previously published data from our laboratory, how heterogenetic and heterospecific dermal/epidermal recombinations have allowed us to distinguish between the respective roles of the dermis and the epidermis. We will then summarize what is known from the work of many different research groups about the molecular signaling which mediates these interactions in order to introduce the following articles of this Special Issue and to highlight what remains to done.

KEY WORDS: *chick, mouse, lizard, Ottawa naked, scaleless*

Introduction

The integument, that is, the skin and cornea, is the only organ that is immediately visible to external examination. Any deviations from wild type are immediately detectable, which explains the large number of studies that have appeared in the last few years using transgenic and K.O. mice. In amniotes, cutaneous appendages are exclusively composed of epidermal cells and during many years by the past, it was generally considered that epidermis is the effector tissue and that its morphogenesis depends to a large extent upon dermal influence (Sengel, 1976). Dhouailly (1977, 1984) first pinpointed that both components of the skin should be considered as donors and receptors of information: skin morphogenesis depends on a continuous dialogue between its two components. At each step of this dialogue, attention needs to be paid not only to the responses of one tissue to the other by the way of diffusible signaling factors, but also primarily to the activation of transcription factors and intratissue interactions, as has been beautifully shown for teeth formation (Pispa and Thesleff, 2003).

The cellular interactions, as we saw in the previous chapters of this issue, start before the formation of an embryonic skin. Indeed, before skin morphogenesis, various cellular interactions occur, which specify first the formation of dermal progenitors (Olivera-Martinez *et al.*; Fliniaux *et al.*, 2004) and then their densification within the sub-ectodermal space, i.e. the establishment of the future cutaneous appendage fields or macropattern. These two first steps lead to the formation of a homogeneous embryonic skin, composed of an epidermis overlying a dense dermis. The next step, i.e. the initiation and organization of regular repetitive appendage primordia, or micropattern, is one of the most fascinating problems in development (Jiang *et al.*, 1999, 2004; Bardot *et al.*, 2004). The final step, the organogenesis of the epidermal primordia (placode) in a complete, mature appendage, is the most complex to elucidate and this has recently been done beautifully in the case of feather (Yu *et al.*, 2004), the most complicated epidermal structure yet evolved (Wu *et al.*, 2004).

Many results have been obtained from experiments where dermis and epidermis were separated by enzymatic or chemical (EDTA) methods and recombined in various conformations. The recombination of tissues that have varying degrees of difference allows us to dissect out the successive steps and cell interactions involved. The comparison of results obtained in heterospecific recombinants from species belonging to the same class or to two different classes of amniotes led to the classic concept of the two steps in dermal induction (Dhouailly, 1977), firstly to initiate placode formation and secondly to direct appendage organogenesis. Other information, derived from heterogenetic recombina-

Abbreviations used in this paper: OT, Ottawa naked.

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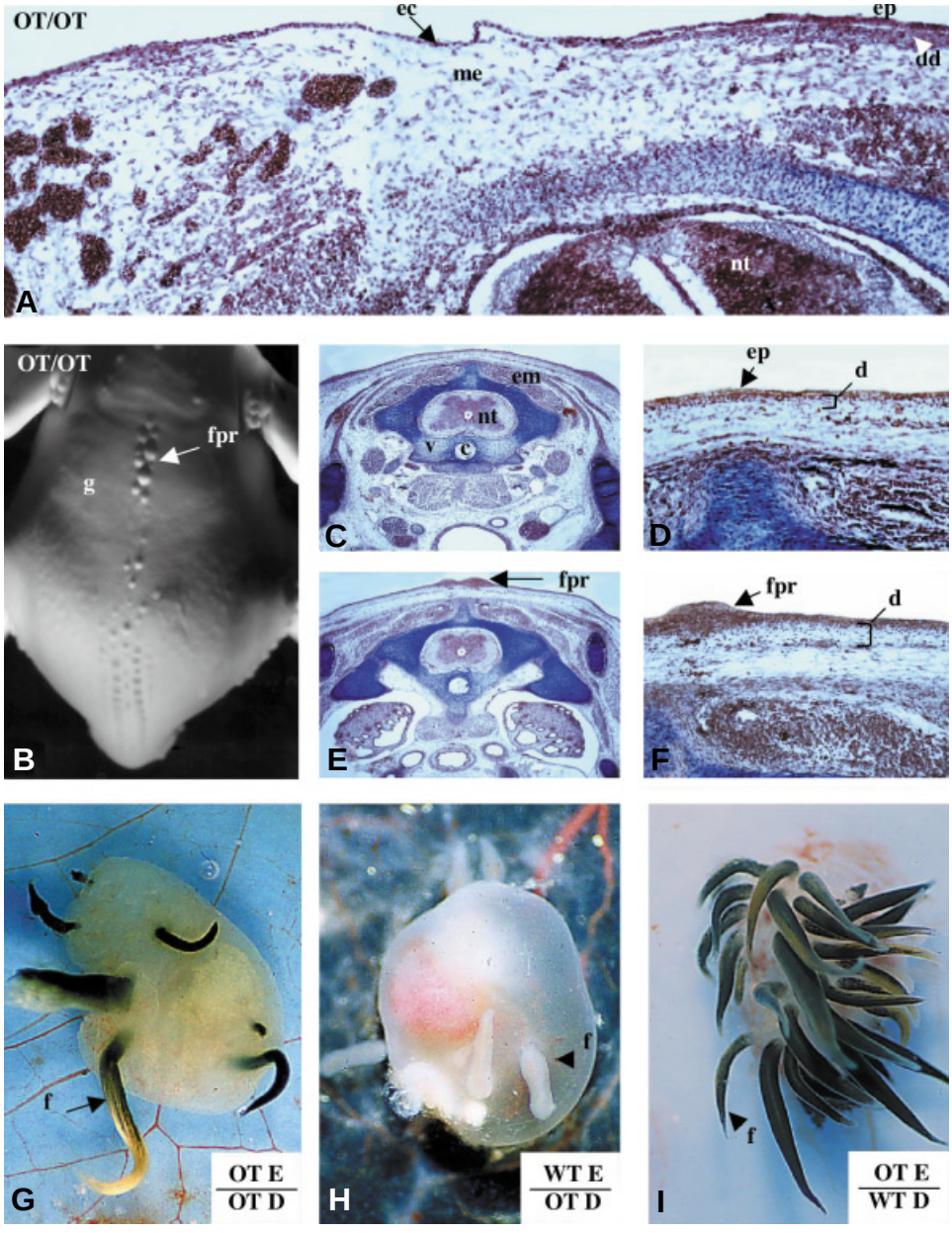

