Growth factors and their role in wool growth: a review

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ABSTRACT

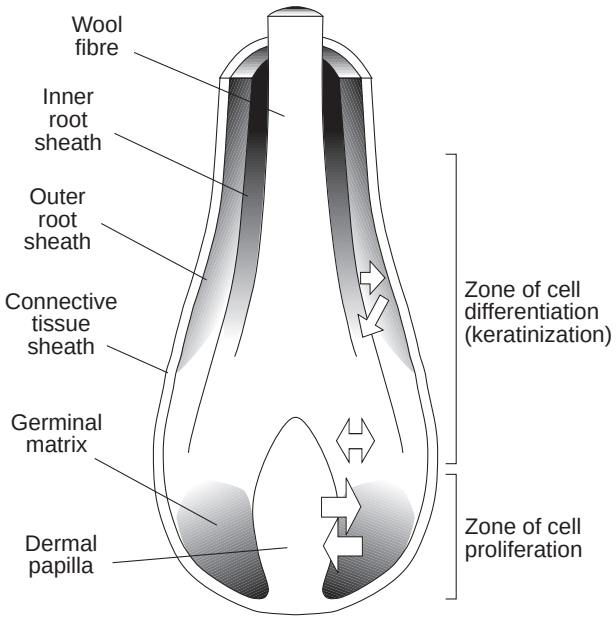
Wool follicles develop as a consequence of interactions between cells of the epidermis and dermis during intrauterine life. Fibre growth in mature follicles is believed to be governed predominantly by exchanges of signals between these cells and the matrices in which they are embedded. Over the last decade, increasing numbers of growth factors have been implicated in such communications. In particular, these include members of the epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), and transforming growth factor-beta (TGF-β) families. Their actions have been shown in studies of skin cells in culture, gene manipulation and physiological experiments with isolated follicles or whole animals. A picture is beginning to emerge of orchestrated morphogenic, mitogenic and differentiative influences between follicle cells, and between cells and their extracellular matrices. When combined with growing technological capability, increases in our understanding of cellular signalling will present novel opportunities for the industry to usefully alter wool growth and fibre characteristics.

Keywords: epidermal growth factor; fibroblast growth factor; hair cycle; insulin-like growth factor; sheep; transforming growth factor-beta; wool fibre; wool follicle.

INTRODUCTION

Fibre production begins at the base of the follicle with proliferation of the matrix cells. This activity is regulated by interactions with a dermal component of the follicle called the dermal papilla (Figure 1). The cells flow away from the follicle bulb and begin to synthesise the hard keratin proteins which are the main components of the fibre shaft. The activities of the follicle and the particular characteristics of the fibre produced are directly affected by a variety of local and systemic factors. The influences of circulating hormones and nutrients on follicle function have been dealt with elsewhere, and the effects on hair growth of a wide variety of local signalling molecules have been summarised (Hardy, 1992; Stenn *et al*., 1994; Paus, 1996). This review deals with effects in wool follicles of some better known growth factors. These are usually secreted proteins that function by binding to and activating cells via their cell surface receptors (Figure 2). Interactions are viewed as almost exclusively local, as growth factors from one cell may bind to receptors on itself or related cells (autocrine action) or to adjacent cells of a different origin (paracrine action). Many growth factors also bind to the extracellular matrix (ECM) consisting of fibrous proteins, glycoproteins and proteoglycans. Some growth factors influence ECM synthesis and degradation, and others are activated by ECM components. These types of interactions are important for the wool follicle cells that are embedded in ECM, or are separated by basement membranes.

FIGURE 1: Growth factor communication within and between cell populations of the wool follicle bulb. Single arrows show paracrine and the double arrow autocrine interactions referred to in the text.



