Animal and *in vitro* Models for the Study of Hair Follicles

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Since the way in which the hair follicle functions is not well understood, many hair disorders are poorly controlled. A range of *in vitro* and *in vivo* models have therefore been developed to investigate the cell biological and biochemical mechanisms involved in the organization of this complex tissue. These range from cultures of a single cell type, such as those of the regulatory, mesenchyme-derived dermal papilla, through organ culture of isolated follicles to natural or genetically manipulated animal models. Each system has advantages and disadvantages for studying particular aspects of follicular function and some are potentially useful for the development of novel treatments for hair disorders. ***Key words: human/mouse/genetically engineered mice/dermal papilla cells/organ culture. JID Symposium Proceedings 8:39–45, 2003***

Human hair growth differs from that of most other mammals in its greatly reduced thermoregulatory role and the much more dramatic effects of androgens with the follicle response varying according to body site. Androgens stimulate much body hair growth, e.g., beard and axillary hair, while frequently inhibiting scalp hair growth causing balding. In addition, many other mammals have synchronized growth cycles which produce waves of new hair growth passing over the body unlike the generally mosaic human pattern. These differences mean that it is important to synthesize information from the various models carefully. The advent of molecular biological methods in combination with the wide range of models now available should facilitate greater understanding of how hair follicles function in health and disease.

WHY IS THERE A NEED FOR MODELS TO STUDY HAIR GROWTH?

How the human hair follicle functions in health and disease is not fully understood. This is why many hair disorders are poorly controlled. The mammalian hair follicle is a complex organ with a complicated structure and function. It involves several cell types from various embryological origins, e.g. keratinocytes, melanocytes and specialized fibroblasts known as dermal (follicular) papilla cells. These form the highly organized, multilayered hair follicle which produces the hair (reviewed in Camacho, Randall & Price, 2000). Follicles may alter the type of hair they produce allowing them to co-ordinate with climate changes, e.g., the different winter and summer coats of many arctic mammals or stages in an individual's development, e.g., the tiny almost colorless facial hair of a child which is replaced by the dark, long and thick hairs of a man's beard. To do this they pass through cycles involving phases of active growth (anagen), regression (catagen) and rest (telogen) (Dry, 1926; Kligman, 1959). When a new anagen begins, a new lower follicle is regenerated, apparently recapitulating the later stages of follicular embryogenesis; the new hair grows up through the skin and the old hair is lost. Currently the biochemical mechanisms involved in these processes are not characterized, although this area is currently the focus of much research (see Camacho, Randall and Price, 2000).

Human hair follicles are even less well understood than those of other mammals because human follicles mainly function independently of their neighbors, unlike the waves of co-ordinated growth cycles in many other mammals (discussed in Ebling *et al*, 1991). Nevertheless, there is some seasonal co-ordination of human hair follicle activity in many parts of the body (Orentreich, 1969; Randall and Ebling, 1991). The other main difference between human beings and many other mammals routinely available to study is the major role of androgens in determining the types of hairs produced in many parts of the body both in health and disease. Androgens normally stimulate the production of terminal hairs in many areas of the body after puberty, e.g., the beard of men and the axillary regions in both sexes (Hamilton, 1958) while appearing to have no effect on other areas such as the eyelashes and nonbalding areas of the scalp, e.g., the occipital regions. However, in genetically predisposed individuals, androgens cause a gradual transformation to vellus follicles on the scalp producing a slowly progressing, patterned balding, androgenetic alopecia (Hamilton, 1951; Ludwig, 1977; Randall, 2001). Androgen-dependent conditions such as hirsutism, excessive hair growth in women in a male type pattern, and androgenetic alopecia in men and women, have a negative effect on a person's quality of life (reviewed in Randall, 2001), probably due to the important role played by human hair in social and sexual communication. This also applies to other hair loss conditions, e.g., alopecia areata.

Human hair follicles should be the best tissue to investigate human mechanisms. However, it is not ethically possible to carry out much research in people unless it is noninvasive observations, e.g., the study of seasonal changes (Orentreich, 1969; Randall and Ebling, 1991) or a clinical trial of a new therapeutic regimen. Limited amounts of tissue are available for *in vitro* studies, but this is frequently a surgical by-product and may not be appropriate to answer particular questions such as how androgens act in follicles from different sites. Unfortunately, as well as ethical constraints, there are greater difficulties when considering animal models for

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human hair follicle function than the subtle species differences seen in many other organs. This is because human beings are the "naked apes"; our hair no longer has a major thermoregulatory role, the prime function of most mammalian hair, and androgens have a much greater effect with the body site of a follicle determining what type of response the follicle will exhibit. This means that an extensive variety of *in vitro* and *in vivo* models have been developed to study hair growth which offer the possibilities of investigating specific aspects of hair follicle function.

