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Review

Deer antler: a unique model for studying mammalian organ morphogenesis

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Abstract. It is now widely accepted that organ morphogenesis in the lower animals, such as amphibians, is encoded by bioelectricity. Whether this finding applies to mammals is not known, a situation which is at least partially caused by the lack of suitable models. Deer antlers are complex mammalian organs, and their morphogenetic information resides in a primordium, the periosteum overlying the frontal crest of a prepubertal deer. The present paper reviews (1) the influence of morphogenetic information on antler formation and regeneration, and proposes that antlers are an appropriate organ for studying mammalian organ morphogenesis and (2) the storage, duplication and transferring pathways of morphogenetic information for deer antlers, and outlines a preliminary idea about how to understand the morphogenesis of mammalian organs through an involvement of bioelectricity. We believe that findings made using the deer antler model will benefit human health and wellbeing.

Additional keywords: antlerogenic periosteum, bioelectric code, pedicle periosteum, regeneration.

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Introduction

Morphogenesis of body or organs is a mystery that can be, but has not been, resolved in life science (Sheldrake 1981). Resolution of this mystery has been considered to be the first step for totally revealing the 'life phenomenon' as a whole (relative to behaviour, thinking, evolution and origin). Currently, studies on the genetic code and the central dogma have failed to explain 'morphogenesis'. A new breakthrough is needed to reveal the underlying mechanism.

In recent years, a major discovery was made in the field of bioelectricity by the Levin Laboratory, and this group advanced a theory that bioelectricity encodes morphogenesis (Tseng and Levin 2013). 'Bioelectricity' is the electric potentials and currents that are generated by a variety of biological processes within living organisms. Bioelectricity as described in this review represents the resting state, which changes slowly. Bioelectricity regulates morphogenesis at several levels. At the cell level, the resting potentials of cells serve as a highly conserved, information-bearing pathway that regulates cell proliferation, migration, differentiation and cell : cell communication (Adams and Levin 2012*a*, 2013). The transmembrane potential (V_{mem}) plays an important role in coordinating cellular activities that contribute to the large-scale patterning needs of the host during *in vivo* morphogenesis (Pineda *et al*. 2006; Levin 2012*a*; Levin and Stevenson 2012). At the tissue level, an applied electric field (EF) improved regeneration of spinal-cord transection in larval lampreys, induced recovery of the accompanying spinal reflex of the spinal cord when a dorsal part was cut in the adult guinea-pig, stimulated significantly regenerative axonal growth in adult mammals, and induced the recovery of some sensory function in human quadriplegic patients (Borgens *et al*. 1980, 1981, 1987; Borgens 1999). Organogenesis is controlled by bioelectricity also. Patterns of hyperpolarised cells drive craniofacial patterning in *Xenopus laevis* (Vandenberg *et al*. 2011). Miss-expression of specific ion channels (ion channels are pore-forming membrane proteins whose functions include establishing membrane potentials by gating the flow of ions across the cell membrane) induces the formation of beating, ectopic hearts next to the original one in *Xenopus* (Levin 2012*b*; Tseng and Levin 2013). Artificially setting the resting potential in embryonic frog cells *in vivo* shows that manipulating the V_{mem} of non-eye cells can induce well formed ectopic eyes that are morphologically and histologically similar to endogenous eyes, and that these can be induced far outside the anterior neural field (e.g. in gut or mesoderm cells) to form complete eyes (Pai *et al*. 2012). Beyond single organs, manipulation of V_{mem} controls the regeneration state of *Xenopus* tadpole tail and hind limbs (Tseng *et al*. 2010; Tseng and Levin 2013). Artificially altering gradients of V_{men} resulted in ectopic limb formation with normal bone structure in *Xenopus* (Levin 2012*b*). The polarised state of amputated *Planaria* fragments determined their regeneration pattern, that is, whether the regeneration part is head or tail (Beane *et al*. 2011).

All the discoveries made in this field are thus far solely based on primitive animals. It is not known whether the bioelectricity theory also applies to mammals, because primitive animals are phylogenetically distinct from mammals, and no appropriate mammalian models have been found that can be used to investigate the role of bioelectricity in mammalian organ morphogenesis. However, revealing the mechanism underlying mammalian morphogenesis would undoubtedly help advance our knowledge of human birth defects, organ regeneration, organ senescence and tumour formation. Consequently, identification of a pertinent model for studying mammalian organ morphogenesis would greatly advance this field.