Fig. 1. In the chick *Ottawa naked* mutant, dense dermis formation is affected. (A) *Transversal section of the thoracic region at stage HH 30. In this case, the formation of a dense dermis (dd) and the subsequent differentiation of the ectoderm (ec) into an epidermis (ep) occurs on the right side, while the mesenchyme (me) remains loose over the neural tube.* **(B)** *Dorsal view at E10. Most parts of the spinal pteryla have not formed and the skin stays glabrous (g). In this case, a few abnormal feather primordia form along the middorsal line and on the shoulders. Their formation is delayed in comparison with the wild type. It should be noted that the skin pattern varies in each homozygous embryo.* **(C,D)** *Transversal section at E10 in the upper thoracic region of the previous embryo. Two different magnifications showing the formation of a sparse dermis (d), while the epaxial muscles (em) and the vertebra (v) differentiate around the neural tube (nt) and the cord (C).* **(E,F)** *Transversal section at E10 in the lower thoracic region of the previous embryo. Two different magnifications showing the formation of abnormal feather primordia (fpr).* **(G-I)** *A group of three skin explants, using equivalent left and right pieces of the same* Ottawa naked *embryo, cultured for 6 days on the chick chorioallantoic membrane (G) control left Ottawa naked explant, with a few feather filaments (f). (H) Heterogenetic recombinant of Ottawa (OT) dermis (D) and wild type (WT) epidermis. Formation of only two abnormal feather filaments. (I) The reverse recombinant of wild type dermis and Ottawa epidermis. Formation of several normally patterned feather filaments (Experiments and photographs by I. Olivera-Martinez).*

tions between the spontaneous chick scaleless mutant and wild type, first drew attention to the inability of the mutant epidermis to respond to dermal induction (Goetinck and Abbott, 1963; Sengel and Abbott, 1963). In fact the scaleless epidermis was not only unable to form a placode, but the main defect was its inability to transmit signals that are required for dermal organogenesis (Dhouailly and Sawyer, 1984).

Here we will present these pioneering contributions obtained by using heterotypic, heterogenetic and heterospecific dermal/epidermal recombinants, including the older and more recent results of our laboratory, which have allowed us to determine the origin of most of the different informative or permissive signals from one or the other of the two skin components. We will then discuss what are the molecular signals that could mediate these interactions from the results obtained by many different research groups, in particular from Dr. Chuong's laboratory, in order to highlight what remains to done. In particular, we want to draw attention to the understudied problem of how the cells maintain the memory of their developmental settings, for example how skin cells are able to maintain their ability to establish a micropattern even if this is disrupted.

When the dense dermis formation is perturbed: the chick *Ottawa naked* mutant

The Ottawa naked (OT) is an autosomal recessive mutation that has not been genetically characterized. Chicks are almost totally naked at hatching. Frequent webbing on toes II and III is associated with the naked skin condition. Moreover, embryos examined at 6 days have frequent neural tube abnormalities or even a total absence of the caudal region. The chicks rarely reach the adult stage. When they survive, the adults might develop a few down feathers but for the most part are totally naked. The eggs from heterozygotes were obtained from Dr. J. L. Pierro (Center for Environmental Health, University of Connecticut, Storrs). At E6.75 (stage HH 30) the dermis formation is very irregular in the dorsal region and, in most parts, the subectodermal mesen-

chyme remains loose (Fig. 1A). At E8.5 a few feather primordia sometimes occur, albeit delayed two days with respect to the wild type. At E10 (Fig. 1B), when a few feather buds are formed, their number, diameter, location in the dorsal field (macropattern) and their arrangement (micropattern) are very variable, but are almost symmetrical on each side of the middorsal line. A dense dermis is not present in most dorsal regions (Fig. 1 C,D), although it forms and subsequently gives rise to abnormally sized dermal condensations in some (Fig. 1 E,F). Heterogenetic dermal/epidermal recombinants were performed at stage HH 31 between these mutant (OT/OT) and wild type (WT/WT) embryos and then grafted for 6 days on the chick chorioallantoic membrane. The skin was dissected on each side of the middorsal line and for each mutant embryo, a group of three grafts was done: one control; one OT/OT dermis/WT/WT epidermis; and one WT/WT dermis/OT/OT epidermis. The results were consistent for each group of three (n=6) that survived. The controls and the recombinants involving an Ottawa naked dermis were featherless or poorly feathered. In addition, in those rare cases where a few feathers formed (Fig. 1 G,H), they were delayed by 2-3 days with respect to the recombinants involving a wild-type dermis. The recombinants involving a wild-type dermis produced large numbers of feathers (Fig. 1I), with the corresponding primordia differentiating rapidly, as soon as the day after the recombination.