OVERVIEW OF MODELS FOR STUDYING HAIR FOLLICLE FUNCTION

Current model systems range from cultures of specific cell types isolated from human follicles to normal and genetically modified mammals. The *in vitro* models generally offer the best opportunity to investigate human follicles since isolated cells, follicles or skin samples can be can be readily cultured from small samples of human skin. Primary cultures of various individual cell types have been studied. These include: dermal papilla cells (Jahoda *et al*, 1984; Messenger, 1984), outer root sheath cells (Limat *et al*, 1993), connective tissue sheath cells (Reynolds *et al*, 1999) and more specialized cell types, such as rodent germinative epithelial cells (Jahoda and Reynolds, 1996). These allow assessment of the features of individual cell types with, or without, manipulation *in vitro*. They also enable investigation of the interactions of the various cell types *in vitro* or re-implanting the cells *in vivo* in rats, nude mice or even people. Such studies have facilitated the identification of the inductive roles of dermal papilla cells, studies of mesenchyme–epithelial interactions (Jahoda *et al*, 1984; Jahoda and Reynolds, 1996) and the recent demonstration that human connective tissue sheath can take over the inductive roles of the dermal papilla (Reynolds *et al*, 1999). One serious disadvantage of these cultured cells is the short-lived nature of primary cultures, although different methods have improved this recently in some cell types (Matsuzaki and Yoshizato, 1998). Another is that the cells in culture are studied without being subject to the normal cellular interactions, physical constraints or their natural environmental supplies of nutritive and regulatory factors.

Some of these disadvantages are overcome by organ culture of isolated follicles (Kondo *et al*, 1990; Philpott *et al*, 1990) which involve more normal cellular interactions between many cell types and can synthesize new hair *in vitro*, while still retaining the ability to be manipulated *in vitro*. In addition to human studies follicles have now been successfully cultured from neonatal rodent vibrissae (whisker) (Buhl *et al*, 1989), red deer (Thornton *et al*, 1996a) and goat skin (Ibraheem *et al*, 1993). However, these are still short-lived, not subject to the normal physical constraints and it has not been possible so far to manipulate human follicles to cycle fully (discussed below). Culture of whole skin biopsies theoretically overcomes the limitations on cellular interactions and physical constraints, but there are difficulties in ensuring penetration of nutrients, etc. Skin samples supported on gel-foam have been notably used for investigating liposome delivery of genes to the follicle (Hoffman and Li, 1995).

The use of animal *in vivo* models overcomes many of these disadvantages since the follicles are in their natural physiological environment and undergo normal cyclic activity. Although conditions can be manipulated *in vivo* in these models within ethical constraints, there may be species differences in follicular function and there are only a limited number of androgen-sensitive models. A wide range of mammals have been studied in natural conditions or in the laboratory (reviewed in Ebling *et al*, 1991). Using the Wistar laboratory rat, John Ebling, Elizabeth Johnson and colleagues carried out a series of significant studies into many aspects of growth waves and their hormonal control (reviewed in Ebling *et al*, 1991). Another popular model is the black mouse, C57BL/6, whose skin lacks pigment enabling the pigmented tips of newly growing anagen hairs to be observed at an early stage in shaved skin (Chase *et al*, 1951; Paus *et al*, 1989). Unfortunately, mice have the significant drawback of patchy growth once the second wave of hair growth has been completed when the animals are still young.

Although useful for studying normal hair cycling, these mammals are not suitable for investigating androgen action. Nude ($Foxn1^{nu}/Foxn1^{nu}$), severe combined immunodeficiency ($Prkdc^{scid}/Prkdc^{scid}$) and recombinase activating gene ($Rag1^{-/-}$) mutant mice have T cell deficiencies or T and B cell deficiencies making them unable to reject foreign skin. Xenografts have been used to accept human skin grafts (Sundberg *et al*, 1999; Sundberg and King, 2001). Although difficult to work with, since they need special aseptic environments, these mice are contributing greatly to our understanding of alopecia areata as an autoimmune disease (Gilhar *et al*, 1999) and are also useful for androgenetic alopecia studies (Van Neste and de Brouwer, 2000). Specialized androgen-sensitive follicles have also been studied including those of the hamster costovertebral gland, a secondary sexual tissue involved in social communication (Adachi and Kano, 1972). The androgen-dependent manes of lions (*Panthera leo*) and red deer (*Cervus elaphus*) offer the most likely mammalian parallels for the human male's beard. Since red deer are killed for food, their hair growth has been investigated *in vitro* by Val Randall and colleagues; they have used both organ culture (Thomas *et al*, 1994; Thornton *et al*, 1996a) and dermal papilla cells from sites with different hormonal sensitivities (Thornton *et al*, 1996b; Thornton *et al*, 2001). The large number of follicles available from one individual makes these a useful model. Androgen-dependent loss of hair in both sexes of the androchronogenetic alopecia mouse (Matias *et al*, 1989›) and, particularly, the stump-tailed macaque (Uno *et al*, 2000) have also been harnessed to investigate novel treatments for androgenetic alopecia.

