Gap Junction Remodeling in Skin Repair Following Wounding and Disease

In the present review, we provide an overview of connexin expression during skin development and remodeling in wound healing, and reflect on how loss- or gain-of-function connexin mutations may change cellular phenotypes and lead to diseases of the skin. We also consider the therapeutic value of targeting connexins in wound healing.

Since the 1970s, it has been known that gap junctions establish specialized communication compartments within the epidermis suitable for the exchange of metabolites and secondary messengers. Upon the identification of the connexin family of genes in the 1980s and 1990s, the gap junction community was intrigue to find that upward of nine members are expressed in a very precise temporal and spatial context within the epidermis. Connexin patterning and remodeling within the epidermis was found to be even more complex during wound healing where connexin expression is uniquely correlated with the stages of wound repair that included keratinocyte proliferation, migration, and differentiation. Furthermore, recent studies have shown that the connexin family members expressed in dermal fibroblasts, especially Cx43, may play a more active and essential role during the healing process rooted in fibroblast proliferation, migration, and differentiation. The last 20 years have subsequently uncovered a number of distinct skin diseases that are the result of patients harboring germ-line mutations in the genes that encode six different connexins.

Gap Junctions

Gap junctions are aggregates of protein channels formed between apposing cells that facilitate intercellular communication by allowing the passage of small molecules and metabolites from one cell to another (1). These intercellular channels assemble from connexin (Cx) subunits named after the molecular mass of each family member (e.g., Cx43 has a molecular mass of 43 kD) (69). Six connexins oligomerize within a cell and are transported to the plasma membrane in a configuration known as a connexon. Connexons at the plasma membrane can open and close to exchange molecules with the extracellular environment in a state known as a hemichannel (27). When one connexon docks with a connexon from an adjacent cell, a gap junction channel is formed. These channels laterally diffuse and aggregate into a semi-crystalline state known as the mature gap junction or a gap junction plaque, where they open and close to facilitate intercellular communication (reviewed in Ref. 29). Gap junctions are one of the most interesting and unique structures found between adjoining cells. The importance of these novel structures is highlighted by the fact that virtually all cells found in solid tissues express connexins and assemble gap junctions. As demonstrated nearly 30 years ago, the mature gap junction domain itself consists of tightly aggregated connexin channels that are thought to extrude all other integral membrane proteins (47). However, gap junctions are highly dynamic, subject to rapid remodeling and turnover (39). Nucleation, growth, remodeling, internalization, and turnover of the gap junction domain are all critical for establishing the appropriate physiological levels of intercellular communication in all tissues and organs (reviewed in Ref. 38).

Connexin Expression in the Developing and Maturing Skin

The fully formed epidermis is composed of four distinct layers in thin skin: the stratum basale, stratum spinosum, stratum granulosum, and stratum corneum (FIGURE 1). Together, these layers form a protective coating that blocks many mechanical and environmental insults the human body procures over time. Keratinocytes within the basal layer maintain stem cell-like characteristics and play an important role in the renewal and regeneration of damaged skin (32). Keratinocytes in the stratum basale also rest on a basement membrane and have a high proliferative index (68). As keratinocytes in the stratum basale differentiate, they form the stratum spinosum and stratum granulosum. During the formation of the stratum corneum, differentiated keratinocytes in the stratum granulosum, called corneocytes, undergo cell death and release keratins, loricrin, and involucrin. Paradoxically, although this layer contains no living cells, this layer is enzymatically active. Enzymes such as transglutaminase 1 and 3 cross link many of these proteins to protect the skin from mechanical insults in this layer.
The epidermis is composed of four layers in thin skin: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. In unwounded epidermis Cx43 (red), Cx31.1 (purple), Cx40 (blue), Cx31 (green), Cx37 (brown), and Cx30 and Cx26 (both in yellow) localize to various epidermal layers in the adult rodent epidermis. Dermal fibroblasts also express Cx43, Cx40, and Cx45 (not shown). Twenty-four hours after the epidermis is wounded, the expression of Cx30 and Cx26 shifts from being expressed in the stratum granulosum to all epidermal layers, whereas the expression of Cx31.1 is drastically reduced. Cx40 and Cx43 levels also decrease at the wounded margin, and Cx40 is localized to the stratum basale after wounding. In the dermal fibroblasts however, Cx43 expression is elevated.