Growth factor-mediated interactions have a two-fold impact on wool production. Firstly, they govern the development of skin. The patterns laid down during follicle morphogenesis in the foetus determine the density, size and form of follicles in the adult sheep. These, in turn, are principal determinants of wool production and fibre attributes, such as fineness (Moore *et al*., 1998). Secondly, growth factors modulate output of mature follicles. Cell proliferation and differentiation within the follicle bulb control fibre characteristics such as diameter variability and crimp. On a seasonal scale, wool production in some improved breeds varies by two to four fold and fibre diameter by two fold (Bigham *et al*., 1978). This is a remnant of the periodic coat replacement in ancestral sheep and is characteristic of mammals in general. Such hair cycles consist of phases of fibre growth (anagen) inter-

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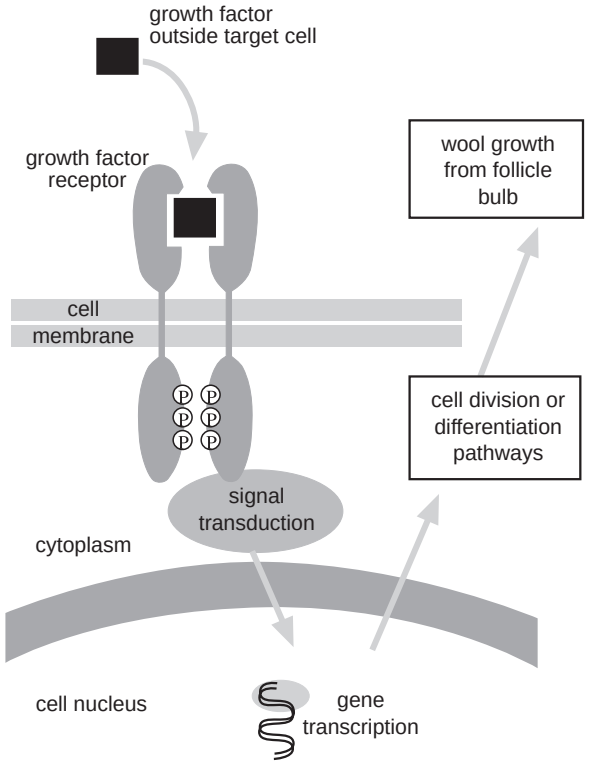
FIGURE 2: Generalised growth factor signalling events in a wool follicle cell. Secreted peptide growth factors bind to the extracellular domains of transmembrane receptors and form receptor dimers. The intracellular domains of many receptors (e.g. those for EGF, FGF and PDGF) can catalyse the transfer of phosphate groups to tyrosine residues of the receptors themselves or to other molecules. This triggers a cascade of signal transduction reactions leading to the activation of genes governing the cell cycle or differentiation pathways, or those encoding further signalling molecules. In wool follicle cells, this chain of events controls proliferation in the bulb, and keratinocyte differentiation, and thereby influencing the rate of growth and characteristics of the fibre.



spersed with follicle regression (catagen), quiescence (telogen) and re-initiation of growth (proanagen). This cyclic growth is regulated by complex intercellular communications and a variety of known peptide growth factors have been implicated (Messenger, 1993). It is highly probable that some key elements of such follicular control mechanisms are retained in sheep.

The evidence for involvement of particular growth factors in wool growth has come from comparing their localisation patterns with known biological activities, or physiological experiments with whole animals, isolated follicles or cells in culture. Further perspectives on the functions of growth factors in skin and hair follicles have been sought either by targeted deletion (knockout) of growth factor genes, or by over-expressing growth factor genes by transgenic procedures. Many of the mutant animals produced by these methods fail to thrive and some show no abnormal phenotype due to compensation by related factors, however others display interesting and revealing effects on development and growth of skin. We have listed peptide growth factors implicated in the functions of the wool and hair follicles (Table 1). Of these, four groups of structurally related molecules are explored in more detail, and we consider how their activities can be manipulated to improve wool production in sheep.