The Ottawa naked defect thus affects the formation of a dense dermis, while the Ottawa naked epidermis functions normally. The formation of the dermis is however perturbed whatever the origin of the dermal progenitors, i.e. the neural crest (Couly and Le Douarin, 1988) for facial dermis, the dermomyotome (Mauger, 1972) for dorsal dermis, or the somatopleural mesoderm (Christ *et al.*, 1983) for ventral and limb skin. This is despite the fact that the molecular mechanisms which result in the specification of the dermal progenitors appear to differ in regions where it has been studied in detail, i.e. the back (Olivera-Martinez *et al.*, 2000, 2001, 2002 and 2004) and abdomen (Fliniaux *et al.*, 2004). Moreover, as the subectodermal mesenchyme formed, we can therefore surmise that only the densification of the dermis is affected. This implies that either the mesenchymal cells are impeded in their proliferation/migration close to the ectoderm, or that they do not receive specific signals from the ectoderm, or that the signals are not at a sufficient level. The answer might come from studies currently in process in our laboratory on the respective roles of the ectoderm and mesoderm in dermal densification in wild type embryos.

When the dense dermis is not redistributed to form dermal condensation: the chick *Scaleless* mutant

Scaleless is an autosomal recessive mutation that has not been genetically characterized, but has been the subject of several scientific studies since the sixties. Scaleless chicks have smooth skin largely free of down feathers and their tarsometatarsus and feet lack scales. Scattered feathers are present in the head, humeral, crural and caudal pterylae (Fig. 2A). The eggs from homozygous SC/SC mutants were obtained from J.L. Pierro (Center for Environmental Health, University of Connecticut, Storrs). Histological observation of embryos at stage HH 30 shows that the dorsal dermis formed normally and is composed of a dense dermis overlying a sparse dermis (Fig. 2B) (Viallet *et al.*, 1998). Thus, in scaleless embryos (Viallet *et al.*,1998; Dhouailly *et al.*, 1998; Widelitz *et al.,* 2000; M. Harris, personal communication), as in wild type em-

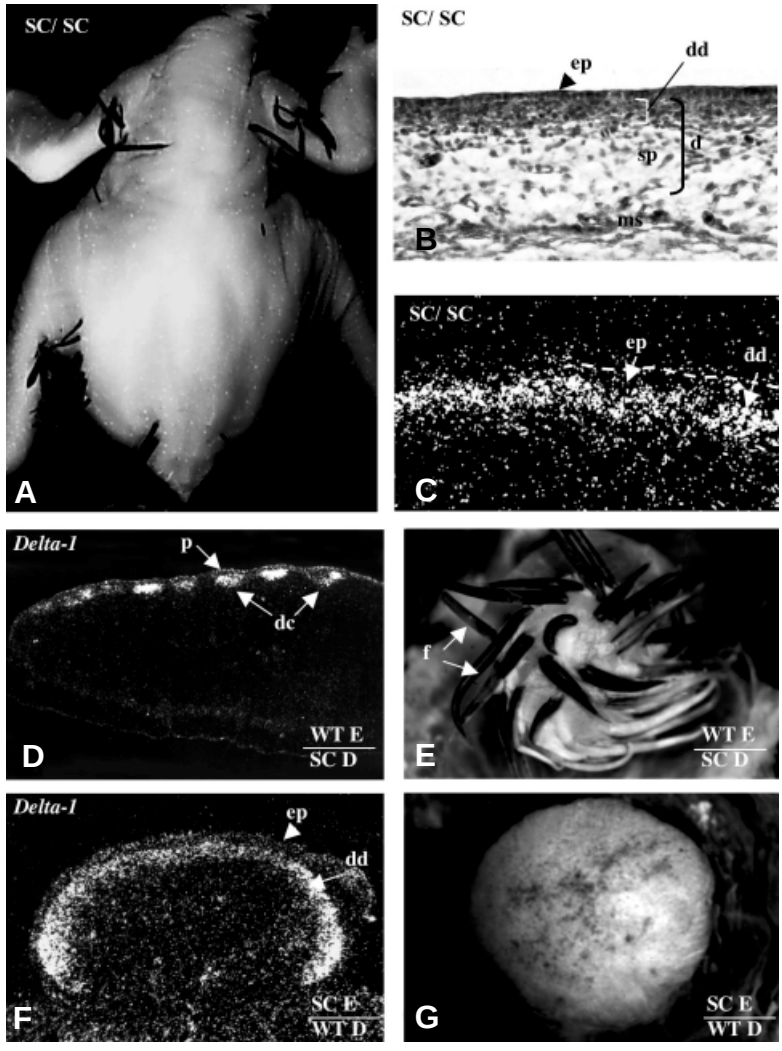

Fig. 2. The chick *scaleless* mutant: the formation of dermal condensations is affected due to a deficiency in epidermal signalling. (A) *Dorsal view at E20. Note the formation of feathers on the humeral, crural and caudal tracts. The spinal tract is entirely devoid of feathers.* **(B,C)** *At E7, the dorsal scaleless dermis reaches the stage of dense dermis (dd) formation (B) and this dense dermis homogeneously expresses* Delta-1 *transcripts.* **(D-G)** *Heterogenetic dermal/epidermal recombinants. After 36 hours, WT epidermis/ SC dermis recombination* **(D)** *leads to the restriction of* Delta-1 *expression to the fibroblasts forming the dermal condensation (dc) of the feather primordia and, after 6 days on the chorioallantoic membrane (E), to the differentiation of feather filaments (f). The converse recombinant of SC epidermis and WT dermis leads to a homogeneous distribution of* Delta-1 *transcripts in the superficial dense dermis (dd) after 36 hours* **(F)** *and after 6 days* **(G)** *to a bare explant. d, dermis; ep, epidermis; ms, muscles; p, placode; sp, spare dermis. (A,B) Photographed by I. Olivera-Martinez; (C-G) courtesy of Elsevier (Viallet et al., 1998).*