Genetic abnormalities, either naturally occurring or manipulated, also contribute to our understanding of hair follicle function. Human mutations, such as the various androgen insensitivity syndromes, have demonstrated the essential nature of functional androgen receptors for all secondary sexual hair growth and male androgenetic alopecia; they have also revealed the importance of 5α-reductase type 2 in many follicles such as the beard, but not axillary and female pattern pubic hair follicles (reviewed Randall, 2000). In addition, various rodents with naturally occurring alopecia areata are contributing to our understanding of the pathogenesis of alopecia areata (reviewed in McElwee, 2002). The advent of molecular biological techniques has allowed the manipulation of specific genes in a range of ways. These include: random or specific overexpression (transgenesis), partial or complete inactivation (dominant negative transgenesis, targeted mutagenesis commonly called "knockouts"), or conditional mutagenesis (various methods to selectively activate or inactivate a gene). These will be discussed below. Initially these involved a total gene defect throughout the organism, which made the roles in specific tissues hard to interpret because direct and secondary effects of the gene manipulation could not be distinguished. Now these can be linked to specific gene promoters so that the manipulation is only expressed in specific tissues, e.g., the keratin 14 promoter is useful for hair follicle studies. At the moment we have access to an exciting range of model systems; three of the most useful will be discussed in more detail below.

DERMAL PAPILLA CELLS FROM FOLLICLES WITH VARIOUS RESPONSES TO ANDROGENS *IN VIVO* RETAIN DIFFERENCES IN CULTURE

The exciting demonstration that cultured rat vibrissa dermal papilla cells retained the ability to stimulate the production of a vibrissa follicle when re-implanted in a rat *in vivo* (Jahoda *et al*, 1984) provoked a great interest in the use of cultured dermal papilla cells. One of the main aims of these studies has been to investigate their role in mesenchyme–epithelial interactions, particularly in recombination experiments with other cell types both *in vitro*

and *in vivo* (Jahoda and Reynolds, 1996). Another major theme has been the examination of androgen action in the hair follicle; this is based on the hypothesis that androgens act on the epithelial and melanocyte components of the hair follicle indirectly by altering the production of regulatory factors by dermal papilla cells (Randall *et al*, 1991; Randall, 1994). These factors could be soluble paracrine factors or components of the expansive extracellular matrix in which the relatively small numbers of dermal papilla cells are embedded. Cultured dermal papilla cells do secrete soluble factors (Itami *et al*, 1995; Thornton *et al*, 1998) and extracellular matrix components (Messenger *et al*, 1991).

Investigations into androgen action using cells derived from follicles with varying responses to androgens *in vivo* have supported the hypothesis that androgens act on the hair follicle via the dermal papilla. Earlier studies focussed on sites stimulated by androgens *in vivo* including beard, pubis and axillary follicles and compared them with relatively androgen-insensitive, nonbalding scalp cells as a control. More recently cells from the smaller follicles from androgenetic alopecia scalp have been cultured and compared with nonbalding scalp cells (Randall, Hibberts and Hamada, 1996).

Cells from follicles derived from androgen-sensitive sites, including human beard (Randall *et al*, 1992) and balding scalp (Hibberts *et al*, 1998) and red deer mane (Thornton *et al*, 2001) follicles, contained low capacity, high affinity androgen receptors at higher concentrations than those from nonbalding follicles. There were no differences in the binding affinity of the receptors from the different sites for a range of androgenic and other steroids. Studies of the ability of dermal papilla cells to metabolize testosterone intracellularly to its biologically more active form, 5α-dihydrotestosterone, have shown that this capacity reflects the limited beard growth, but strong axillary and female pattern pubic hair growth, in patients suffering from 5α-reductase deficiency (Wilson *et al*, 1993; reviewed in Randall, 2000). Beard dermal papilla cells readily form 5α-dihydrotestosterone (Itami *et al*, 1991; Thornton *et al*, 1993) unlike nonbalding scalp or pubic and axillary follicles (Hamada *et al*, 1996). This marked parallel with hair growth in patients lacking 5α-reductase type 2 provides strong support for the hypothesis that androgens in the follicle act via the dermal papilla; it also supports the usefulness of dermal papilla cells to study androgen action since their gene expression *in vitro* appears to reflect that *in vivo*.