INSULIN-LIKE GROWTH FACTOR FAMILY

Insulin-like growth factors (IGFs) are single chain polypeptides of about 70 amino acids, which act both locally and as circulating hormones. They are ubiquitous in growing tissues and have been ascribed paracrine and autocrine roles as mitogens, morphogens, and differentiation and cell cycle progression factors (Jones and Clemmons, 1995). The role of hepatic IGF-I as a mediator of the anabolic effects of growth hormone is now well known. By contrast, the plasma concentration of IGF-II in adult sheep is about twice that of IGF-I (Hua *et al*., 1993), and its function is less well understood. IGFs and their receptors are expressed in a wide range of cell types, including those of skin and hair follicles (Nixon *et al*., 1997; Rudman *et al*., 1997) and, as in the blood, IGF-II is more highly expressed in sheep skin than IGF-I (Nixon *et al*., 1998) .

Receptors for IGF-I and IGF-II include the type 1 IGF receptor (IGFR-1) and insulin receptor. These are composite transmembrane tyrosine kinases. The type 2 IGF receptor (IGFR-2) is an unrelated molecule. It avidly binds IGF-II, but not IGF-I, and is identical to the mannose-6-phosphate receptor that is involved in lysozyme targeting. It is possible that IGFR-2 has no signalling capability, but can act as a "sink" to moderate IGF-II action (Francis *et al*., 1994). Both receptor types are expressed in wool follicles, and IGFR-2 is particularly abundant. Transient changes in the density of radio-labelled IGF binding have been shown to precede follicle regression, indicating that receptor regulation plays a role in controlling wool follicle growth (Nixon *et al*., 1997).

Keratinocyte proliferation is IGF-dependent both *in vitro* (Neely *et al*., 1991) and *in vivo* (Eming *et al*., 1996), and IGFs prolong the growth phase of hair follicles in culture (Philpott *et al*., 1994). The importance of IGFs in skin has been further demonstrated by hypoplasia of the epidermis and reduced follicle density in mice with null mutations for IGF-I and IGF-II (Liu *et al*., 1993). Conversely, mice over-expressing IGF-II via a keratin promoter (Ward *et al*., 1994) displayed skin hypertrophy. Moreover, increased growth rate and fleece weight have been reported in transgenic sheep carrying IGF-I genes under control of a keratin promoter (Damak *et al*., 1996).

IGF action and half life in the body is modified by high affinity binding proteins (IGFBPs) (Francis *et al*., 1994; Jones and Clemmons, 1995). Some IGFBPs bind IGFs in circulation (mainly IGFBP-3) and transport IGFs out of vasculature and localise them to target cells or ECM. IGFBP-3, -4 and -5 have been identified in skin (Batch *et al*., 1994) and the dermal papilla in particular (Batch *et al*., 1996), suggesting that they modulate IGF effects on fibre growth.

Thus, various elements of the IGF system appear to play a major part in the regulation of wool follicle growth and therefore fibre quality. However, the multiplicity of ligands, receptors and binding proteins, and possible involvement of circulating IGF make the elucidation of the