bryos (Wessells, 1965; see also review by Dhouailly *et al.*, 2004), the formation of a dermis in areas which correspond to the pterylae is characterized by an increase in the cellular density of the fibroblasts. In scaleless embryos, however, the next step does not occur. Feather formation involves the segregation of at least two types of dermal cells, via a redistribution of the cell population that forms the dense dermis (F. Michon, unpublished data). The first type will form the dermal condensation, will then be endowed with morphogenetic properties and participate in feather formation. The second type will form the ordinary sparse dermis underlying the inter-feather epidermis. DiI experiments (Jiang *et al.*, 1999) show that the two fates of dermal fibroblasts could still be reassigned at E8 (HH 33), i. e. when the dermal condensations have already formed. The Notch pathway is known to play a role in binary choices (among others: Artavanis-Tsakonas *et al.*, 1995; Simpson, 1997) and *in situ* hybridization of wild type chick embryos showed that *Delta-1* transcripts are heterogeneously distributed in the forming dorsal dermal condensations at E7/E8 (Viallet *et al.*, 1998). In contrast, in scaleless embryos of the same stages, *Delta-1* transcripts are homogeneously distributed in the dense dermis (Fig. 2C) (Viallet *et al.*, 1998). Moreover, *Delta-1* over-expression using a retroviral infection in wild type embryos led to formation of large, ectopic secondary apteria (Viallet *et al.*, 1998). Pioneering experiments at the beginning of the sixties (Goetinck and Abbott, 1963; Sengel and Abbott, 1963) showed that the scaleless defect is expressed by the epidermis while the scaleless dermis functions normally. More precisely, heterochronic heterogenetic recombinants demonstrate that the scaleless dermis is endowed with appendage-inducing abilities at an early stage and will rapidly lose them due to a lack of interaction with a wild type epidermis (Dhouailly and Sawyer, 1984; Song and Sawyer, 1996). Heterogenetic recombination of scaleless dermis with a wild type epidermis leads, after 36 hours, to the repatterning of *Delta-1* expression in the dermis (Fig. 2D), followed, after 6 days of culture on chorioallantoic membrane, by the emergence of feathers (Fig. 2E) (Viallet *et al.*, 1998). The converse recombinant of a dorsal scaleless epidermis with a wild type dermis leads to a homogeneous distribution of *Delta-1* transcripts in the superficial dermis after 36 hours (Fig. 2F) and, 6 days later, to a smooth explant (Fig. 2G) (Viallet *et al.*, 1998). The group of P. Goetinck (Song *et al.*, 1996) showed that the defect in scaleless embryos is a lack of expression of FGF4 by the epidermis. Their experiments were done *in vitro*, by adding beads overloaded with either FGF2 or 4 on scaleless chick embryo skin explants *in vitro*. They obtained abnormal feather buds which had an abnormal micropattern and some fusions. The same year, our group was engaged in a similar type of experiment, but the pieces of dorsal scaleless embryonic skin, overlaid with FGF2 beads, were grafted on the chick chorioallantoic membrane. Perfectly differentiated feathers appeared (Viallet *et al.*, 1998) and moreover, the feather buds arose sequentially in the vicinity of the loaded beads, suggesting that the beads do not replace an epidermal placode as suggested by Song *et al.* (1996), but instead gave and expanded a general permissive message to the dermis. The endogenous FGF4 signalling of the epidermis to the dermis in wild type embryos is thus a permissive signal, which might interact indirectly with *Delta-1* expression, to allow (Viallet *et al.*, 1998; Dhouailly *et al.*, 1998) the formation of dermal condensations in feather- or scale-forming regions. Dermal scale condensations, as well as the formation of placodes which precedes the organization of the dense dermis are clearly distinguishable in whole pieces of dermis and epidermis after their separation (Dhouailly, 1984). FGF4 does not constitute a “feather-message”, as suggested by a third group (Widelitz *et al.*, 1996). Whereas they obtained supernumerary feather formation in the sub-wing semi-apterium by using FGF2 beads, we did not in the midventral apterium (Dhouailly *et al.*, 1998). The difference between the upper dermis of a semi-apterium and that of an apterium is that the former normally forms a dense dermis, albeit with a delay, as well as a few randomly distributed feathers, while the latter remains loose and totally bare (Sengel *et al.*, 1969; Sengel, 1976). Moreover, FGF2 beads allow the scaleless foot skin to form scutate scales (Dhouailly *et al.*, 1998; Prin and Dhouailly, 2004).

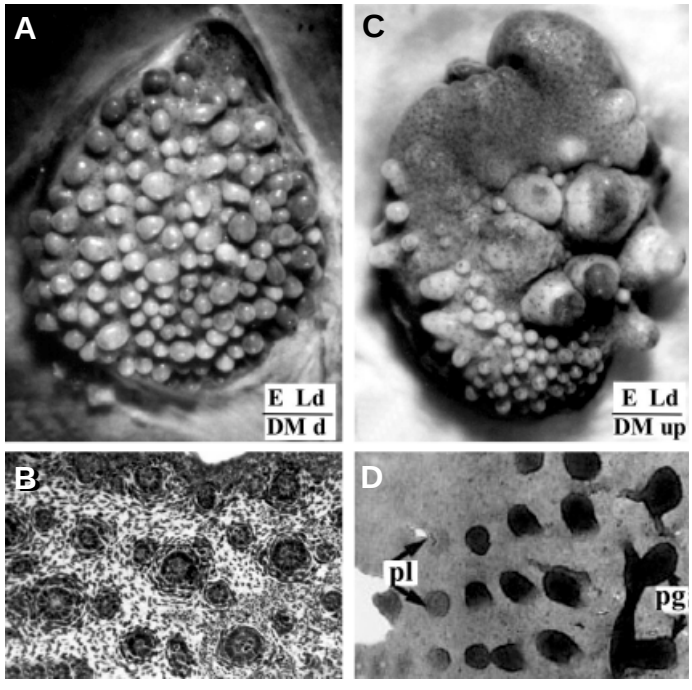

Fig. 3. Formation of scale buds in a hair-pelage or a hair-vibrissa pattern by a lizard embryo dorsal epidermis. (A) *With dorsal mouse dermis: formation of large and small scale buds corresponding to the central and lateral primary hair follicle pattern* **(B)**. **(C)** *With upper-lip mouse dermis: formation of large scale buds arranged in a whisker pattern* **(D)**, *surrounded by small scale buds corresponding to pelage hair pattern. The dermis is thus responsible for the pattern, while the epidermis responds according to its genetic potential (Dhouailly, 1975). pg, peg; pl, placode.*