Factors produced by dermal papilla cells from various sites have been examined, often using conditioned media, i.e., media in which dermal papilla cells have been grown and into which they have released their products. Conditioned media has stimulated the growth of other dermal papilla cells (Thornton *et al*, 1998), hair follicle outer root sheath cells (Limat *et al*, 1993) and epidermal keratinocytes (Hibberts and Randall, 1996). Importantly, the addition of testosterone to the dermal papilla cells increased the mitogenic capacity of beard cell media (Itami *et al*, 1995; Thornton *et al*, 1998), but decreased that from balding scalp from both men (Hibberts and Randall, 1996) and the macaque (Obana *et al*, 1997) This again reflects *in vitro* what would be predicted from observations of hair growth *in vivo*. The current emphasis of research (reviewed in Randall *et al*, 2001) is in identifying factors produced differently by dermal papilla cells in response to testosterone, such as insulin-like growth factor (IGF-1) (Itami *et al*, 1995) and stem cell factor (SCF), a regulator of melanocytes (Hibberts *et al*, 1996).

In conclusion, cultured dermal papilla cells are not an easy model to use and have significant disadvantages in the need for full depth skin samples, their slow growth rate and generally short-lived nature (Messenger, 1984; Randall, 1996). However, they do offer very important advantages. These include: the possibility of investigating the paradoxical differences in androgen responses between follicles from various sites; their ability to induce hair growth when reimplanted *in vivo*; the retention of a gene expression *in vitro* which appears to reflect *in vivo* aspects of hair growth. Overall, this makes them a useful tool to address certain specific questions. However, it is essential that the appropriate types of cells are studied to address particular questions, e.g., only those derived from follicles which are sensitive to androgens *in vivo* to investigate androgen action.

HAIR GROWTH *IN VITRO*

Since methods for the isolation and culture of human hair follicles were first published (Kondo *et al*, 1990; Philpott *et al*, 1990), many researchers have used these model systems to investigate hair follicle biology (reviewed in Philpott *et al*, 1996). The major application of these models has been to investigate the possible role of growth factors in controlling hair follicle growth and differentiation (see Philpott, 2000). The importance of *in vitro* hair follicle culture as a tool to assist hair biology research can best be demonstrated by referring to studies that give an indication of the variety of uses that can be made of these models. In terms of hair growth regulation, Philpott *et al* (1994) have shown that hair follicles in the absence of insulin show premature entry into a catagen-like state, while physiological levels of IGF-I can prevent this. This led to their speculation that IGF-I was an important hair growth regulatory factor and that IGF-I receptor (IGF-IR) may be regulated in a hair cycle dependant manner. Subsequent *in vitro* and *in vivo* studies in murine and human models have shown this to be the case (Rudman *et al*, 1997), and IGF-I is now considered to be one of several key hair growth regulatory molecules. On the basis of these observations, *in vitro* experiments using cultured dermal papilla cells are now being carried out to investigate IGF-I signaling pathways in the hair follicle.

Transforming growth factor-β (TGF-β) was initially proposed as a negative regulator of hair growth (Philpott *et al*, 1990), and subsequent *in vitro* studies have shown that TGF-β2 promotes anagen to catagen transition in cultured human hair follicles (Soma *et al*, 1998). Interleukins 1α and 1β, as well as TNF-α, were identified as potent inhibitors of hair follicle growth *in vitro,* and histological changes in follicle morphology suggest these cytokines play an important role in the pathophysiology of inflammatory hair disease (Philpott *et al*, 1996). This is supported by the observation that the severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene (Tarlow *et al*, 1994).

Cultured hair follicles have been used to investigate aspects of both hair follicle metabolism and the processes of hair fiber formation. Metabolic studies have shown that the human hair follicle engages in anaerobic glycolysis, that is the preferential metabolism of glucose to lactate, despite the presence of oxygen; the follicle also metabolizes significant amounts of glutamine and can therefore be described as a glycolytic glutaminolytic tissue (Williams *et al*, 1993). As well as studying hair follicle intermediary metabolism, *in vitro* cultured follicles have also been used to investigate the role of amino acids in hair fiber synthesis (Matheson *et al*, 1999).