On the basis of more than three decades of research, we believe that deer antlers are a very desirable model for studying mammalian organ morphogenesis. Subsequently, we list some research approaches that, we believe, can be used to track down morphogenetic information in deer antler morphogenesis. All these undoubtedly would provide a basis for using antlers as a morphogenetic model for investigating the possible role of bioelectricity in mammalian organ morphogenesis.

Why deer antlers?

Research on morphogenesis must be carried out *in vivo* using live animals. Recently, Levin (2011) suggested using a computer to model the concept of morphogenesis; however, to model morphogenesis in this way, a large amount of bioinformatic data must be acquired, and these data are currently not available. Deer antlers are living mammalian organs, their morphogenesis has been comprehensively studied, their morphology is species-specific, their regeneration recapitulates process of development, and morphogenetic studies can be conducted by simply manipulating the antlerogenic periosteum (AP) (Gao *et al*. 2012).

Antler morphogenesis and morphogenetic primordia

Antlers are the cranial appendages grown by male deer. They consist of two parts: antler *per se* and pedicle (the antecedent of antlers) (Goss 1983). Pedicles are permanent bony protuberances and outgrowths of the deer frontal bone. Antlers are cast and regenerate from their pedicles once a year. Antler ontogeny includes two separate events, namely, generation and regeneration. These two events can be treated as a whole or as separate processes for studying morphogenesis.

The initial development of pedicles occurs from the frontal crests, in the winter, when male deer approach puberty. When these pedicles grow to the species-specific height, their apical skin starts to become shiny, indicating that antler transformation from the pedicle has taken place. Pedicles and first antlers can be readily distinguished from the difference in skin type; pedicle skin is a typical type of scalp skin, whereas antler skin is unique and velvet-like, which is called velvet skin. For most of the temperate species, such as red deer, fallow deer and sika deer, growth of the first set of antlers falls in a rapid period by the end of spring and early summer; in the autumn, these first antlers become calcified, which causes shedding of velvet skin to expose the bare bony antlers. In the winter, the hard antlers are firmly attached to their living pedicles. The first set of antlers usually does not branch, so they are called spikes. In the next spring, the hard antlers drop off from their pedicles and new antler regeneration immediately follows. From then on, antler growth enters well defined yearly cycles; in the spring, hard antlers are cast and new antlers regenerate from their pedicles; in the summer, antler growth enters a rapid growth period; in the autumn, antlers become calcified and antler velvet skin peels off to expose hard dead bony antlers; in the winter, hard antlers are firmly attached to their pedicles until the spring of the following year (Li and Suttie 2012). The casting of hard antlers in the spring triggers a new round of the antler growth cycle (Li *et al*. 2004). Unlike the first set of antlers, regenerated antlers, for the most of temperate species, are branched.

Research has found that the periosteum in the presumptive pedicle growth region is endowed with the morphogenetic information necessary for antler formation (Hartwig and Schrudde 1974). This periosteum is called the AP. The demarcation between the AP and the surrounding non-antlerogenic counterpart is not clearly delineated; what is known is that away from the centre of the AP, the antlerogenic potential gradually decreases. The actual size of the AP is species-dependent. Goss and Powel (1985) reported that the diameter of the AP in fallow deer is ~15 mm. We found that the AP of sika deer is oval in shape and ~20–30 mm in diameter (C. Li, unpubl. data). Deletion of the AP abrogates future antler formation from the original site, whereas transplantation of the deleted AP elsewhere on the deer body autologously induces ectopic antler formation. This finding was further confirmed by a genetic-marker labelling technique (Li and Suttie 2001*a*). It is obvious that AP cells have considerable power of proliferation, differentiation and self-renewal, as they, from such a small piece of periosteum, can form 10–20 kg of antler tissue mass just within 60–70 days. Li and Suttie (2001*b*) considered that the AP is a piece of postnatally retained embryonic tissue. AP is transiently developed during embryogenesis but this is stopped when the fetus approaches full term, and its development is reactivated postnatally when deer approach puberty.

