Traveling stripes on the skin of a mutant mouse

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In the course of animal development, complex structures form autonomously from the apparently shapeless egg. How cells can produce spatial patterns that are much larger than each cell is one of the key issues in developmental biology. It has been suggested that spatial patterns in animals form through the same principles by which dispatched structures are formed in the nonbiological system. However, because of the complexity of biological systems, molecular details of such phenomena have been rarely clarified. In this article, we introduce an example of a pattern-forming phenomenon that occurs in the skin of mutant mice. The mutant mouse has a defect in splicing of the *Foxn1* (*Whn* or *nude*) gene, which terminates hair follicle development just after pigment begins to accumulate in the follicle. The immature follicles are rapidly discharged, and a new hair cycle resumes. Eventually, the skin color of the mouse appears to oscillate. The color oscillation is synchronous in juvenile mice, but the phase gradually shifts among skin regions to eventually form traveling, evenly spaced stripes. Although the time scale is quite different, the pattern change in the mutant mouse shares characteristics with the nonlinear waves generated on excitable media, such as the Belousov–Zhabotinskii reaction, suggesting that a common principle underlies the wave pattern formation. Molecular details that underlie the phenomenon can be conjectured from recent molecular studies.

There are many periodic patterns in nature that form autonomously from an apparently uniform or random state (1–5). In 1952, Turing (6) presented a pioneering paper that describes the theoretical principle of how spatial patterns self-organize. Later, theoretical and experimental studies were carried out on a variety of systems. To date, the mechanism of pattern formation is one of the key issues of nonlinear physics, developmental biology, and ecology (1–5). It is possible, in a few cases, to generate spatial patterns by simple chemical reactions in a dish. Among them, the most famous is the spiral or concentric wave pattern made by the Belousov–Zhabotinskii (BZ) reaction (7). When spatial phenomena are experimentally induced, the substances inputted are known and the products are detectable. Therefore, it is relatively easy to study the molecular mechanism of how the pattern formed (8).

Biological systems have numerous periodic patterns. The structure of adult animals is extremely complex, although eggs, in their initial state of development, are structurally quite simple. Molecular genetic experiments carried out for two decades have shown that most animal eggs contain a concentration gradient of signaling molecules that activate or inhibit downstream genes. Cascades of the gene activation originated from the initial signals (coordinate genes) are thought to generate the fine localization of the expression pattern of specific genes (9). Therefore, to some extent, positional information for the spatial pattern of animal body is inherent in the oocytes. However, the complexity of the adult body obviously exceeds the amount of positional information that can be generated by coordinate genes. Moreover, some embryos can regenerate their correct structure even when the original pattern is surgically disarranged (9). Therefore, embryos need to produce spatial patterns for proper morphogenesis (6).

How such positional information forms is one of the central problems of developmental biology. In a few cases, spatial patterns made by biological systems are quite similar to that made by nonbiological systems, not only in the resulting patterns but also in the dynamics of pattern change. For example, the motion of cAMP waves made by the gathering slime mold, *Dictyosterium myxamoebae*, is quite similar to that of the BZ reaction (7, 10). An example of a stationary pattern (Turing pattern) is the striping of the tropical fish *Pomacanthus imperator*. When the fish grows, new stripes are inserted exactly in the manner that the Turing model predicts (11, 12). Similar dynamic patterns are formed by a specific chemical reaction: the cholateiodide-malonic acid reaction (13), in a quasi-2D conformation (14). Molecules that are involved in these phenomena are apparently different in the biological and nonbiological systems. From the striking similarity in the dynamics it is highly plausible that the principles at work in these phenomena are identical. Many mathematical models have been proposed to explain how biological patterns are formed. However, because of the exceeding complexity compared with nonbiological systems, the molecular basis of such phenomena have not been clarified.