FIGURE 1. Connexin expression in unwounded and wounded epidermis
The epidermis is composed of four layers in thin skin: stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. In unwounded epidermis Cx43 (red), Cx31.1 (purple), Cx40 (blue), Cx31 (green), Cx37 (brown), and Cx30 and Cx26 (both in yellow) localize to various epidermal layers in the adult rodent epidermis. Dermal fibroblasts also express Cx43, Cx40, and Cx45 (not shown). Twenty-four hours after the epidermis is wounded, the expression of Cx30 and Cx26 shifts from being expressed in the stratum granulosum to all epidermal layers, whereas the expression of Cx31.1 is drastically reduced. Cx40 and Cx43 levels also decrease at the wounded margin, and Cx40 is localized to the stratum basale after wounding. In the dermal fibroblasts however, Cx43 expression is elevated.
(31), whereas various lipids give the skin its waterproof property (30).

Nine different connexin family members have previously been reported to be expressed in the rodent epidermis (Cx26, Cx30, Cx30.3, Cx31, Cx31.1, Cx37, Cx40, Cx43, Cx57) (25, 37, 59), and these connexins are expressed in regional and temporal expression patterns during development. During the embryonic E12–E14 stage of rat epidermis development, the epidermis is composed of two cellular layers: the outer periderm and the inner basal layer. Both Cx43 and Cx26 are expressed in these layers, and, before the periderm layer undergoes apoptosis, Cx26 expression is downregulated (62). Later in rodent epidermis development (E17–E20), Cx43, Cx45, Cx31.1, and Cx37 have all been reported to be expressed in the basal layer, whereas Cx26, Cx37, Cx31, and Cx43 are restricted to the stratum spinosum and stratum granulosum (8, 25, 62).

In the adult mouse epidermis (FIGURE 1), Cx40 and Cx43 have been reported to be expressed within the stratum basale (8, 33). In humans, 10% of the keratinocytes in the stratum basale do not express Cx43, and these cells are reported to have stem cell-like characteristics (50). In the stratum spinosum, Cx43 is highly expressed, whereas in the stratum granulosum, no Cx43 expression is observed (8, 11). Cx37 is expressed in the stratum spinosum and stratum granulosum (25), whereas Cx31.1 was found to be predominantly expressed in the lower basal layer and in the lower stratum spinosum (25). Cx26 and Cx30 expression is restricted to the stratum granulosum in the rodent epidermis; however, one study observed Cx26 to be expressed in the upper stratum spinosum in human epidermis as well (74), whereas an additional study could not localize Cx26 to any epidermal layer (65). In addition, Cx31 was observed to be expressed in the stratum granulosum and in the upper stratum spinosum (36). Immunolocalization of other connexins, however, has not been investigated to date due in large part to the lack of available high-quality antibodies.

In the dermis, human dermal fibroblasts are reported to express Cx43, Cx45, and Cx40 (76). Cx43 has also been reported to be expressed in the arrector pili muscle, sweat glands, sebaceous glands, and hair follicle (9). In the development of the human hair follicle, both Cx26 and Cx43 expression could be observed within the hair peg (2). As the hair follicle grows, Cx26 was identified within the outer root sheath and the inner root sheath but absent in the hair matrix. Cx43, however, was prominently expressed with the inner root sheath and hair matrix and weakly expressed within the outer root sheath. Later in development, intense Cx26 expression was found in the outermost layer of the outer root sheath, whereas Cx43 was highly expressed within the innermost layer of the outer root sheath (2). In addition, Cx30 has also been described to be expressed within the outer root sheath of the adult human hair follicle (21). Cx43 expression during the anagen stage of rodent hair follicle growth has also been reported to be expressed in the inner and outer root sheath, the dermal papilla, and the proliferating matrix. However, during catagen, Cx43 expression is absent in the inner root sheath (10, 62).