Following the casting of the first set of antlers, the most important biological phenomenon, regeneration, is initiated. Antlers regenerate from the pedicle stumps, and at this time, the AP no longer exists (it has formed a pedicle). Now, the question is 'what is the histogenesis of the regenerating antlers'? The answer to this question is not straight-forward, and has been controversial. Goss (1995) stated that it is the healing pedicle skin that gives rise to regenerating antlers, while others have not been so convinced by the idea (Kierdorf *et al*. 2007). The controversy was settled when Li *et al*. (2007*a*) proved experimentally that the pedicle periosteum (PP) is the tissue base for antler regeneration: deletion of the PP abolished subsequent antler regeneration.

Species-dependence

Generally, spike antlers (the first set of antlers) do not branch, and branching starts from the second set of antlers, i.e. the first set of regenerated antlers. Antler shape, generally, is species-specific and has been used for deer species classification. Differences in antler shape among species are mainly reflected in branch shape, type and number, as well as symmetry. Differences in antler shape can be quantitatively determined by the following three axes: anterior and posterior (head and tail direction), distal and proximal (from antler tip to base) and lateral and medial (antler outer face and inner face). Pocock (1933) standardised the terms for antler parts, such as 'main beam', 'brow tine', 'bez tine', 'trez tine' and royal tines (total number of top tines). Generally speaking, (1) most deer antlers possess brow tines, but only some have bez tines, such as red deer and wapiti; (2) the maximum number of branches in certain deer species is fixed, for example, sika deer has four tines, sambar has three; (3) some deer species have palmate antlers, for example, moose and fallow deer; (4) some species have a unique antler shape, for example, antlers of milu grow their tines posteriorly, instead of anteriorly, and tips of all well developed tines of an antler reach the similar level, and, hence, can be stably placed on a levelled surface upside down. Milu has been called 'four unlikes' and is regarded as an auspicious animal, one of the reasons for that is its unique antler shape.

Additional control

The three axes of regenerative antlers have much commonality. On the basis of Thompson (1917), the spatial shape of a branched antler is spherical. Pocock (1933) thought that the royal (topmost) tines of some deer species, such as red deer, constitute a 'cup' shape. Symmetry seems to be a 'general rule' in the morphogenesis of even-numbered organs in a body. In the most deer species, the two antlers are symmetrical, just like our left and rights hands (Goss 1995). However, some deer species do not follow this rule. For example, the two brow tines of reindeer antlers are intentionally asymmetrical (Goss 1995). Normally, the brow tine on one side has a branched or palmate shape, whereas the one on the other side is unbranched or missing. The larger brow tine normally grows forward and bends towards the middle line. The choice of the dominant side is random, but once this is determined, the opposite side will give way to the dominant side and forms only a small brow tine or none at all (Goss 1980, 1995). Likewise, the F1 hybrid between sika deer and wapiti inherit antler characteristics from both species, and sometimes show asymmetry, with one side having the wapiti antler shape (with a bez tine) and the other side the sika deer antler shape (absence of a bez tine) (Li *et al*. 1988). More interestingly, this antler shape allocation is not fixed, suggesting that the morphogenetic primordia for antlers are endowed with the full information needed to develop antlers of either side or species, but this full information is not expressed at the same time, i.e. the final shape of an antler does not possess characteristics of both sides or species. Consequently, there must exist an additional control mechanism. This mechanism must control the final antler shape microscopically and macroscopically. This control must not only regulate the asymmetry described above, but also fix antler shape as 'cup', 'ball' or 'crown'. The nature of this control is currently unknown.

Regeneration

One of the criteria for a suitable model for morphogenetic study is the retention of the ability to periodically recapitulate the specific morphogenetic process. Thus far, almost all models for morphogenetic research have been primitive animal species, as only these species have the ability to regenerate, although to variable degrees. Deer antlers, the mammalian organs that can regenerate annually, are unique in that aspect and meet this criterion.