TABLE 1: Actions of selected growth factors in skin

Growth Factor	Receptor(s)	Action in wool follicle or skin	References
Epidermal growth factor (EGF)	EGFR	Cell proliferation inhibited in wool follicle but promoted in other skin cells by EGF infusion. A brief catagen-like follicle regression results. Used for biological wool harvesting.	Moore *et al*., 1985; Wynn *et al*., 1989; King *et al*., 1990; Nanney *et al*., 1990; Luetteke *et al*., 1994; Bond *et al*., 1996
Transforming growth factor-alpha (TGF-α)	EGFR	TGF-α, EGFR mutants show enlarged follicles and wavy fur.	Luetteke *et al*., 1993; Mann *et al*., 1993; Sutton *et al*., 1995; Nixon *et al*., 1996a
Insulin-like growth factor I (IGF-I)	IGFR-1 insulin receptor	Both local and systemic action. IGFBPs are expressed in hair follicles. IGF expression and receptor distribution varies with wool follicle cycle. Required for follicle growth in culture.	Batch *et al*., 1994; Little *et al*., 1994; Philpott *et al*., 1994; Sutton *et al*., 1995; Damak *et al*., 1996; Dicks *et al*., 1996; Nixon *et al*., 1996b
Insulin-like growth factor II (IGF-II)	M-6-P/IGFR-2 IGFR-1	IGF-II mRNA and receptor abundant in wool follicles	Ward *et al*., 1994; Nixon *et al*., 1997
Acidic fibroblast growth factor (FGF-1, aFGF)	FGFR-1 (4 variants)	Associated with differentiating keratinocytes of the wool follicle	du Cros *et al*., 1993; Bond *et al*., 1998
Basic fibroblast growth factor (FGF-2, bFGF)	FGFR-2 (4 variants)	Associated with basement membrane of wool follicle. Inhibits hair development in mouse.	du Cros, 1993; Bond *et al*., 1998
Fibroblast growth factor 5 (FGF-5)	FGFR-1 variant	Inactivation of gene prolongs anagen. Cycle dependent expression in peripheral follicle cells.	Hébert *et al*., 1994; Pethö-Schramm *et al*., 1996; Rosenquist and Martin, 1996
Keratinocyte growth factor (FGF-7, KGF)	FGFR-2 variant	Required for normal follicle development and hair growth. Dermal papilla to germinal matrix communication.	Guo *et al*., 1993; Danilenko *et al*., 1995; 1996
Hepatocyte growth factor (HGF)		Stimulates keratinocyte growth and motility. Expressed in dermal papilla.	Jindo *et al*., 1994; Shimaoka *et al*., 1994
Parathyroid hormone related protein (PTHrP)	PTHR	PTHR antagonists stimulate hair growth in the mouse	Holick *et al*., 1994; Whitfield *et al*., 1996; Schilli *et al*., 1997
Platelet derived growth factor (PDGF-B and PDGF-A)	PDGF-Rα PDGF-Rβ	Mitogenic. Induced by other growth factors. In connective tissue sheath of foetal mouse and human hair follicles	Orr-Urtreger and Lonai, 1992; Akiyama *et al*., 1996
Transforming growth factor-beta (TGF-β); three mammalian genes	TGFβR-I TGFβR-II variants	Follicle morphogens and inhibitors of cell proliferation. Inner- outer root sheath communication. Associated with follicle regression.	Blessing *et al*., 1993; Sutton *et al*., 1995; Paus *et al*., 1997; Thomas *et al*., 1997; Welker *et al*., 1997
Vascular endothelial growth factor (VEGF)		Autocrine action in human dermal papilla cells	Lachgar *et al*., 1996

regulatory mechanisms affected a challenging task. It is noteworthy that neither whole body nor skin patch infusion of IGF-I (Hocking Edwards *et al*., 1995) had a measurable effect on wool growth. Greater knowledge of the extent to which receptors and binding proteins govern IGF signalling in skin is required to interpret such results and is likely to provide fruitful avenues for altering wool growth.

EPIDERMAL GROWTH FACTOR FAMILY

Epidermal growth factor (EGF) was first described as a 53 amino acid peptide containing three disulphide bridges. Later, it was discovered that this peptide is cleaved from a larger precursor (Mroczkowski *et al*., 1989) and that EGF can be active not only as a small, diffusible effector but also as a pro-hormone when anchored to the cell membrane with the EGF sequence located in the extracellular domain.

EGF acts on the cell by binding to the EGF receptor (EGFR) lodged in the cell membrane as represented in Figure 2. EGFR is predominantly associated with epidermal cells of the skin throughout hair follicle development (Green and Couchman, 1984) and with mature follicles, sweat glands and sebaceous glands. Generally, receptors are sparse or absent in dermal fibroblasts *in vivo* and are

not present in the dermal papilla of the follicle (Wynn *et al*., 1989; 1995).

Skin and hair follicles also synthesise a number of other EGF-like factors, all of which bind to and activate EGFR. These include transforming growth factor alpha (TGF-α), heparin-binding EGF, betacellulin and amphiregulin (Pisansarakit *et al*., 1991; du Cros *et al*., 1992; Prigent and Lemoine, 1992). EGF and TGF-α are the best characterised of the family and share a close structural relationship. Interestingly, Sutton *et al*. (1995) were unable to detect EGF mRNA in sheep skin or kidney, whereas TGF-α was found in foetal and adult sheep skin, and localised to follicle epithelia (Nixon *et al*., 1996a).