Regulation ofConnexin Expression and Localization During Wound Healing

When the skin is injured, the epidermis and dermis must reform the wounded area. Within the first 24 h of wounding, Cx26 and Cx30 expression increases in all strata (FIGURE 1). In addition, there is a drastic decrease in the expression of Cx31 and Cx31.1 with Cx31 expression becoming evident in the stratum basale (26, 36). Cx43 levels in the stratum basale and spinosum proceed to decrease at the wound edge (6, 26, 36, 40). Keratinocytes residing in the basale layer also selectively express the phosphorylated S368 species of Cx43, suggesting that the phosphorylational state of this connexin responds to the onset of wounding (61). Additional studies should be instrumental in determining how connexin phosphorylation events modulate wound healing. To that end, it has recently been shown that CASK predominantly binds to the hypophosphorylated state of Cx43 and that this interaction influences cell migration (49). The differential expression and phosphorylation state of connexins during wound healing may also allow the selective passage of transjunctional molecules to promote either the proliferation or differentiation of keratinocytes. This is supported by an older study that demonstrated that when keratinocytes were induced to differentiate under high calcium condition, Cx43 and Cx26 levels decreased, whereas the levels of Cx31 and Cx31.1 increased, suggesting that a new composition of intercellular channels were formed (7). In the future, determining which molecules selectively pass through various heterotypic and heteromeric gap junction channels should be critical to our understanding of how changes in connexin expression and regional localization can affect the intercellular signaling networks.

The overall mechanisms of how the multitude of connexins regulate wound healing remains only partially understood, but some studies suggest that wound healing is enhanced when Cx43 levels are lowered. Since Cx43-null mice die from right ventricular outflow tract abnormalities (58) and Cx26-null...
Connexins in Keratinocyte Proliferation and Linkage to Disease

During the initial stages of wound healing (days 1–3 postwound), keratinocytes at the wounded edge increase their proliferation rate (15) and migrate under the coagulum. Since Cx43 levels decrease and Cx26 increase at the wounded edge, these connexins are suggested to play distinct roles during proliferative and migrational events in the early stages of wound healing. Mechanistically it is not surprising to observe a reduction in the plasma membrane pool of Cx43 since it has been documented to play a role in cell-to-cell adhesion (56). With a net reduction in adhesion, cells would be permitted to proliferate and begin their migration into the wounded area. In parallel, persistent Cx26 expression is thought to maintain the wounded epithelium in a hyperproliferative state (19). Cx26 and Cx30 have also been found to be highly expressed in non-healing chronic wounds (6) and in keratinocyte-derived skin tumors (28). In addition, the expression of Cx30 was upregulated in human psoriatic skin, irradiated skin, as well as primary keratinocytes subjected to irradiation, further supporting the role of Cx30 in hyperproliferation (44). Although both Cx26 and Cx30 are spatially and temporally co-regulated during wound healing and these connexins can form heterotypic channels, it is possible that autosomal-dominant and/or -recessive mutations in the genes encoding these connexins may result in a similar skin disease phenotype. This turns out to be the case in Clouston syndrome and Vohwinkel’s syndrome (41, 42).

Clouston’s syndrome is a disease caused by mutations in the GJB6 (Cx30) gene. Patients with Clouston’s syndrome develop nail dystrophy, palmar plantar hyperkeratosis, and slow growing, fine, dry, brittle, and sparse hair (14). Mutant Cx26 expression also causes several skin diseases including Vohwinkel’s syndrome (46). Vohwinkel’s syndrome results in starfish-like acral keratoses, papular and honeycomb keratoderma, palmar plantar hyperkeratosis, constricting bands around the digits, and moderate hearing loss. One symptom shared by both Clouston’s syndrome (Cx30 mutations) and Vohwinkel’s syndrome (Cx26 mutations) patients is an epidermal thickening in the palmar and plantar regions of the hands and feet (palmar plantar hyperkeratosis). This phenotype could also be observed in the tail epidermis of a mouse model of Vohwinkel’s syndrome where the D66H mutant Cx26 was expressed in the epidermis under the control of the keratin 10 promoter (4). Although it is tempting to correlate the epidermal expression of Cx26 and Cx30 with the proliferation of keratinocytes and palmar plantar hyperkeratosis, palmar plantar hyperkeratosis is also observed in patients with oculodentodigital dysplasia (ODDD), which is caused by mutations in the GJA1 gene that encodes Cx43 (71, 72) and in patients with erythrokeratodermia variabilis, which is linked to Cx31, Cx37, and Cx31.1 mutations (60, 70). Since mutations in the Cx26, Cx30, Cx31, Cx37, Cx31.1, and Cx43 gene can result in palmar plantar hyperkeratosis, a full complement of functional GJIC throughout the stratified compartments of the epidermis is likely necessary to maintain proper skin homeostasis.