The first dermal induction and the initiation/patterning of appendages

Heterospecific recombinations between dermis and epidermis were performed thirty years ago by using skin tissues from lizard, chick and mouse embryos, thus from the three different classes of amniotes (Dhouailly, 1973, 1975, 1977). They all yielded consistent results. In brief, class specificity of cutaneous appendages, i.e. the formation of scale- feather- or hair-type buds is epidermis dependant, whereas their initiation/ patterning and outlines are dermis dependant. The dermis, as it transmits its

initial triggering influence to initiate appendage morphogenesis in the overlying epidermis, also specifies their size and distribution pattern and the epidermis responds according to its genetic potential. These buds do not, however, give rise to mature appendages. For example, mouse pelage hair-forming dermis induces a large quantity of two size classes of scale buds in a lizard epidermis (Fig. 3A) and feather buds in a chick epidermis, corresponding to central large and lateral small primary follicles of the mouse pelage (Fig. 3B). Likewise, mouse vibrissa-forming dermis induces a small number of giant scale buds in a lizard epidermis (Fig. 3C) and giant feather buds in a chick epidermis, according to the mouse upper-lip pattern (Fig. 3D). A number of signaling factors have been shown to be expressed in the placode as well as in the dermal condensation. Bmp 2, 4 and 7 and follistatin (for a review: Chuong, 1998) are expressed in the feather primordia during pattern formation while gremlin is only expressed in the interbud dermis (Bardot *et al.*, 2004). The ectopic expression of Bmp2 and 4 leads to the inhibition of the feather primordia and, reciprocally, to the over-expression of noggin, follistatin and gremlin induce the formation of ectopic or enlarged feathers (Noramly and Morgan, 1998; Patel *et al.*, 1999). Interestingly all the feather activators (BMP antagonists, FGF, Shh) and most of the inhibitors (BMP2, 4) are expressed in the primordia. This has led to the proposal of a model based on diffusion of repressor/activator signals (Chuong, 1998).

The dynamical patterning of embryonic chick dorsal skin can be followed by detection of *BMP-2* transcripts (Noramly and Morgan, 1998) first in the nascent placode, then in the dermal condensation. The entire dorsal lumbar skin was recovered from stage HH 31 chick embryos and the explants were cultured *in vitro* and harvested after 8, 12 and 18 hours. At time 0 (HH31) the expression of *BMP-2* in the placode cells allows the identification of 5/7 distinct rows of feather primordia (Fig. 4A). After 8 hours of culture, *BMP-2* expression is difficult to detect (Fig. 4B) and is no longer detected after 10 hours, when there is a complete loss of the periodic patterning. After 12 hours, this patterning is re-established, with some modifications such as fusions that occur along the lines of chevrons (Fig. 4C). It should be noted than in dorsal pteryla, the micropattern of feather buds is grossly hexagonal, so that each feather is surrounded by six other feathers. Inter-feather distances are larger longitudinally than laterally, so that the feathers appear to be arranged in oblique transverse rows or chevrons. In the trunk, the chevrons open towards the tail (Sengel, 1976; Dhouailly *et al.*, 2004). After 18 hours, almost the entire skin explant is patterned *de novo* (Fig. 4D), albeit with some abnormalities such as lateral fusions and heterogeneity of the diameter of the feather primordia. These experiments (F. Michon, unpublished data) show that the skin has the ability to autonomously rebuild a new periodic pattern when this has been disrupted by the mechanical stress of dissection/ organotypic culture. It should be noted than in this experiment, the axial row reappears first. However, the presence of the skin corresponding to the initial primary row is not necessary for the re-patterning of the integument: when it is eliminated by excision, a new dominant row appears, lateral to the initial one (Novel, 1973). The appearance of epidermal placodes precedes the organization of the dense dermis into dermal condensations (Sengel and Rusaöuen, 1968; Dhouailly, 1984) and we can suggest than the first dermal induction initiates this placode formation, which in turn allows and