EGF has a variety of morphogenetic and developmental actions in skin associated with the widespread distribution of EGFR (Wynn *et al*., 1989; Nanney *et al*., 1990; 1995). Mice injected with EGF showed reductions in body and hair growth rates (Moore *et al*., 1981). The inhibition of hair growth was related to a reduction in the size of the proliferating cell population of the follicle bulb and a decline in the rate of follicle development (Moore *et al*., 1983). At the end of the hair cycle, hairs were shorter and finer than those of littermate controls and were curved, giving the coat a waved appearance.

In sheep, the effect of EGF injection was more dramatic. There was a rapid, transient and synchronised regression of follicles, most entering a catagen-like phase (Hollis *et al*., 1983). This indication that EGFR action might have a role in the follicle cycle was supported when Murillas (1995) reported that entry of pelage hair into catagen was abolished in transgenic mice expressing a mutant form of the EGFR. Presumably, the inability of the mutant EGFR to transduce a signal was sufficient to block normal progression through the hair cycle.

Inactivation of the TGF-α gene in knockout mice (Luetteke *et al*., 1993; Mann *et al*., 1993) was non-lethal and the development of the mouse was substantially normal. The most striking effects were on the skin and hair; follicle development was abnormal and the coat wavy. These features are also characteristic of natural mutations of this pathway (Luetteke *et al*., 1994). Transgenic animals over-expressing TGF-α (Vassar and Fuchs, 1991), consistent with EGF treatments mentioned above, exhibited thickened epidermis and stunted hair growth.

Whilst the multiple roles of EGF in the skin continue to unfold, there have been some practical benefits for the producer. The observed effects of EGF on wool growth has led to the development of a radical approach to shearing called biological wool harvesting (Moore *et al*., 1982; 1985).

FIBROBLAST GROWTH FACTOR FAMILY

The fibroblast growth factors (FGF) are a large family of growth and differentiation factors (Basilico and Moscatelli, 1992), the two best known members being acidic FGF (FGF-1) and basic FGF (FGF-2). FGFs bind to heparin and can therefore act when attached to components of the ECM. High affinity receptors for FGFs (FGFRs) are encoded by four genes which can be alternatively spliced to give a range of receptor proteins. These possess up to three immunoglobulin-like extracellular domains, and intracellular tyrosine kinase domains (Coutts and Gallagher, 1995).

Although originally considered to act exclusively on cells of mesenchymal origin, FGFs are now recognised as potent mitogens for derivatives of the ectoderm and mesoderm. Cultured sheep skin cells, both keratinocytes and dermal fibroblasts, proliferate in response to FGFs, and an FGF-like peptide was found in immunoblots of ovine skin keratinocytes (Pisansarakit *et al*., 1990).

Basic FGF has been immunolocalised to the outer root sheath of the mature wool follicle. Its distribution was superficially similar to that of EGF, although it appeared to be associated more with the ECM between the outer root sheath and the dermis (du Cros *et al*., 1991; 1993). Its presence in the outer root sheath and more particularly, in the proliferative zone of the follicle bulb at maturity, may indicate a mitogenic function. If bound to the basal lamina in an active form, basic FGF could provide a source of a continuous proliferative stimulus to the bulb cells.

Acidic FGF was shown to be particularly concentrated in upper bulb cells displaying early signs of fibre differentiation (du Cros *et al*., 1993)and, like EGF, has localised regulatory functions specifically associated with its distribution. The distribution of acidic FGF is indicative of a differentiation rather than a proliferation factor. In this context it may be significant that EGF partially inhibits FGF-stimulated cell growth whilst FGF inhibits the binding of EGF to its receptor. The bulb cells have a number of fates, forming concentric sheath layers in the follicle. It is possible that differing concentrations of acidic FGF in these cells modulates their functions, directing them into these alternative differentiation pathways. Evidence for this proposition was recently obtained using isolated wool follicles in culture (Bond *et al*., 1998). Whilst FGF had no effect on fibre growth, 2D gel electrophoresis revealed that the patterns of proteins synthesised by the follicle differed from those cultured in the absence of the factor. By affecting the expression of particular keratin genes and thus the patterns of differentiation of the follicle, such growth factors may determine aspects of fibre quality.