The transient and systematic decrease in Cx43 levels at the wound edge may suggest that Cx43 expression acts to slow the proliferation of keratinocytes. Consistent with this notion, in mice expressing 85% lower epidermal Cx43 levels (tamoxifen-induced Cx43Cre-ER(T)/fl), a compensatory increase in Cx30 was observed, which appeared to support cell proliferation (36). Likewise, mouse wounds treated with an antisense oligonucleotide against Cx43 also led to an increase in cell proliferation at the wound edge (51). Furthermore, when a Cx43 mimetic peptide (Gap27), which blocks connexon docking, was applied to porcine and human keratinocytes, an increase in proliferation was observed (55). The ability of Cx43 to modulate keratinocyte proliferation is further supported in a study that demonstrated that keratinocytes derived from the Cx43 G60S genetically modified mouse model of oculodentodigital dysplasia were enhanced in their ability to proliferate (11). Collectively, strategic reductions in Cx43 levels
appear to promote keratinocyte proliferation and assist in the initial stages of wound healing.

Although in vivo studies suggest that downregulation of Cx43 promotes the proliferation of keratinocytes, perplexing studies have shown that sparsely plated human keratinocytes express Cx43, and it is only after cell confluency is reached that a downregulation of Cx43 is observed (24). In addition, undifferentiated primary mouse keratinocyte cultures also express high levels of Cx43, and these levels go down after Ca\textsuperscript{2+}-induced differentiation (7). The in vitro vs. in vivo differences observed in the expression of Cx43 suggest that monolayer keratinocyte cultures may not truly represent the basal keratinocyte phenotype present in the in vivo environment. The reasons for the paradoxical differences in the role of Cx43 in cell proliferation is not entirely clear, but it may be rooted in the fact that in vitro studies fail to take into account the basement membrane signaling events seen in vivo and the complex stratification of the epidermis. Future studies need to consider the complex microenvironment of keratinocytes and employ more elaborate organotypic epidermal cultures.

**Cx43 in Dermal Fibroblast Proliferation**

Although the majority of connexin-linked wound healing studies have focused on the role of connexins in keratinocytes, the role that dermal fibroblasts play in wound healing and connexin-linked skin diseases is far less clear. Unlike keratinocytes, Cx43 is surprisingly upregulated in dermal fibroblasts at the wound edge (15). Since fibroblasts proliferate in the wounded area, an increase in Cx43 expression might suggest that it enhances proliferation. In addition, dermal fibroblasts isolated from ODDD patients were shown to have a decrease in Cx43 expression, and these fibroblasts were shown to have slower proliferation (13). However, the proliferation rate of fibroblasts derived from Cx43-null and heterozygote mice did not differ from fibroblasts derived from wild-type mice (77). In contrast, fibroblasts derived from the murine cardiac tissue from Cx43\textsuperscript{+/-} mice demonstrated an increase in cell proliferation (78), suggesting that differences may exist depending on the tissue origin of the fibroblasts.