The antler regeneration cycle is well defined and its timing is very similar among most deer species. However, there are some exceptions, such as the Père David deer (milu), whose antlers are cast and regenerate in winter (we have an extra term in the winter solstice in China, called 'Mi Jiao Jie', which means that it is the season for milu to cast their hard antlers), their growth period occurs in spring, and calcification in summer. In most deer species, antler regeneration takes place immediately following the previous hard-antler casting, but moose and white-tailed deer are exceptions to this rule. In these species, there is a gap of few weeks between previous antler casting and new antler regeneration. Compared with the ordinary mammalian organs that form only once in an animal's life time, periodic regeneration of deer antlers is in yearly cycles and its difference among species provides us an unique opportunity to investigate the 'temporal attribute' of mammalian organ morphogenesis (morphogenesis only takes place at a specific time).

Periodic regeneration of deer antlers provides a useful model for studying the epigenetics of mammalian organ development. The 'memory' phenomenon is involved in antler regeneration. Bubenik and Pavlansky (1965) called this phenomenon 'trophic memory'. Wounding on the surface of a growing antler can cause an extra tine to form on the site. This extra tine is remembered by the deer as it reappeared at the same location in antlers subsequently grown by the deer for the next 4 years. No extra tine was detected on the contralateral side antler. Obviously, no genetic change was involved in this incidence, but the phenomenon was displayed for several years and then gradually disappeared. Two aspects of biological information might be involved in this, namely, (1) epigenetic phenomenon, and (2) maintenance of a three-dimensional structure. There are numerous advantages in using the antler model to track epigenetic control of mammalian organ morphogenesis through this wounding approach and to study the mechanism underlying the maintenance of organ morphogenesis, such as short cycle and low costs. We do not know whether a relationship between the 'trophic memory' and species-specific antler branching exists. If it does, establishment of the antler branching process must have gone through the following steps: during the course of evolution, 'trophic memory' was gradually fixed genetically under the pressure of both initial impetus and selection, and was then formed into a stable genetic trait. This stable genetic trait was expressed as the pattern of species-specific branching.

Manipulation

Deer antler morphogenesis can be altered artificially by manipulating, for example, the AP and sex hormones. Therefore, we can readily explore the morphogenesis of mammalian organs using the antler model.

Discovery of the AP has been hailed as the hallmark of antler research history, as all the morphogenetic information is endowed in the AP (Gao *et al*. 2012). Because of this, AP cells can grow ectopic antlers where they are subcutaneously implanted on the deer body (Hartwig and Schrudde 1974; Goss 1987). Even more surprising, if xenografted on a nude mouse, deer AP can induce pedicle-like or even antler-like bony protuberances (Li *et al*. 2001, 2009). The morphogenesis of these ectopic antlers on the deer body is very similar to that of the original antlers: every phase of an ectopic antler growth cycle is synchronised with that of the original antlers, suggesting that the AP is endowed with the full information needed for antler morphogenesis (Goss 1990). Goss and Powel (1985) reported that if the area is same, the half-round AP has greater potential than the full-round AP for antlerogenesis, suggesting that antler morphogenetic information may not distribute evenly. Before initiation of the pedicle and antler generation, if the AP is rotated 180° and then placed back to the original site, polarity will change accordingly and branching occurs posteriorly (Goss 1991). This provides direct evidence that the distribution of morphogenetic information is regionalised in the AP. To confirm this, Gao *et al*. (2012) conducted the following experiment: the entire piece of AP was divided into four halves, namely, anterior half, posterior half, medial half and lateral half. Subsequently, each half of the AP was transplanted subcutaneously to the forehead. The results showed that morphogenetic information mainly resides in the anterior half and the medial half of the AP. The anterior half controls the brow tine formation, and the medial half controls the formation of the rest of the tines. Antler generation fully relies on the AP; hence, we can directly work on this target tissue when we study antler morphogenesis, which thus greatly simplifies the complicated system.

On the basis of the reports using the antler model, some conclusions can be made. (1) Morphogenetic primordia have a certain capacity to readjust their polarity to reach normal morphology if being altered. For example, the minced AP tissue (pieces arranged 1–2 mm in size) from fallow deer could still initiate almost normal antler formation when grafted back autologously; a whole piece of AP when grafted back to the original place upside down (no change in anterior–posterior polarity, but change in medial-lateral polarity) could form normal antlers (Goss 1991; Gao *et al*. 2010). These findings indicate that when the morphogenetic information in the AP is disturbed by external factors, the AP itself has certain regulative power. (2) Morphogenetic information can be superimposed. When the AP from the left side was grafted upside down on top of the AP from the right side, the grafted site would form a normal antler. Compared with the singularly upside-down transplanted AP, the formed antler was bigger, being 53% longer and having 38% more tines (Goss 1991), suggesting that morphogenetic information can be augmented in a certain extent. Therefore, when the AP launches antler morphogenesis, the regionalised and relatively independent morphogenetic information in the AP must coordinate in this process.