In this article, we report on the traveling wave pattern that develops on the skin of a mutant mice strain. The mutation causes follicular development to terminate just after it starts accumulating pigments, then the immature follicles are rapidly discharged and a new hair cycle resumes; eventually, the skin color of the mouse appears to oscillate. The color oscillation is synchronous in juvenile mice, but the phase of the oscillation shifts gradually through skin regions to eventually form evenly spaced, traveling stripes. Although the time scale is quite different, the pattern change in the mutant mouse shares some characteristics with the nonlinear waves generated in excitable media, such as the BZ reaction (7) or gathering slime mold (10). The same basic principle probably underlies wave pattern formation in both systems. From recent experimental studies, molecular details that underlie the phenomenon can be conjectured.

Materials and Methods

Animals. The mutant is a hybrid strain between 129 and C57BL/6. The mutant allele is autosomal recessive. The colonies were maintained by intercrossing between the homozygous male and the heterozygous female. Photographs of the mouse were taken with a Nikon digital camera DH1. Genomic and cDNA sequencing were carried out with the standard methods described in *Molecular Cloning: A Laboratory Manual* (45).

Complementation Test. The mutant male mice were crossed with the albino KSN nude ($foxn1^{nu}/foxn1^{nu}$) mice purchased from Japan SLC (Hamamatsu, Japan). The phenotypes of all of the

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Abbreviation: BZ, Belousov–Zhabotinskii.

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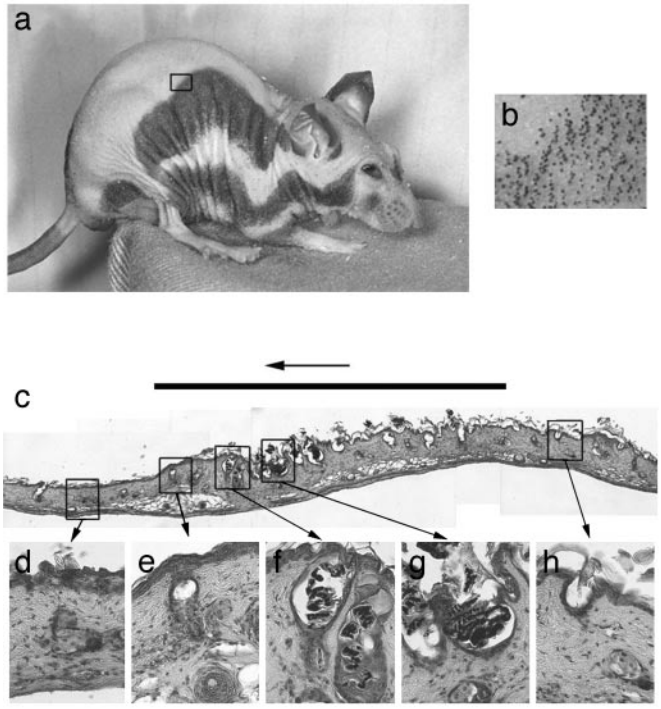

Fig. 1. Stripe patterns of homozygous *Foxn1*^{tw} mouse and skin sections. (*a*) Homozygous *Foxn1*^{tw} mouse at 120 days after birth. (*b*) Close-up of the border of the pigmented region. The region shown is indicated by the square in *a*. (*c*) Paraffin section (×100) of skin cut from the side of the body and along the direction in which the wave runs. The thick line over the picture shows the region of a black stripe. The stripe is running leftward at a speed of ≈1.5 mm per 30 days. (*d–h*) Paraffin section (×400) of each position of the wave. (Scale bars: 50 μm.)