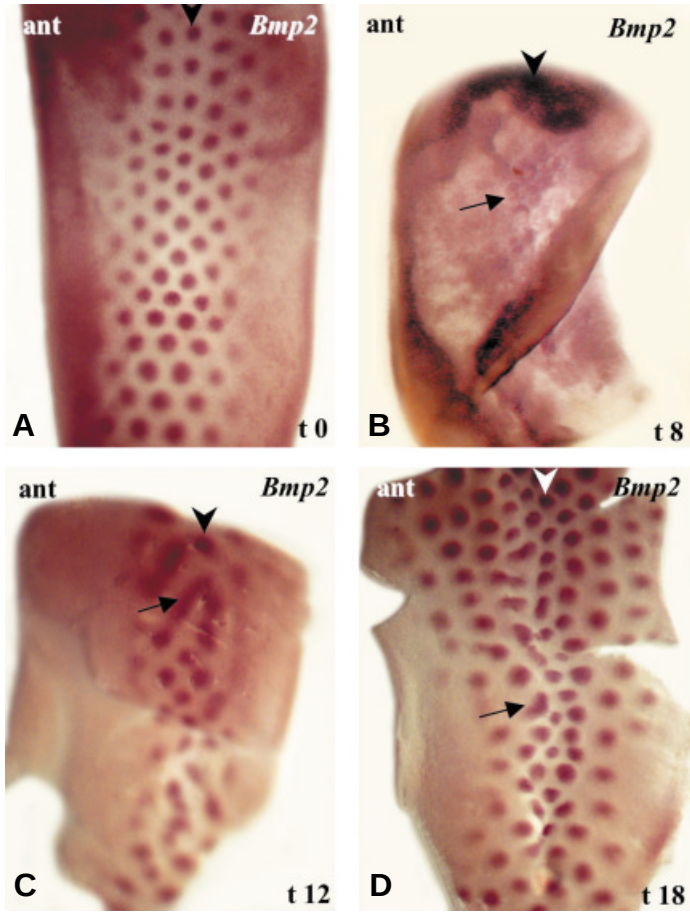

Fig. 4. Lability of the dorsal feather pattern in organotypic culture, as revealed by *in situ* hybridization with a BMP2 probe. *Forty pieces of dorsal lumbar pteryla were dissected at stage HH 31. Ten were immediately fixed and processed for hybridization, while the rest were cultured and fixed after 8, 12 or 18 hours.* **(A)** *At time 0, five to seven rows of feather placodes have formed.* **(B)** *After 8 hours, it is very difficult (arrow) to detect heterogeneity in BMP2 expression.* **(C)** *After 12 hours, feather formation has resumed and fusions occur laterally leading to well-defined chevrons (arrow).* **(D)** *After 18 hours, the skin explant is again entirely patterned, albeit with some abnormalities (arrow). The arrowhead indicates the dorsal midline, ant, anterior. Experiments and photographs by F. Michon.*

directs the cell migration in the dermis, leading to the redistribution of the fibroblasts from the dense dermis. As the first dermal messages, responsible for the initiation and patterning of appendages have remained understandable between the three classes of amniotes, it was proposed (Dhouailly, 1977) that they were mediated by the same type of molecules that are evolutionarily well-conserved. This was indeed the case as was shown several years later in the case of chick and mouse embryos (for a review: Chuong, 1998). That these signals belong to the Wnt family was recently confirmed via experiments involving the Wnt inhibitor Dickkopf-1 (Dkk-1). Dkk-1 is a soluble Wnt inhibitor that inhibits Wnt activation of the Frizzled receptor by binding the co-receptor low-density-lipoprotein-receptor-related protein 6 (LRP6) (Semenov *et al.*, 2001; He *et al.*, 2004). When dissociated chick dermal cells were transduced with RCAS-*Dkk-1* and then re-

aggregated and overlaid by an epidermis, feather formation is inhibited (Chang *et al.*, 2004). Dkk does not, however, differentiate between different Wnts and specific antagonists for each member of this large family do not exist yet.

The second dermal induction and feather organogenesis

The association of chick epidermis with hair-forming mouse dermis produces arrested feather buds, the epidermis of which forms ingrowing thickenings which can be recognized as abnormal barb ridges (Dhouailly, 1973). These hypomorphic barb-ridges are formed by stacking of cells corresponding to future barb and barbule cells. Nevertheless, although the cytodifferentiation of feather cells is independent from dermal signaling once feather formation has been triggered by the first dermal messages originating from the mouse dermis, the outgrowth and typical architectural organization of the feather filament did not occur.

In chick neoptile feathers the number of barbs varies from 11 to 16 and the rachis, formed by the fusion of the anterior barbs, is rudimentary. In contrast, in duck neoptile feathers, the number of barbs varies from 18 to 26 and the anterior rachis is well developed. Moreover, the teleoptile remiges appear soon after birth in chick but later in duck. The number of barb ridges in the embryonic feather filament, which corresponds to that of barbs in the neoptile feather; their partial fusion at the base of the feather to form a rachis; the timing of the formation of the second generation, the juvenile feathers and their shape, are species variable characters, which are all governed by the second dermal messages (Dhouailly, 1970). The experiments to show this consisted of heterospecific recombinations of wing bud ectodem and mesoderm between chick and duck, grafted on chick or duck hosts. The results were clear-cut. The morphological characters of the feathers were all determined by the dermis, except the number and shape of barbule cells, which conformed to the species origin of the epidermis (Fig. 5 A-C). Moreover, the time of renewal of the appendages was also dependent on the dermis (Fig. 5 E,F). The corresponding dermal/epidermal interactions must occur in the feather follicle, between the dermal papilla and the ring (wall) of undifferentiated epidermal cells. The later proliferate and subdivide into barb ridges, which fuse with the anterior part of the ring to give rise to the rachis, with additional barb ridges forming in the posterior part of the ring. Rachis formation, as well as the addition of new barb ridges, is more pronounced in case of the duck than chick neoptile feather and occurs through the entire outgrowth of the teleoptile feather in both species.