These studies outline some of the uses that have been made of *in vitro* cultured follicles. However, it is important to point out that to maximize these models it is important to observe several guidelines. Hair follicles should be isolated within several hours of skin being removed from the patient; follicles isolated from skin kept refrigerated overnight will grow, but variability between follicle growth rates is markedly increased. Even slightly damaged follicles should be discarded. The follicles must not be allowed to dry out during isolation and the isolation medium, Earle's balanced salt solution: phosphate buffered saline (EBSS:PBS), should not become alkaline. Moreover, the saline must contain calcium and magnesium (i.e., PBS^{+}). In addition, fetal calf serum is inhibitory, and its use can result in marked variation of growth. If these simple guidelines are followed, optimum rates of follicle growth can be achieved. Finally, it is important to emphasize that not all facelift biopsies are suitable for hair follicle isolation. It is particularly difficult to isolate intact hair follicle bulbs from skin in which the follicles only just reach into the subcutaneous fat, with their bulbs close to the dermis. In Michael Philpott's laboratory only a third of facelift samples are

normally suitable for optimum hair follicle isolation and growth. This can be a serious drawback for laboratories with only limited access to such material.

Although human hair follicles can be switched from anagen to catagen *in vitro* by insulin/IGF-I starvation or culture with TGF-β, full human hair follicle cycling is not achieved. To address this problem the culture of murine vibrissae follicles has been investigated. Robinson *et al* (1997) found that vibrissae follicles isolated at different stages of the murine hair cycle grow *in vitro*, at rates that reflect their *in vivo* status. Recently, Philpott and Kealey (2000) reported that murine hair follicles cultured *in vitro* retain cyclical activity. These studies suggest that murine vibrissae follicles may represent an excellent *in vitro* model system for investigating the cyclical nature of hair growth.

The future of isolated hair follicle culture One of the major disadvantages of human hair follicle culture is the difficulty many researchers have in obtaining sufficient human samples. However, if sufficient human skin can be obtained, the *in vitro* cultured follicle is a powerful tool for the hair biologist. In addition, alternatives to human follicles are available and certainly mouse or rat vibrissae follicles may become the model of choice for studying the hair cycle. When using cultured hair follicles it is important to appreciate the limitations of the model. They have a very limited lifespan *in vitro* when compared to *in vivo*. However, as already discussed, within their narrow window of viability, cultured follicles are highly responsive to *in vitro* manipulation, be it by cytokines, nutrients or trace elements. Moreover, with the advances in antisense technology and development of chimeric proteins, cultured hair follicles represent an excellent model in which to carry out neutralization or gene inactivation studies. In addition, real time reverse transcriptase polymerase chain reaction (RT-PCR) technology can now determine changes in gene expression in individual hair follicles. A second drawback is that catagen hair follicles are rarely obtained from human scalp skin because of their long anagen phase. Moreover, although human hair follicles can be switched from anagen to catagen *in vitro*, modeling of the human hair growth cycle *in vitro* is still not possible. Therefore, murine follicles are the best model system to study cyclical changes *in vitro*. Finally, difficulties in obtaining sufficient skin from androgen responsive sites means that human follicles do not readily lend themselves as *in vitro* whole follicle models for investigating the role of androgens on hair growth. For these studies dermal papilla cell culture is the only viable option at present (Randall *et al*, 2001), although extrapolating data from such cell cultures to the whole organ is not always easy. However, the red deer mane follicle offers a useful *in vitro*, androgen responsive model (Thornton *et al*, 1996a).

In conclusion, *in vitro* cultured follicles have been widely used for investigating hair biology. The challenge for the future is to develop models that reflect the cyclical nature of hair growth and also permit follicle neogenesis to occur *in vitro*.

Future directions for *in vitro* models In order to understand the precise biochemical function of single molecules, or groups of molecules, in hair follicle biology the ideal model system would be one in which either *de novo* hair follicles could be induced *in vitro* or in which hair follicles could be reconstructed from their component cells. It would then be possible to reconstitute hair follicles from cells in which individual genes of interest were conditionally expressed, i.e., either inactivated or overexpressed, and to investigate the effects on hair follicle function.