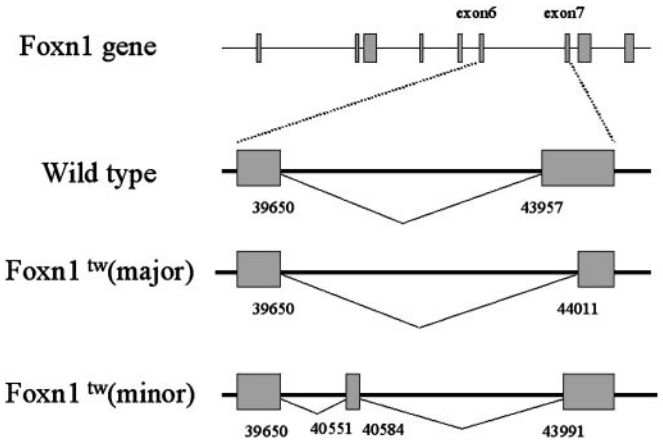

Fig. 2. Two types of aberrant alternative splicing between exons 6 and 7 of *Foxn1*^{tw} mRNA. (*Top*) Normal splicing between exons 6 and 7 of *Foxn1* mRNA. The nucleotide numbers of 5′ and 3′ of intron 6 are 39651 and 43956, respectively. The nucleotide numbers are referred to in the reported *M. musculus whn* gene sequence, GenBank accession no. Y12488. (*Middle*) The major form of the mutant mRNA. This type, 77% of the total of *Foxn1*^{tw} mRNA, has a 54-bp deletion 5′ in exon 7 (43957–44010), generating an 18-aa deletion in the center of the DNA binding domain. (*Bottom*) The minor form of the mutant mRNA. This type, 23% of the total of *Foxn1*^{tw} mRNA, has a 44-bp cryptic exon (40541–40584) and a 34-bp deletion 5′ in exon 7 (43957–43991), generating a translational termination (43992–43994).

pups were identical to the nude phenotype except that they had traveling stripes on the skin.

Histology. Skin samples were fixed in 10% paraformaldehyde, dehydrated, and embedded in paraffin. Sections (8 μm thick) were examined after hematoxylin-eosin staining.

Results and Discussion

A new mutant mouse with traveling stripes (Fig. 1 *a* and *b*) arose spontaneously in our colony of 129 C57BL/6 mice. Most of the phenotypic characteristics of the mouse are similar to those of the nude (*Foxn1*^{nu}/*Foxn1*^{nu}) mouse (15–20), except for an adult skin pattern. Complementation tests against the nude (*Foxn1*^{nu}/*Foxn1*^{nu}) mouse (21) have shown that this mutant is a new allele of the *Foxn1* gene (data not shown). We named this allele *Foxn1*^{tw} (tw: traveling wave). Genomic and cDNA analysis of the mutant revealed that alternative splicing causes small deletions in the cDNA of the *Foxn1* gene (Fig. 2). We could not detect any normally spliced mRNA of the gene from the homozygous *Foxn1*^{tw} mouse, but instead identified two types of abnormally spliced mRNA. A major spliced product from the homozygous *Foxn1*^{tw} mouse lacks 54 bp at the 5′ region of the seventh exon that corresponds to the 18 aa in the presumptive DNA binding domain of the gene (20) (Fig. 1). As the deletion is small, it is expected that the protein from this mRNA has some residual activity. In the minor splicing product, 34 bp at the 5′ region of the seventh exon are deleted, and 33 bp from the intron are inserted. Because a frameshift occurs to the downstream sequence that contains the DNA binding domain, this mRNA should be functionless. The abnormally spliced products are isolated from heterozygous mice at a ratio of 50%, suggesting that the splicing abnormality is a cis effect of the mutant allele. We could not detect any mutation around the splicing donor and acceptor site of the intron between the sixth and seventh exons. It has been reported that a single base substitution 10 kb downstream of the splice site causes an abnormal splicing of the transcript of the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene. Although we found three base substitutions in the middle of the intron, at present it is not clear whether they are responsible for the abnormal splicing (22).