Another member of the FGF family, FGF-7, also called keratinocyte growth factor (KGF) has been shown to be a key paracrine regulator of follicle development and fibre growth. KGF is synthesised within the dermal papilla, whilst its receptor, a splice variant of FGFR-2, is expressed by epithelial follicle cells, especially in the bulb (Danilenko *et al*., 1995; Rosenquist and Martin, 1996). Treatment with recombinant KGF stimulated hair growth in mice, whereas over-expression or receptor blockade inhibited follicle morphogenesis (Guo *et al*., 1993). KGF knockout mice were surprisingly normal except for their matted and greasy coats, analogous to the naturally occurring *rough* mutation (Guo *et al*., 1996). This phenotype indicates that while KGF is redundant for many mesenchymal/epidermal interactions, it is required for normal hair growth and has specific effects on the fibre shaft. KGF is also regulated during the wool follicle cycle in sheep,

although the expression pattern differs from that reported in mice (A.J. Nixon and C.A. Ford, unpublished).

The FGF-5 gene has also been shown to influence hair growth cycling. Expression of this growth factor was localised to the outer root sheath (Hébert *et al*., 1994), and increased during anagen and declined after catagen (Pethö-Schramm *et al*., 1996). Inactivation of FGF-5 by knockout technology produces a homologue of the *angora* mutation. These animals possess an exceptionally long coat resulting from prolonged growth (Hébert *et al*., 1994) and provide evidence that FGF-5 is one of the factors that trigger follicle regression.

In summary, communications are indicated from the dermal papilla to the surrounding matrix mediated by KGF, from outer root sheath by FGF-5, from inner root sheath and differentiating keratinocytes by acidic FGF and from around the basement membrane outer root sheath and matrix by basic FGF. These interactions were reiterated in an analysis of the expression patterns of nine FGF family ligands and their four receptors conducted by Rosenquist and Martin (1996). FGF family members are therefore involved in continuous and varied cellular crosstalk required for the maintenance and control of fibre growth (Figure 1).

TRANSFORMING GROWTH FACTOR-BETA SUPERFAMILY

Some other central players in hair follicle development and cycling belong to a large group of signalling molecules that share similarity with transforming growth factor-beta (TGF-β). In addition to three known mammalian TGF-β isoforms, this superfamily includes the bone morphogenetic proteins (BMPs) and inhibins/activins (Kingsley, 1994). TGF-βs are synthesised as a larger precursor that is cleaved to create a 112 amino acid growth factor moiety and a binding protein. When bound together, they form an inactive or "latent" complex (Massague, 1990).

Receptors for TGF-βs are transmembrane serine/threonine kinases. Signalling requires interaction of the growth factor with members of two classes of receptors, designated type I and type II (Wrana *et al*., 1994). Initial binding of the ligand is to a type II receptor and subsequent phosphorylation of the type I receptor activates intracellular signalling.

TGF-βs regulate cell growth and differentiation during development and remodelling processes in a wide range of tissues and organ systems, including skin and hair follicles (Pelton *et al*., 1991; Hogan, 1996). *In vivo*, they generally inhibit growth by suppressing epithelial mitosis, directing differentiation, or inducing apoptosis (Wrana *et al*., 1994). For example in skin, hyperproliferative disorders can result from loss of sensitivity to TGF-β inhibition. Paradoxically, TGF-βs can also stimulate fibroblast proliferation and chemotaxis, and induce ECM elaboration: key events in follicle development and function.