The first approach involves the establishment *in vitro* of cell cultures, possibly in skin equivalents that mimic the embryonic environment and will support embryonic follicle development. Currently, little has been published with regard to *de novo* hair follicle synthesis *in vitro*. This lack of data is interesting. Histiotypic culture of skin equivalents is well established. Moreover, it has been demonstrated that when primary mouse keratinocytes are combined with cultured dermal papilla cells and grafted onto nude mice, hair follicles develop (Lichti *et al*, 1993; Kishimoto *et al*, 2000). What is not known or has not been reported is whether such recombinants would support follicle development *in vitro*. However, one can presume that these experiments have probably been carried out and that the lack of publications probably reflects failure of follicles to develop.

The second approach involves culturing isolated cells from hair follicles and then attempting to recombine these cells into hair follicle like structures. Colin Jahoda and Amanda Reynolds have carried out most of this pioneering work (reviewed in Jahoda and Reynolds, 1996). However, although at present it is possible to recombine different hair follicle derived cell populations and regenerate hair follicle-like structures, these are only really successful when the recombinants are implanted into animals and allowed to regenerate *in vivo*. When these experiments are carried out *in vitro*, follicle regeneration is not as marked. However, their data suggests that the information required for hair growth is intrinsic to the follicular cell populations (Jahoda and Reynolds, 1996).

MOUSE MODELS FOR HAIR BIOLOGY RESEARCH

Mice with hair abnormalities have been maintained and/or studied for centuries; the most notable of these is the rhino mutation (Gaskoin, 1856; Sundberg *et al*, 1999). Spontaneous mutant mice, or those caused by radiation or chemical mutagenesis, have provided background information on hair diseases, but more importantly, they have also provided information on mouse genetics, biology, and husbandry. Transgenic technology has grown into targeted and conditional mutagenesis resulting in a flood of novel mutant phenotypes due to a variety of changes in known genes. Many of these new genetically engineered mice are allelic mutations with well-characterized, spontaneous models allowing rapid comparisons with specific human genes (Sundberg *et al*, in press). Powerful web based tools provide myriads of information on mice including phenotypes, genetics, sequences, and other types of information with direct comparisons with data on human beings and many other species.

A titan in mouse genetics, Elizabeth S. Russell, died recently. She was a pioneer in the genetics of pigmentation (Russell, 1939, 1946, 1948, 1949a) and had the profound foresight to establish a repository for mutant laboratory mice. This in itself provided the model on which all mouse repositories are now built. More importantly, the repository she created transferred mutated genes onto the C57BL/6 J background to create congenic strains. These methods standardized a critical aspect of mouse biology, the genetic background, making reproducible experiments possible using multiple mutations in the same animals. Another critical discovery of Dr Russell's was that two seemingly unrelated gene products interacted to cause the same, or nearly the same, phenotype. This was done using mice that had coat color abnormalities and anemia, namely dominant spotting and Steel-Dickie mutant mice. She was the first to recognize the interaction of a receptor (cKit-receptor in the Steel-Dickie mice) and its ligand (cKit in the dominant spotting mutant mice) that preceded the modern era of signal transduction (Russell, 1949b).

Phenotypic mimics, single gene mutations that are located on unrelated genes (complementary), but cause similar or identical clinical disease have proven to be very important in understanding normal organ function. Breakthroughs in hair biology have come from understanding and utilizing the unique features of these mice. Initially transforming growth factor alpha (*Tgf*-α) null mutations (Luetteke *et al*, 1993; Mann *et al*, 1993) were found to be mimics of the spontaneous mouse mutants called waved 1 (*wa1*). These findings were confirmed by two groups independently by allelism testing, crossing the null mutant mice with the *wa1/wa1* mice to yield affected offspring. The waved 2 (*wa2*) mimic was subsequently shown to be due to a mutation in the *Tgf*-α receptor, epidermal growth factor receptor (*Egfr*) (Luetteke *et al*, 1994). Several other waved mutant mice have subsequently been discovered and are currently under investigation.

Table I. Genes identified through development of spontaneous and genetically engineered mutant mouse models that affect hair follicles and the fibers they produce (condensed and modified from Sundberg and King, 1996; Sundberg and King 2000; Nakamura *et al*, in press). Details on each mutation can be found by entering the gene symbols into the Mouse Genome Informatics Database (www.informatics.jax.org/menus/marker_menu.shtml)

Abnormally low numbers of hair follicles

Soluble Factors and Receptors:
Tg(FGF7)2Efu, Tg(FGF7)2Efu, Fgf10^{tm1Ska}, Tg(FGFR2)1Saw, Inhba^{tm1Zuk}, Ingfr^{tm1Arge}, Nog^{tm1Amc}, Tg(PTHLH)7Wmp, RORa^{sg}, RORa^{tm1Mba}, Tg(Tgfb1)1Der, Tgfb2^{tm1Doe}