**Cx43 as a Regulator of Keratinocyte and Dermal Fibroblast Migration**

Without doubt, Cx43 is the most well studied connexin when migrational properties of keratinocytes and dermal fibroblasts following wounding are considered. In a scratch wound assay, Cx43 mimetic peptides Gap26 and Gap27 (that reduce the plasma membrane levels of Cx43) increased the migration rate of both human keratinocytes and dermal fibroblasts (76). In addition, Gap27-treated mouse keratinocytes exhibited enhanced migration into a wounded epidermis (34). Mechanistically, some evidence suggests that this is linked to cell adhesion (37) or communication properties (53) rather than to effects on the connexin interactome (54). For instance, the antisense targeted knockdown of Cx43 results in enhanced mouse dermal Swiss 3T3 fibroblast migration (51). Reciprocally, the chronic absence of Cx43 (Cx43\textsuperscript{-/-}) in mouse embryonic fibroblasts resulted in a decrease in fibroblast migration (22), which was thought to be due to a disruption of the microtubule organization center and adverse effects on cell polarity. In one of our studies, dermal fibroblasts derived from a mouse model of ODDD harboring the G60S Cx43 mutant as well as dermal fibroblasts obtained from patients with ODDD revealed that the expression of mutant Cx43 and overall reduction in GJIC delayed the migration of fibroblasts (13). It remains to be determined whether chronic and acute reductions in Cx43 levels or function have reciprocal roles in regulating dermal fibroblast migratory properties. Since migrating keratinocytes are not exposed to basement membrane proteins (43) and keratinocytes at the wounded edge have reduced Cx43 levels (76), it is possible that, only when keratinocytes are able to synthesize and deposit basement membrane proteins, a decrease in keratinocyte migration (52) and an increase in Cx43 expression will be observed. This notion is supported in a study that demonstrated that human keratinocytes adhered to laminin 5 had greater Cx43 levels and gap junctional coupling compared with keratinocytes plated on collagen or fibronectin (6, 40).

**Cx43 in Keratinocyte Differentiation**

Once keratinocytes have migrated into the injured epidermis as part of the reepithelialization process, keratinocytes must differentiate to reform the epidermal barrier. Since connexins are differentially expressed during keratinocyte and fibroblast differentiation, they may also play an important role in regulating this differentiation process. Mice lacking Cx31 (Gjb3\textsuperscript{-/-}) develop an epidermis and did not show any skin defects (54). In addition, mice lacking both Cx31 and Cx43 were not found to have any epidermal differentiation defects at fetal day 17.5 as defined by histological assessment and evaluation of keratins 6, 10, and 14, loricrin, and filaggrin (35). However, in mice expressing mutant forms of Cx26 [S17F (67), D66H (4)] and Cx31 [F137L (66)], severe epidermal defects are
observed. This may be due to the dominant effect that mutant connexins have on wild-type connexins (12, 18, 63). Although skin development abnormalities have not typically been identified in embryonic connexin-null mice, many connexin-linked diseases of the skin have later onsets ranging from birth through to adulthood; thus connexins must play a substantial role in epidermis maintenance and renewal.