Members of the TGF-β superfamily are amongst the earliest signals detected during skin follicle development. BMP4 is transiently expressed in the mesenchyme, marking sites of incipient follicle formation in mouse whisker pads (Jones *et al*., 1991). BMP2 occurs in the epidermal placode as the follicle develops and continues in the germinal matrix of the mature anagen follicle (Lyons *et al*., 1990). TGF-β1, -2, and -3 have also been observed during the formation of epidermis and hair follicles in rodents (Lehnert and Akhurst, 1988; Pelton *et al*., 1991). Similarly in the skin of sheep, mRNAs for all three isoforms have been examined through foetal development and TGF-β1 in particular is induced immediately prior to wool follicle formation (Sutton *et al*., 1995). TGF-β1 was localised by immunocytochemistry in the developing epidermis and in the inner and outer root sheaths of primary, but not secondary, wool follicles (Thomas *et al*., 1997). Although the individual roles of the TGF-βs are not yet clear, it is evident that they participate in multiple autocrine and paracrine signalling events throughout follicle morphogenesis. Their receptors, type II in particular, are restricted to follicle matrix and outer root sheath indicating that these cells are the main targets of TGF-βs (Paus *et al*., 1997). The focal appearance of both receptor types before placode formation also suggests that TGF-βs are follicle morphogens.

Transgenic over-expression of BMPs in the outer root sheath (Blessing *et al*., 1993) and of TGF-β1 in the epidermis (Sellheyer *et al*., 1993) resulted in shutdown of epithelial cell proliferation and marked impairment of follicle formation. Together with the localisation patterns, these responses corroborate both the inhibitory and the morphogenic properties of BMPs and TGF-βs in developing skin and follicles.

There are strong indications that TGF-βs are equally important in hair growth cycling, particularly at the onset of catagen. Two recent studies of TGF-β expression through a murine hair cycle showed highest mRNA concentrations immediately before or through catagen (Seiberg *et al*., 1995; Welker *et al*., 1997). Similar cycle-dependent variations in receptors for TGF-β are shown by immunohistochemistry (Paus *et al*., 1997). These patterns of changes accord with the proposed regulatory functions of TGF-β in controlling cell proliferation in the follicle through an inhibitory feedback mechanism. It is tempting to speculate that induction of similar, but attenuated, effects of TGF-βs in the sheep are involved in modulating wool growth.

DEVELOPMENT OF APPLICATIONS FOR WOOL PRODUCTION

The examples from these four growth factor families illustrate our state of knowledge of some intercellular communication pathways used by the follicle. It is clear that the signalling molecules involved are numerous, although some might play a permissive or secondary rather than a regulatory role. There is also much evidence of interactions between growth factor pathways (eg. (Krane *et al*., 1991). Pieces of the puzzle are thus rapidly accumulating, but it is as yet unclear how they fit together to form an overall picture of the follicular growth mechanism. By participating in this task, wool biologists have the opportunity to capitalise on advances made in the wider field and

develop aspects which can be applied within the wool industry.

The first step in utilising growth factors to alter fibre production is to confirm expression of the candidate molecule in the wool follicle and conduct experiments to test its proposed regulatory roles and interactions. Test systems in which either follicle activities are exaggerated, such as synchronised cycles of shedding sheep (Nixon *et al*., 1997), or relationships simplified, as in follicle cell culture (Pisansarakit *et al*., 1991), are useful tools for determining growth factor functions. Once actions on fibre growth are established in these models, it may be possible to resolve the more subtle control in continuously growing fleeces of sheep in commercial flocks.

In addition to the candidate approach, wool growth controlling genes can be isolated on the basis of their differential expression under altered states of fibre growth (Rufaut *et al*., 1997). Such methods represent a broad screening of genes involved in modulated wool growth, and permit an open-ended search for novel molecules or pathways. Identified growth-controlling genes can likewise be examined in production stock.