Several signaling factors are expressed in the developing feather, including BMP2, BMP4, Noggin and Shh (Yu *et al.*, 2002; and 2004). *BMP4* is expressed mainly in the dermal papilla, while Noggin is expressed in its posterior region, where barb ridges start to form. *Shh* is expressed in the internal sheath and is essential for inducing apoptosis and thus the splitting of barb ridges. The Chuong group, in a series of elegant experiments showed that, by adding RCAS viruses carrying these genes or dominant negative genes to the follicles of plucked teleoptile feathers, abnormal feathers were formed during regeneration. Over-expression of *Noggin*, a BMP antagonist, increases the barb number and even causes them to become split, while over-expression of *BMP2* and *BMP4* caused the formation of a giant rachis and barb fusions. Suppression of Shh leads to the forma-

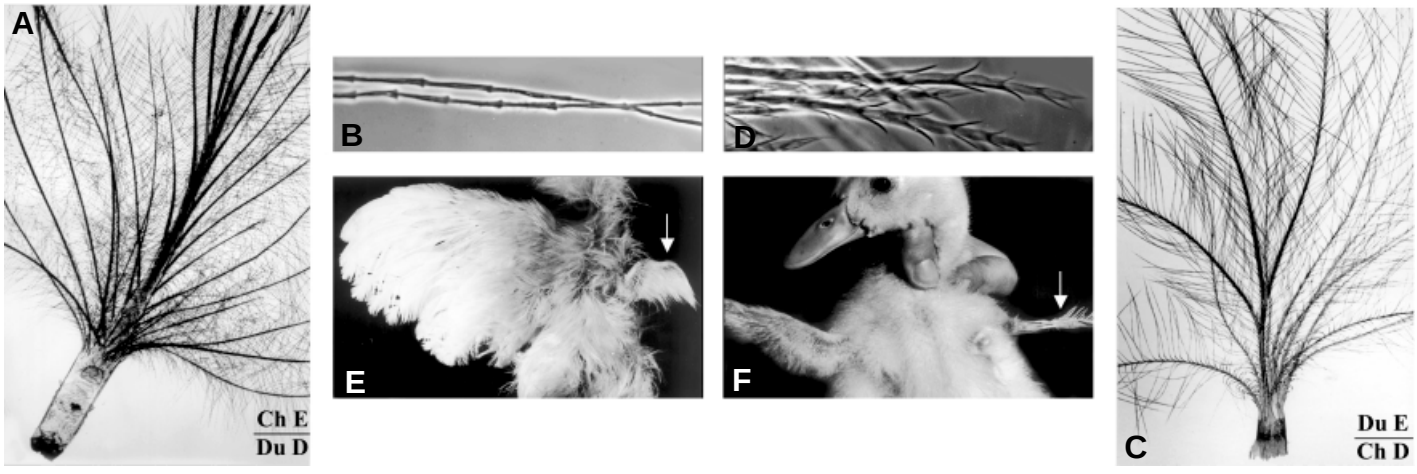

Fig. 5. Chimeric neoptile feathers produced by heterospecic duck/chick forelimb ectoderm/ mesodermal pulp recombinants. *The dermis is responsible for the feather architecture and the time of replacement of the neoptile by the teleoptile feather, while the shape of epidermal barbule cells is conferred by the epidermal species.* **(A)** *A typical duck-type neoptile feather obtained from the association of chick epidermis and duck dermis. Note the formation of 26 barbs, most of them being attached to a well developed rachis.* **(B)** *Detail of the barbules of the feather shown in (A), showing a typical chick-type morphology, i.e. a succession of cylindrical cells, slightly swollen at their distal tip.* **(C)** *Typical chick-type neoptile feather obtained from the association of chick dermis and duck epidermis. Note the formation of 12 barbs.* **(D)** *Detail of the barbules of the feather shown in (C), showing a typical duck-type morphology, i.e. a succession of cylindrical cells, with two spiny protrusions at their distal tip.* **(E)** *A chick host, two weeks old, bearing a chimeric right wing composed of a duck mesenchymal pulp associated with a chick ectoderm. The chimeric wing is perfectly developed, but is covered only with neoptile duck-type feathers, while the left host wing is covered by the second generation, the juvenile teleoptile feathers.* **(F)** *A duck host, two weeks old, bearing a chimeric right wing composed of a chick mesenchymal pulp associated to a duck ectoderm. The chimeric wing is in this case poorly developed, because the microsurgery was not perfect, but in the second generation, the juvenile teleoptile chick-type feathers had formed (Dhouailly, 1970).*

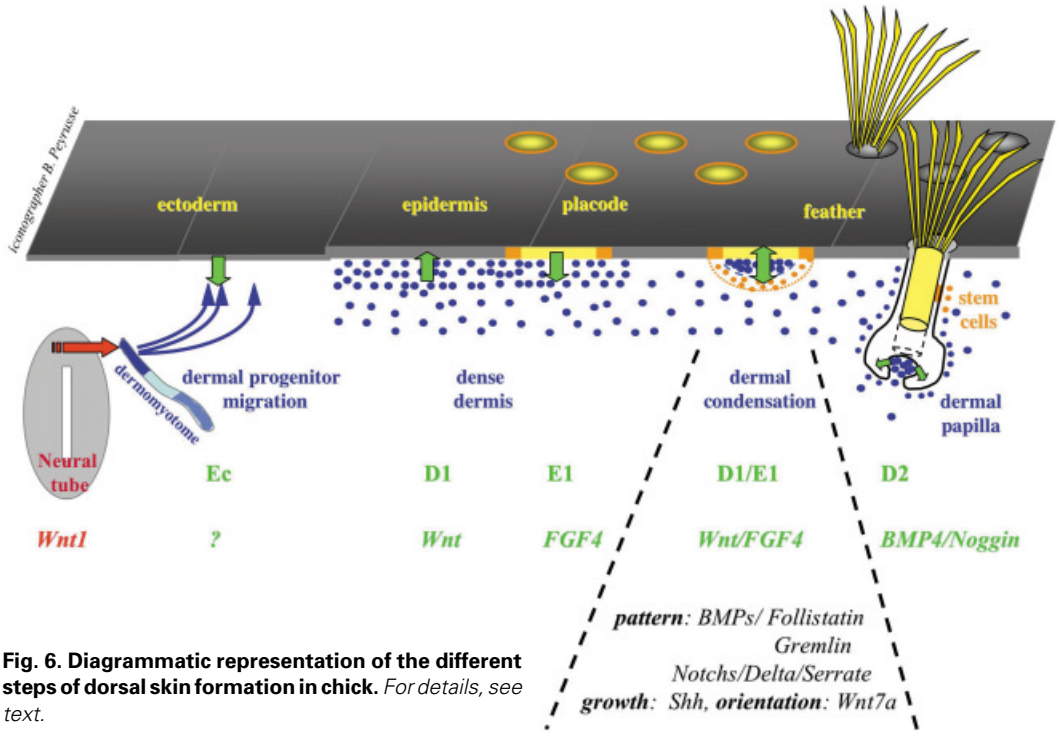

Fig. 6. Diagrammatic representation of the different steps of dorsal skin formation in chick. *For details, see text.*