An additional advantage for using antler as a morphogenetic research model is that antlers belong to a male secondary sexual appendage, which is strictly under the control of sex hormones (Bartos *et al*. 2012). This attribute provides us a simple control means for investigating organ morphogenesis. Both males and females possess AP, but only males (except in reindeer) form pedicles and antlers; this is because the AP initiates pedicle growth only when it is stimulated by high levels of androgen (Goss 1983; Suttie *et al*. 1995; Li *et al*. 2003). Females also have the potential to launch pedicle and antler growth, and this potential can be evoked by administrating exogenous androgen hormones (Jaczewski *et al*. 1976; Suttie *et al*. 1995; Li *et al*. 2003).

Advantages

Antler morphogenesis is very similar to limb formation during embryogenesis (Li and Suttie 2001*b*). (1) Both originate from discrete primordia, the lateral plate mesoderm (LPM) and AP, respectively. If LPM is deleted, limbs fail to form, whereas if the LPM is transplanted along the body plane and away from the presumptive limb-formation region, an ectopic limb will form. Rotation of the LPM when grafted back will form a polarity-reversed limb (Kieny 1968). (2) Limb formation is initiated by the activation of a group of mesenchymal cells resident in the LPM (Todt and Fallon 1984). Formation of a pedicle and antler is also achieved through activation of a group of mesenchymal cells in the AP (Li and Suttie 1994). To study limb formation, one has no choice but to use early developing embryos. Because these embryos are tiny and reside in the uterus, their manipulation requires dedicated facilities and skillful hands. In contrast, to study antlers, one only needs to find a source of deer and to wait for right season, as antlers are gigantic (relative to embryos) and external organs, and so can be easily manipulated.

Levin (2012*b*) considered that mammalian teratoma would be an ideal model for studying modularity of morphogenetic information (relative to the regionalisation of morphogenetic primordia), but teratoma is very rare, occurring at an incidence of 0.0053% in China (Dai *et al*. 2002). Deer are farmed all around world; hence, they can be accessed almost anytime and anywhere. Therefore, the antler model can greatly complement the teratoma model in this aspect.

Research approaches

The slow progress of morphogenetic research on mammalian organs could be due to the following two limiting factors: (1) lack of a suitable model for conducting the study and (2) appropriate techniques not being available. The achievements made in the field of bioelectricity (discovery of the result that bioelectricity encodes morphogenesis in primitive animals) will undoubtedly speed up the progress of morphogenetic research, and, hence, will highlight the importance of the antler model for morphogenetic research in mammals. We predict that the successful establishment of the antler model will be the first point of breakthrough for understanding the bioelectric code of mammalian organ morphogenesis, ultimately benefitting human health and wellbeing.

The most important step for understanding and utilising the bioelectrical code is to find a way to track down bioelectric information. In this aspect, we may consider the AP as an internal memory box, within which morphogenetic information is stored (here, we presume that morphogenetic information is made up of bioelectric elements in mammals, just like in primitive animals). The key aspects of the present study are to find a way to read and understand the code information. To detect the dynamic value of bioelectricity in the AP before antler formation is the basis for being able to read bioelectric information. Modern technology has made this possible; for example, application of fluorescent bioelectricity reporters (Adams and Levin 2012*a*, 2012*b*) allows us to record dynamic changes in bioelectricity in live animals. To understand the antler morphogenetic code, the following steps should be conducted: (1) analyse the relationships between bioelectric parameters and morphogenesis; (2) identify the pattern of bioelectric coding (binary, or similar to but higher pattern than binary); and (3) identify the general rule, i.e. the bioelectric pattern that encodes antler morphogenesis.