Some distinctive and potentially useful associations of growth factor actions with fibre traits are beginning to emerge. For example, FGF-5 and TGF-β have been identified as markers of catagen. If they inhibit follicle growth, they represent avenues for ameliorating variable fibre tenacity, perhaps by uncoupling parts of the follicle cycle machinery. Fibre characteristics can be influenced by EGF and FGF action. Fibre curvature may also be related to factors which generate asymmetric patterns of keratinocyte production in the follicle bulb (Nixon *et al*., 1996a). If convenient means of modifying these biological processes were devised, they might allow short term adjustments to be made to fibre characteristics within a season. Thereby, wool growers could respond more rapidly to changes in market demand without having to alter the genetic base of their flock.

Possible routes for technology developments utilising growth factor action include pharmacology, transgenics, or genetic selection. The most prominent example of the pharmaceutical approach is use of EGF for defleecing or biological wool harvesting (Moore, 1985). Transitory wool follicle shut down in response to a single dose of EGF produces a synchronised interruption in wool growth that allows the whole fleece to be readily peeled from the animal. The advance has been assisted by developments in recombinant DNA technology which permit the synthesis of large quantities of EGF-like proteins (Allen *et al*., 1987). The procedure has value-adding features, including uniform wool fibre length and absence of second cuts (Panaretto *et al*., 1989). A drawback of biological wool harvesting is the increased incidence of abortion in ewes. Furthermore, at dose rates required to overcome variable responses, a net is required to hold the fleece in place during a 4-6 week period of post-treatment regrowth before harvest.

Growth factor signalling can also be perturbed by immunological means. Vaccination with peptide fragments such as used to alter reproductive performance might be applied to growth factor control of wool growth. Similarly, antibodies directed at either hormone or receptor, can have either stimulatory or inhibitory effects. For example, the enhancement of IGF effects on somatic growth with anti-IGF-1 (Hill and Pell, 1998) indicate that passive immunisation might be applied to growth factors active in skin.

The existence of mutations with fibre growth effects, such as *angora*, *waved*, and *rough* encourage the view that other growth factor related polymorphisms might exist which could be exploited by traditional or marker assisted selection. Permanent genetic improvements can also be achieved by the transgenic approach. Transfer of recombinant DNA is currently being pursued in sheep to improve wool production (Ward and Nancarrow, 1992). The first sheep carrying growth factor transgene constructs have demonstrated the technical feasibility of this approach (Damak *et al*., 1996). Refinements to the timing and the site of ectopic expression, in particular with follicle bulb specific promoters, could provide greater advantage. The identification of early keratinocyte differentiation markers by survey of differential expression could make a useful contribution in this regard.

Initial steps with growth factor technologies have highlighted some obstacles. The general growth and development effects of known growth factors necessitate treatments directed specifically at wool follicles or even particular cell populations if undesirable consequences elsewhere in the animal or to its foetus are to be avoided. A key element in the success of future applications is therefore likely to be the development of technologies for directing gene expression or targeting molecules with a higher degree of precision.

CONCLUSION

In the past few years, we have seen the continued discovery of growth factors that operate in skin follicles and an increasing appreciation of their co-ordinated roles in skin development and growth cycle regulation. The examples explored in this paper illustrate interactions between and within all major cell compartments of the follicle, and influencing all aspects of cell growth and differentiation. This represents, in principle, a variety of effects on the fibre.

To date, the molecular mechanisms shown to control fibre growth have been general developmental and growth regulatory pathways. They are common not just to different tissues within an animal but to diverse species, including invertebrates. Thus, the agricultural scientist can gain much from advances made with other species, in other fields, or with varied organ systems. However, this common biochemistry also presents the challenge of achieving specific effects on wool follicle cells whilst avoiding unwanted side effects in other tissues.

As the molecular basis of fibre growth unfolds and more refined genetic or physiological tools become available, new opportunities will arise to modify follicular growth by affecting growth factors, their binding proteins, receptors, or intracellular signalling reactions. Such tech-

nologies have potential to provide quantum leaps that are independent of the genetic variation extant in sheep flocks. The aim is to achieve the level of consistent control over a naturally variable growth process and thus equip producers to meet market requirements for high quality products.

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