Fig. 1*a* shows the appearance of a homozygous *Foxn1*^{tw} mouse that has typical stripes. Although this mouse is hairless, microscopic inspection (Fig. 1*b*) reveals that the hair follicles accumulate black pigment, resulting in an apparent black color in the area. Fig. 2 *c–h* shows the skin section of an adult homozygous mouse that is sectioned parallel to the direction stripes move. The stripe region (represented by the thick bar in the upper portion of Fig. 2*c*) is moving to the left. In advance of the wave front (Fig. 1*d*) the skin is relatively thin and the hair follicles appear undifferentiated, showing that they are in the resting phase (telogen). At the wave front (Fig. 1*e*) follicles located deeper in the thickened dermis contain many fatty cells. Follicles are in the activated phase (anagen) and accumulating pigment. Behind the wave front follicles accumulate bent or coiled hair shafts that move toward the surface and discharge debris form the skin surface. Eventually the skin returns to an unpigmented state (Fig. 1 *f–h*). Behind the wave skin and follicles assume a resting phase. As the next wave arrives soon hair formation does not cease as long as the mouse lives.

It is known that hair formation in the rodent skin has a periodic cycle (23) and that the region of the activated hair growth (anagen phase) spreads as a transient traveling wave (24). Militzer (25) used nude mouse of the pigmented strain (C57BL/6-*Foxn1*^{nu}) to visualize regions in the anagen phase of the hair growth and recorded the pattern change for up to 400 days. He found that young *Foxn1*^{nu} mice have a few broad traveling bands of anagen phase on the skin, but later (>200 days), those patterns are replaced by one or a few periodically appearing,