Transcription Factors:
Msx1^{tm1Rilm}, Msx2^{tm1Rilm}, Lef1^{tm1Rug}, Sox18^{Ra}, Sox18$^{Ra\text{-}op}$, Tg(Zfp38)A4Htz

Enzymes:
Chuk^{tm1Salk}, Chuk^{tm1Aki}

Adhesion Molecules:
Tg(ITGA5)0794Fmw, Tg(ITGB1)0840Fmw, Itgb1^{tm1Ref}, Itgb1^{tm1Efu}, Tg(Cre)1Efu, Tg(KRT16)10Cou

Others:
Tg(KRT16)10Cou, Tg(rv-CYCLIN)29Dlh, Slc30a^{lm} Trp63^{tm1Brd}

Unknown genes:
ap, Bda, fd, Hpt, Hk, lgh, pf, Er

Hair follicle morphogenesis abnormalities

Soluble Factors and Receptors:
Tg(EGFR)0Jlj, Egfr^{tm1Cwr}, Egfr^{wa2}, FGF7^{tm1Efu}, Fst^{tm1Zuk}, Tg(Fst)4Zuk, Tg(Hgf)1Paus, Tg(IGF1)1Hys, Tg(IGF1)Jdg, NGFb^{tm1Gne}, Tg(Ngf)47Kma, Ngfr^{tm1Jae}, Ntrk3^{tm1Bbd}, Ntf3^{tm1Jae}, Ntf3^{tm2Jae}, Pdgfa^{tm1Cbet}, Shh^{tm1Chg}, Tgfa^{tm1Ard}, Tgfa^{wa1}, Tg(Tgfa)1Efuv

Transcription Factors:
Cutl1^{tm1Ejm}, Cutl1^{tm2Ejn}, Ets2^{tm1Rgo}, Gli2^{tm1Alj}, Tg(Msx2)1Rem, Tg(LEF1)1Efu

Enzymes:
Adam17^{tm1Imx}, Atp7a^{Mo}, Atp7a$^{Mo\text{-}br}$, Atp7a$^{Mo\text{-}brJ}$, Atp7a$^{Mo\text{-}dp}$, Atp7a$^{Mo\text{-}ml}$, Atp7a$^{Mo\text{-}ms}$, Atp7a$^{Mo\text{-}to}$, Atp7a$^{Mo\text{-}vbr}$

Adhesion Molecules:
Tg(CTNNB1)1Efu

Others:
Tg(CTNNB1)1Efu Tg(erbb2)1Jek, Gab1^{tm1Thir}, Ca, Ca^{d}, cph, cpy, cu, cw^{thd}, fld, fld^{2J}, fr, gs, rhg, ro, scb, Scr, soc, wal, Wc, Wf

Hair Cycle Abnormalities

Soluble Factors and Receptors:
Tg(BMP4)6Blh, Tg(Bdnf)1Paus, Bdnf^{tm1Tbn}, Fgf5^{tm1Mrt}, Fgf5^{go}, Gfra1^{tm1Jmi}, Gfra2^{tm1Msa}, Tg(Ngf)47Kma, Ntf3^{tm1Jae}, Ntf3^{tm2Jae},Tg(Ntf3)1Kma, Ntf5^{tm1Jae}, Tgfb1^{tm1Doe}, Vdr^{tm1Ska}, Tg(Wnt3)7Gsb

Transcription Factors:
hr, hr^{ba}, hr^{N}, hr^{rh}, hr^{lgN5053Mm}, Tg(Msx2)1Rem, Tg(Cre)1Tak, Stat3^{tm1Aki}

Enzymes:
Terc^{tm1Rdp}

Adhesion Molecules:
Icam1^{tm1Bay}, Icam1^{tm1Jcgr}, Tg(JUP)4Pac

Others:
Bcl2^{tm1Mpin}, Bcl2^{tm1Sjk}, Tg(BCL2)1Tsk, Tg(BCL2L1)1Cbt, Tg(Dvl2)7Gsb, Tg(bK6-E6/E7)M8

Unknown Genes:
acd, Al, al, ao, Frl^{a}, Frl^{b}, Frl^{K}, hl, Eh, jb, Koa, tf

Hair Structural Abnormalities

Soluble Factors:
Tg(Il6)1Efu, Tg(Notch1)1Anc, Tg(Notch1)A3Rko

Transcription Factors:
Foxn1^{nu}, Foxn1$^{nu\text{-}str}$, Foxn1$^{nu\text{-}Y}$, Foxn1^{tm1Tbo}, Tg(Foxn1)1Jlb, Tg(Foxn1)G2Hon, Hoxc13^{tm1Mrc}, Ovol1^{tm1Efu}