tion of a remnant membrane between the barbs. It is thus very probable that a difference in the level of expression of *Noggin* and *BMP4/2* exists between the dermal papillae of the neoptile feather follicle in chick and duck, but this would be difficult to quantify.

Conclusion: the molecular events which mediate dermal/epidermal interactions

The molecular events underlying the cellular interactions during skin morphogenesis have thus been partially documented by several laboratories using expressions pattern studies and ectopic treatment using loaded beads or over-expression using retroviruses. Many of the recently described developmental signalling pathways have been implicated in one or all of these interactions (summarised in figure 6). Among these signals, the Wnt pathway is associated first in the formation of the dorsal dermis. Wnt1 from the dorsal neural tube has been shown to induce the specification of dermal progenitors from the dorsal dermomyotome (Olivera-Martinez *et al.*, 2001, 2002 and 2004). The dermal precursors express *Wnt 11*, which might be implicated in their migration to the subectodermal space (Olivera-Martinez *et al.*, 2002, 2004). Wnt 1, 3a and 5a in the feather primordia and Wnt 11 in the interbud dermis region control the shape of the feather bud while Wnt7a is involved in the anterior-posterior orientation during the outgrowth of the feather bud (Chang *et al.*, 2004; Widelitz *et al.*, 1999; Chuong *et al.*, 1996). At early stage the level of β-catenin is homogeneous throughout feather field epidermis and then is restricted to placodes (Widelitz *et al.*, 2000). We can thus propose that this general *β-catenin* expression is the response of the epidermis to a general Wnt message originating from the dermis. Then, this message appears to become restricted to the primordia in wild-type embryo, whereas it remains as a smear over the tract fields in the scaleless embryo (Widelitz *et al.*, 2000). During pattern formation nuclear β-catenin staining increases in the placode and is lost in the ectoderm that adopts interfollicular fate, in addition the forced expression of *β- catenin* induces the formation of ectopic feathers (Noramly *et al.*, 1999; Widelitz *et al.*, 2000). The restriction of *β-catenin* expression, as well as that of *Delta-1* expression might be a consequence of FGF4 expression in the epidermis (Viallet *et al.*, 1998; Song *et al.,* 1996). The Notch pathway may serve to stabilize the patterning of feather primordia (Viallet *et al.*, 1998).

The coupled BMP4/BMP-antagonist is first observed during feather field specification in the abdomen (Fliniaux and Viallet unpublished data). During pteryla formation transient *BMP2* expression is observed in the epidermis while the BMP antagonists gremlin and follistatin are expressed in the underlying dermis and the epidermis, respectively. (Noramly *et al.*, 1998; Bardot *et al.*, 2004; Patel *et al.*, 1999). When the patterning occurs, BMP2, 4 and 7 and the BMP antagonists excepted gremlin are expressed in the primordia. This observation leads to a model based on activation via differential diffusion of activators and inhibitors for the formation of the periodic patterning (Chuong, 1998). Later, at the step of feather organogenesis, the BMP/antagonist pair (in this case Noggin) is also involved in the patterning of the barb ridges (Yu *et al.*, 2002, 2004). What is the role of Shh? It is expressed at 2 days by the chick endoderm (Watanabe *et al.*, 1998) and could play a role in inhibiting *BMP4* expression in the somatopleure in the abdomen and thus in allowing feather field formation. At the early stage of pattern formation *Shh* is expressed in the placode while its receptor Ptc is expressed both in the placode and the dermal condensation (Ting-Berreth and Chuong, 1996; Jung *et al.*, 1998; Morgan *et al.,* 1998). In our point of view, Shh is involved in activating cell proliferation both in the dermis and epidermis. Forced expression of *Shh* (or Shh treatment) causes ectopic feather formation in pteryla, semi-apteria and even in the midventral apterium (Fliniaux *et al.,* 2004) and appears to be sufficient to enhance dermis density over a critical thresholds. In fact, *Shh* is strongly expressed in feather buds that elongate into feather filaments, is slightly expressed in overlapping scutate scales and is barely detectable and short lived in reticulate scales that did not overlap (Prin *et al.*, 2004). Inhibition of Shh signaling in chick (Prin and Dhouailly, 2004) can lead to feather growth arrest. Likewise, *Shh* knock-out mice (Chiang *et al.*, 1999) have arrested hair buds. Together all these results lead to the conclusion that Shh is a

general growth activator of cutaneous appendages. Moreover Shh also intervenes later, to allow apoptosis of the internal epidermal sheath and consequently the splitting of barb ridges during feather organogenesis (Yu *et al.*, 2002, 2004).

Thus, after more than thirty years of research, the cellular interactions and their mediators become clearer. However, the formation of a dense dermis and particularly the nature of the signal originating from the ectoderm, as well as the migration of dermal cells to from the dermal condensations deserve further research work. Moreover, we need to complete our preliminary diagram (Fig. 6), by adding which transcription factors and what type of intra-tissular interactions are triggered at each step. Only then we will obtain an overview of feather- and of course, hair-forming skin morphogenesis as complete as that already detailed for tooth organogenesis (for a review, see Pispa and Thesleff, 2003).

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