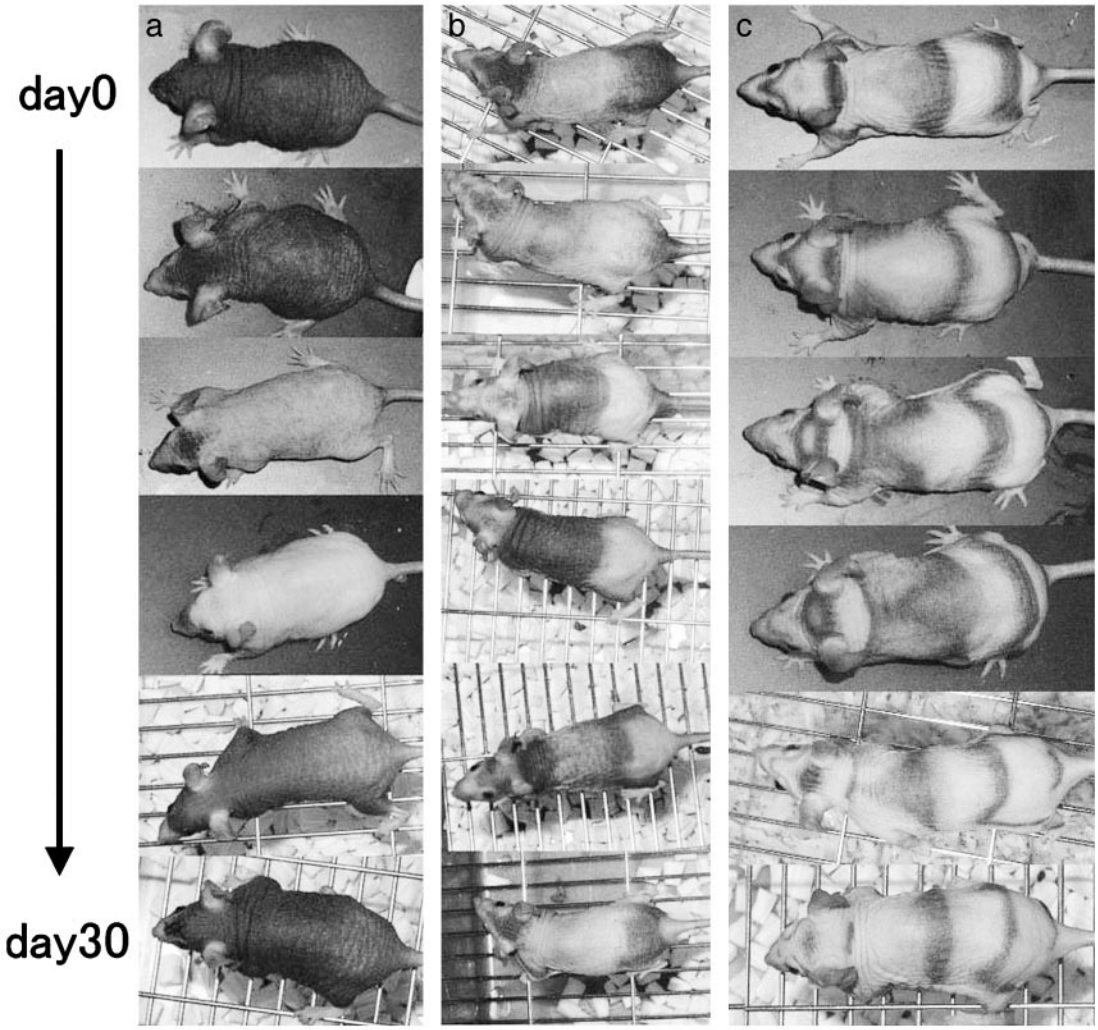

Fig. 3. Pattern change of a homozygous *Foxn1*^{tw} mouse during the 30-day cycle. (*a*) Days 30–60. (*b*) Days 90–120. (*c*) Days 210–240 after birth. Pictures are taken at 5-day intervals with a Nikon digital camera. The pattern change shown here is typical for a homozygous *Foxn1*^{tw} mouse.

broad bands that are arranged sagittally or longitudinally along the body axis. Compared with the *Foxn1*^{nu} mouse, aged *Foxn1*^{tw} mice form a number of regularly arranged, narrow bands that enable us to elucidate the dynamics and mechanisms of wave formation. In 1971, Mann (26) reported a mutant line, named *cryptothrix* (*crh*), that had similar waves. The *crh* mutation is not allelic to any known hairless mice and the target gene has not been cloned yet. Reports on *crh* were concentrated on the abnormalities of individual hairs and very poorly described patterns of movement. It was simply reported that the stripes move anteriorly (27, 28).

To investigate how the stripes are generated in *Foxn1*^{tw} mice we recorded the time course of the pattern changes. Fig. 3 shows how the pigmentation pattern of the *Foxn1*^{tw} mouse changes at different ages. Pictures of the skin pattern of 50 homozygous *Foxn1*^{tw} mice were taken at 5-day intervals. Pattern changes of an identical mouse during days 30–60, 90–120, and 210–240 are shown in Fig. 3. The first cycle of the color oscillation takes place almost synchronously in most skin areas (Fig. 3*a*). However, from the second cycle the phase of the color cycling begins to differ gradually across the skin regions to form broad traveling bands (Fig. 3*b*).

At the third month from birth a very broad band appears at the back. The band splits into two broad traveling bands, one of which moves anteriorly and the other posteriorly. The bands narrow as the mice age, and finally, at ≈7–10 months after birth form narrow and evenly spaced traveling bands (waves) (Fig. 3*c*). Fig. 4 *c* and *d* shows how the waves travel in an adult *Foxn1*^{tw} mouse. Each wave arises around the armpit region (Fig. 4 *c* and *d*, line 1) and travels in all directions. Apparently, waves run regardless of the lie of hair. Waves moving along the fore leg form rings around the leg (Fig. 4 *c* and *d*, lines 2 and 3 on the leg). The wave moving along the trunk spreads with a rounded wave front and meets another wave coming from the other side of the body (Fig. 4 *c* and *d*, line 3). The intersecting wave fronts form a cross shape then form two parallel waves that move in opposite directions (Fig. 4 *c* and *d*, line 4). Waves traveling along the neck and face area finally reach the forehead, there meeting another wave coming from the back and then disappearing (Fig. 4 *c* and *d*).