To investigate morphogenetic information (bioelectric elements) of deer antlers through bioelectricity, we need to find out where antler morphogenetic information is stored, where it comes from and where it is applied. In the early period of embryo development, AP has already acquired the species-specific antler morphogenetic information, but this information remains dormant. Only at the time after the deer is born and is approaching puberty is the expression of this information initiated and the pedicle begins to develop. Eventually, the morphogenetic information in the AP is transferred to and stored in the pedicle, specifically the PP, into which the AP develops. Subsequently, a small proportion of the cells of the distal part of the PP give rise to a regenerating antler (Li *et al*. 2007*a*) under the guidance of morphogenetic information which is re-accessed year after year, suggesting that all antler morphogenetic information is permanently stored in the PP.

With regard to the way through which the AP transfers the stored morphogenetic information to the PP, we infer that (1) the AP duplicates a few copies of the morphogenetic information, and these copies are stored in the PP along the longitudinal axis of a pedicle. One copy is activated per year for each round of antler regeneration, (2) the AP does not duplicate its morphogenetic information, but directly transfers the information as a whole to the PP of the developing pedicle, and then the PP gives rise to one copy to the regenerating antler at the right season. These hypotheses can be experimentally tested through several means, as discussed below. (1) Before the initiation of antler regeneration, identify the demarcation between the potentiated and the dormant regions along the longitudinal axis of a pedicle (Li *et al*. 2007*b*), and divide these two regions circumferentially with a scalpel; carefully peel off the potentiated PP and rotate it 180° around the bony core, and observe the morphogenesis of the subsequent regenerative antler. If morphology of the subsequently regenerated antler is changed following the rotation, that means that the morphogenetic information is already stored in the potentiated PP, otherwise it would mean that the morphogenetic information is duplicated from the proximal part of PP only at the time when the antler starts to regenerate. If antler regeneration fails to take place, this could mean that the surgery was not successful. (2) Before initiation of antler regeneration, the PP is evenly divided and cut into several equally distant rings; retain or delete a PP ring, and observe the morphogenesis of the subsequent antler growth. If a retained PP ring within the dormant region can initiate antler regeneration with its species-specific shape, this would indicate that antler morphogenetic information is stored in a segmented fashion, or *vice versa*. No matter which way it may be, the duplication of morphogenetic information would have to have been carried out to achieve the final goal. Compared with the development of limbs during embryogenesis, when the LPM gives rise to limbs, no PP-equivalent tissue is involved; hence, there is no tissue carrier for storing morphogenetic information. Perhaps one of the main reasons why antlers can regenerate is because they have the ability to duplicate their morphogenetic information and possess the necessary tissue carrier (possibly the PP) needed for storing the information.

Conclusions and prospects

Dynamic transferance and duplication of morphogenetic information during antler development offers us an opportunity to track the passage of morphogenetic information and to investigate the possible role of bioelectricity (note that it would be difficult to investigate morphogenetic information if the information is fixed and does not transfer). Exploration of the correlation between bioelectricity and antler morphogenesis would further our understanding of, for example, (1) how additional control works on organ morphogenesis and (2) how bioelectricity influences organ regeneration in mammals.

Sex hormones control the morphogenesis of secondary sex characters. What is the relationship between sex hormones and bioelectricity? Do sex hormones locate upstream of bioelectricity and work as dose-dependent 'on/off switches' to activate bioelectricity code? Perhaps we can get the answer from the investigation of a possible role of bioelectricity in deer antler morphogenesis.

Antlers and tumours have some similarities; both are derived from stem cells and share numerous commonalities, including the distribution of rich blood vessels and extreme growth rate. The only factor that distinguishes antlers from tumours is that antler growth is under the guidance and control of morphogenetic information, and, hence, forms orderly tissue types, whereas tumorogenesis is not guided by morphogenetic information, and forms disorderly tissue types. The results of research into bioelectricity and antler morphogenesis may help us understand the underlying morphogenetic mechanism as to how a tissue can grow normally and does not go cancerous under the condition of extreme growth rate.

We wish, one day, the bioelectrical code that controls organ morphogenesis can be cracked so that we can use the unique deer antler model to investigate ways of duplicating and storing morphogenetic information during mammalian organ development. On the basis of the results of these investigations, we may be able to duplicate the morphogenetic information that is normally unable to be duplicated in non-regenerable organs, and to store the duplicated information in the proper tissue carrier. This stored information could be then activated at the plane of arm or leg stumps, which can deflect wound healing towards the direction of regeneration.

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