Enzymes:
Nsdhl$^{Bpa\text{-}1H}$, Nsdhl$^{Str\text{-}1H}$, OdcTgN(K6ODCtr)55Tgo, Tg(Otc)94Mori, Otc^{spf}, Otc$^{spf\text{-}ash}$

Adhesion Molecules:
Dsg3^{tm1Stan}, Dsg3^{bal}, Dsg3^{bal2J}, Dsg3^{balPas}

Others:
Tg(Krt2-6a)1Der, Tg(Av-E1A)901Gpd, Tg(KRT2-9)1Grog, Ebp^{Td}

Unknown Genes:
at, ba, cls, crhx, dep, exf, fr, fz, fz^{fy}, fzt, Hct, hid, Hq, ic, jd, lah, lah^{1}, lt, ma, Ng, N, olt, pk, Re, Re^{den}, Re^{wc}, rc, ruf, sa, sch, Sha, spc, thf, Uncv, we

Immunologic related hair abnormalities

Soluble Factors:
Fas^{lpr}, Tg(Il1a)1.1Tsk

Enzymes:
Hcph^{me}, Hcph$^{me\text{-}v}$

Adhesion Molecules:
Itgb6^{tm1Des}, Itgb2^{tm1Bay}, Itgb2^{tm2Bay}

Unknown Genes:
cpdm, fsn, nkt

Sebaceous Gland Abnormalities

Soluble Factors and Receptors:
Eda^{Ia}, Eda$^{Ia\text{-}J}$, Eda$^{Ia\text{-}Z3H}$, Eda$^{Ia\text{-}c}$, Edar^{dl}, Edar$^{dl\text{-}3J}$, Edar$^{Dl\text{-}slk}$

Enzymes:
Scd1^{ab}, Scd1^{abJ}, Scd1^{ab2J}

Others:
Tg(APOC1)1Lmh

Unknown Genes:
Bsk, cr

Human diseases, once thought to be just variations in disease due to environmental or genetic background variability, can also vary due to this principal of mimicry. For example, anhydrotic ectodermal dysplasia mice can be due to mutations in the ligand (Tabby, $Ecda^{Ta}$), its receptor (downless, $Ecdar^{dl}$) and even more genes, such as crinkled ectodysplasin-A receptor-associated death domain ($Eduradd^{cr}$) are likely to be involved. Human patients with this disease have mutations in the same groups of genes explaining similar phenotypes but different linkage patterns (Sundberg and King, 2001). Other hair related genes are providing similar insights. Now that the mouse hairless mutation phenotype has been confirmed to be a homolog of human papular atrichia (Sundberg *et al*, 1999), other mimics, such as transgenic mice for overexpression of ornithine decarboxylase (Panteleyev *et al*, 2000) and spermine/spermidine N^1-acetyltransferase (Pietila *et al*, 2001) or null mutations for vitamin D receptor (Li *et al*, 1997;Sundberg unpublished observations), are explaining why some patients with papular atrichia do not have mutations in their hairless gene. This trend will only continue to explain clinical variability between patients with genetically based diseases.

Many genes are now known whose product directly or indirectly affects hair biology in health and disease. Numerous spontaneous and genetically engineered mutant mice can be used to refine our knowledge of human hair diseases (**Table I**) and help continue to define the pathways and interactions of genes involved in hair biology; these advances also promise to provide insight into diseases affecting other organs.

CONCLUSION

In summary, a wide range of both *in vitro* and *in vivo* models have been developed to study the function of the hair follicle. These range from individual cultures of one type of human follicle cell type, e.g., dermal papilla cells through follicle organ culture to natural and genetically manipulated animal models. Many of these models also have an important role to play in the development of new drugs to correct common hair disorders such as hirsutism, androgenetic alopecia and alopecia areata. Each of the systems has advantages and disadvantages and can provide information about specific aspects of hair growth; it is important to synthesize information gained from the various models. The advent of molecular biological methods in combination with the wide range of models available should enable the secrets of how follicles function in health and disease to be unlocked in the near future.

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CONTRIBUTORS TO THIS WORKSHOP

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The session was chaired by VA Randall (UK) and cochaired by S Arase (Japan), P Hynd (Australia) and JP Sundberg (USA).

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