We observed the pattern changes of 50 homozygous *Foxn1*^{tw} mice. As juveniles (<100 days) all of the mice showed identical pattern changes. Later, however, individual differences emerged. At 200 days half of the mice had typical sharp traveling lines, as shown in Fig. 3*c*. Others had irregular (for example, asymmetric, fragmentized, or very wide band) patterns (data not shown). Because the ratio of mice with the typical pattern increases when kept individually in a cage, we

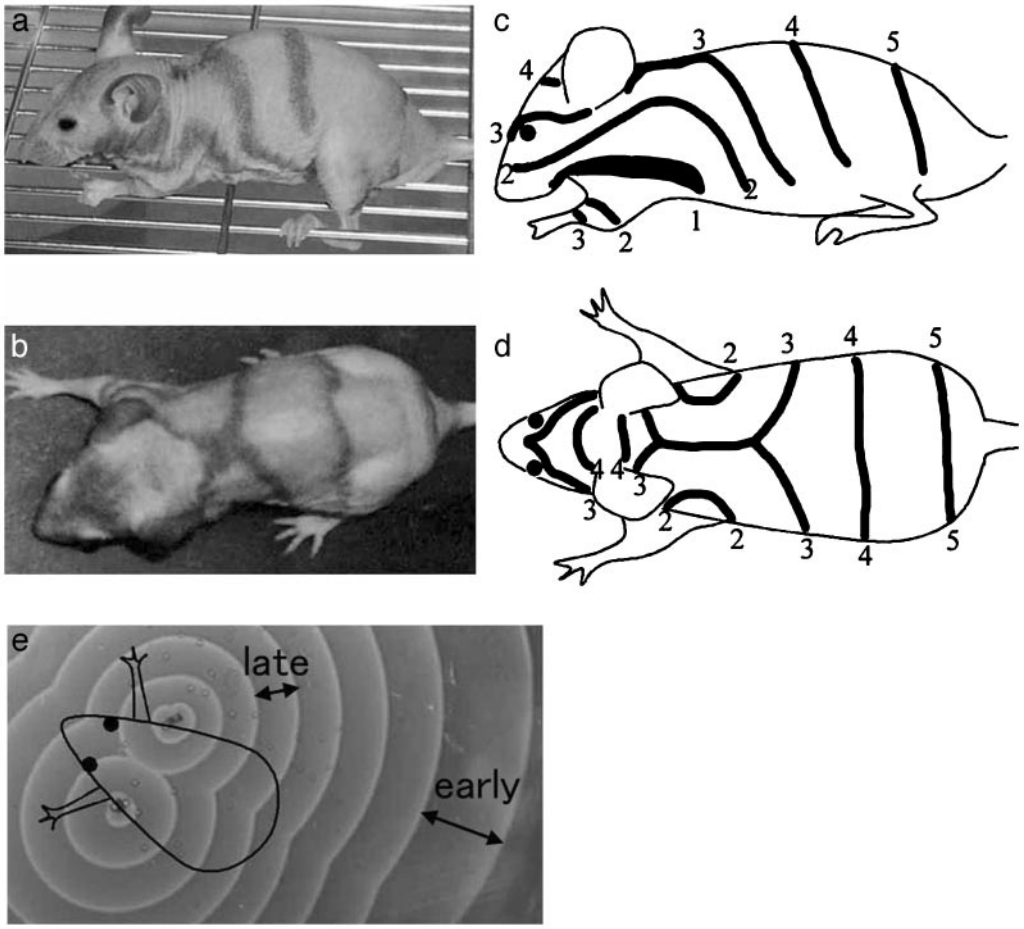

Fig. 4. Summary of stripe movement. (*a* and *b*) Side and top views of the adult *Foxn1*^{tw} mouse, respectively. (*c* and *d*) Schematic drawing of typical wave movement in an adult *Foxn1*^{tw} mouse. The numbers by each line represent the time course of a wave. The time interval between the numbers is ≈30 days. (*e*) Traveling waves formed by the BZ reaction. The shape of the mouse is superimposed. The outermost wave is the first wave. Intervals between waves are relatively wider for the first a few waves, then become shorter and constant. BZ waves are made by the standard method described in ref. 44. To indicate the centers of concentric waves, two iron grains were put on the dish.

conjecture that scuffles among cage mates disarrange the regular pattern. Physical stimulation is known to activate the hair cycle (16).

Despite large difference in time scales the behavior of the waves described above shares the common characteristics of self-organizing, nonlinear waves, such as the BZ reaction (7) or the cAMP pattern of gathering slime mold (10, 29). Fig. 4*e* shows typical concentric waves made by the BZ reaction. Supposing that the pacemakers located at the armpit region the BZ reaction forms a pattern with stripes very similar to the *Foxn1*^{tw} mouse. It is notable that the spacing between the early waves is much larger than that of late waves in both *Foxn1*^{tw} mice and the BZ reaction (Figs. 3*c* and 4*e*). It is obvious that the molecular mechanism generating waves on the mouse skin is totally different from that of the BZ reaction or cellular slime mold. However, theoretically, any kind of system can form very similar waves if it satisfies some simple conditions: oscillating reaction, lateral activation, and pacemaker region (5, 30, 31).

In the *Foxn1*^{tw} mice, the constant oscillation is induced by early termination of the hair formation. It is known that the *Foxn1* gene is required to maintain hair formation activity through stimulating keratin synthesis (32, 33). Loss of the gene causes termination of hair formation. Then, the immature hairs are quickly discharged and the new hair cycle starts immediately. Therefore, the skin of nude and *Foxn1*^{tw} mice can act as an oscillating media. Molecular genetic studies (reviewed in refs. 20 and 34) on hair formation have identified many molecular mediators that control hair formation. There are some candidates for the lateral activator functions in the formation of waves. Sato *et al.* (34) have proved that localized, but transient, enhanced expression of the *sonic hedgehog* (*Shh*) gene (35, 36) in postnatal skin can initiate the onset of a hair formation cycle. As endogenous *Shh* is normally expressed at the hair bulb in early anagen (34), *Shh* is an ideal candidate for the lateral activator of wave formation. Another candidate is *FGF7*. *FGF7* is also expressed during early anagen in the cells of dermal papilla and proved that its activity is required for the early development of hair follicles (37, 38). As both molecules are expressed almost simultaneously in the course of hair formation, it is not easy to find out which plays a key role in the wave formation from the temporal pattern of their gene expression. One of the possible means to determine the key molecule would be skin implantation of tumor cells that express one of the candidate genes. If the key molecule is released continuously from a spot of mouse skin, a concentric wave should be formed.

Movement of the waves suggests that the pacemakers locate at the dorso-ventral border around the armpits of the mouse. This means that the hair cycle is slightly quicker in the ventral

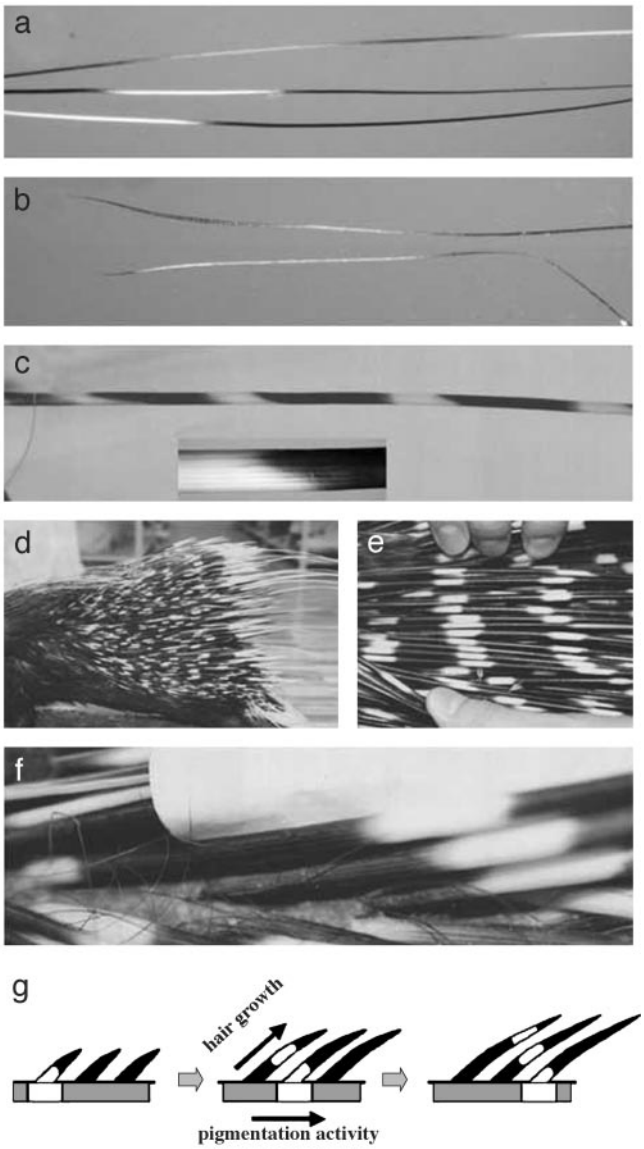

Fig. 5. Color patterns of cats, mice, and porcupines. (*a–c*) Periodic color pattern of house cat, mouse, and African porcupine. (*d*) Outlook of African porcupine. (*e*) Synchronized hair pattern of quills of African porcupine. (*f*) Skin and the roots of quills. (*g*) Schematic drawing explaining the reason the hair pattern looks synchronized.

skin than in the dorsal skin. In many animals, ventral hairs are shorter and pigment is lighter compared with the dorsal hair. Therefore, there must be some difference in the environmental condition or differentiation state (39–43). It is not surprising that the cycling time of hair formation is different between the two regions. As described above, the skin of *Foxn1*^{tw} mice can satisfy the basic requirements for consecutive traveling wave formation. The stripe pattern of the *Foxn1*^{tw} mouse is an example that the principles of the nonlinear system work in biological systems despite the large difference in time scale.

The waves of *Foxn1*^{tw} mice show that animal skin can act as good media for traveling wave formation. To date, hundreds of molecular mediators have been isolated. Theoretically, any of them can generate waves if they stimulate neighboring cells to release the same molecule. Therefore, waves of similar dynamics can occur regardless of hair formation cycling. One of the examples we found is the color pattern recorded in the quills of porcupine. Fig. 5*c* shows the typical color pattern of porcupine quill. A periodic black and white pattern shows that the pigmentation activity oscillates at a shorter period than the cycle of quill (hair) formation. The pigment of quills originates from the melanocytes distributed around the quill follicle. Therefore, if the quills grow continuously, then the color pattern of each hair represents a record of temporal pattern of local melanocyte activity. Fig. 5*d* shows the general outlook of an adult porcupine. The color at the tip of each quill is white in most areas. This means that the oscillation of melanocyte activity is synchronized in the whole body when the animal is an infant. By contrast, quill pigmentation near the skin is apparently not in the same phase. Although the pigmentation activity is not synchronized, the color pattern of the quills looks synchronized (Fig. 5*e*) in many areas. This is probably because the region of high pigmentation activity is moving like the wave in the *tw* mouse (Fig. 5 *f* and *g*). As the hairs grow at a similar speed to that of the wave, the resulting pattern looks well synchronized. As the hair formation is not involved in the color pattern of porcupine quill, the underlying molecular mechanism should be different from that of the *tw* mouse. However, the overall dynamics deduced from the quill color is almost identical. Many rodents and carnivores (Fig. 5 *a* and *b*) have similar color patterns in their hair although most of them are less distinct than that of porcupine. It would be possible that the traveling wave of pigmentation activity is a common phenomenon among mammals.

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