To investigate whether a loss-of-function Cx43 mutant would impact the differentiation of the epidermis, we cultured mouse keratinocytes derived from G60S genetically modified mice that mimic ODDD and induced partial differentiation by the addition of high calcium. Although keratinocytes expressing mutant Cx43 exhibited higher levels of the terminally differentiation markers loricrin and involucrin upon partial differentiation, it is important to note that primary mouse keratinocytes do not differentiate well in culture (11). In a more in situ-like situation, we found that rat organotypic cultures formed from keratinocytes expressing various ODDD-associated mutants developed a thinner stratum corneum layer compared with cultures derived from keratinocytes expressing wild-type Cx43. In addition, organotypic cultures expressing a mutant Cx43 reported to cause palmar plantar hyperkeratosis (fs260) also resulted in parakeratosis in the stratum corneum layer (12). Langlois et al. further demonstrated that a shRNA-targeted reduction in Cx43 expression could disrupt the organotypic architecture and reduce the production of loricrin and involucrin (42). This would suggest that not only is the expression of Cx43 important for maintaining epidermal tissue architecture but disrupting Cx43 expression in the epidermis may also damage the epidermal barrier and thus activate the differentiation pathway to reform the epidermal barrier. Interestingly, the shRNA-targeted reduction of Cx43 also resulted in a decrease in Cx26 levels in organotypic cultures. Consistently, Cx26 levels were also lower in the adult skin of mutant mice selected for their heterozygous expression of the Cx43 G60S mutant (42). Thus the transdominant effect of Cx43 on Cx26 or some sort of cross talk between these connexins may underlie ODDD in patients that harbor one of two frame-shift mutant linked to the syndromic aspect of the disease resulting in the comorbidity of palmar plantar hyperkeratosis. Since Cx26 is absent in uninjured forearm skin but weakly expressed in the basale layer of plantar skin region (45), the palmar plantar phenotype may be due to the transdominant nature of Cx43 on Cx26 (45, 64). In addition, although only two frame-shift ODDD-linked Cx43 mutants lead to skin defects, Cx26 mutants are strongly associated with human and mouse skin disease. Mice expressing the D66H Cx26 mutant were shown to have a propensity for premature cell death. This premature cell death may weaken the epidermal barrier of the skin and activate the basale keratinocytes to reform the epidermis. Overstimulation of the keratinocytes may also increase the thickening of the epidermis (4) and lead to palmar plantar hyperkeratosis. It is interesting to note, however, that others have shown that Cx26 D66H mutant did not interfere with the formation of the epidermal water barrier during late embryonic development (5) and that the deafness-associated mutant human Cx26 (R143W) was shown to improve the epithelial barrier in vitro (48). The characterization of additional mutant mouse models of connexin-linked skin diseases are necessary to unravel the complexities of how individual connexin mutations lead to various skin diseases that may have early or late onset in life.

**Cx43 in Fibroblast Differentiation**

Although dermal fibroblasts are embedded in the collagen mesh of the dermis, ~30% of these fibroblasts are thought to have processes that connect with other fibroblasts where Cx43 mediates intercellular communication (41). Since fibroblast differentiation into myofibroblasts is an active process in wound healing, it has been proposed that Cx43 and GJIC play an active role in this differentiation step as well as in the secretion of collagen (17). In Mori et al., knockdown of Cx43 was shown to enhance fibroblast-dependent wound healing (51). In this study, mouse wounds treated with Cx43 antisense had significant increases in the levels of hydroxyproline and mRNA for collagen type α1 and TGF-β1 compared with control-treated wounds. An increase in myofibroblast differentiation and wound contraction was also found when Cx43 was reduced by antisense treatment (51). In addition, antisense targeting Cx43 was shown to decrease the amount of granulation tissue and scarring in the wound healing of burns (16). Surprisingly, we found that dermal fibroblasts derived from patients with ODDD expressed a decrease in the phosphorylated species of Cx43 and expressed lower levels of the myofibroblast marker smooth muscle actin after TGF-β1 treatment (13). In addition, fibroblasts derived from Cx43-null mice demonstrated a reduced ability to contract as well as a failure to elongate (20). This suggests that TGF-β1 signaling may require normal Cx43 expression to differentiate fibroblasts into myofibroblasts. If this is true, mutant Cx43 expression in fibroblasts may impair the ability of dermal fibroblast to contract a wounded area.
Final Thoughts and Future Considerations

It is clearly apparent that the connexin status affects skin physiology leading to changes in wound healing and susceptibility to skin diseases. Although there are many connexins expressed within the skin, the fundamental role that each connexin plays is still not well understood. Given that gap junctions can pass small molecules from cell to cell, identification and manipulation of these transjunctional signaling molecules should be instrumental in our understanding of how cutaneous wounds heal. In addition, future studies that identify connexin phosphorylation events that influence connexin localization, function, and interaction should undoubtedly enhance our understanding of their role in wound healing. Ultimately, by studying how connexin expression modulates the function of keratinocytes and dermal fibroblasts, connexin manipulation in the skin may be important in mitigating connexin-related skin diseases and enhancing wound healing. In addition, abnormal connexin expression has been associated with other skin disease pathologies such as diabetic ulcers (3, 55, 75), which are known to have poor healing responses.

Given that the skin is a readily accessible organ, connexin levels could in principal be manipulated with the topical administration of therapeutic creams. When considering how to approach connexin-related skin disorders, it is important to contemplate whether it is more appropriate to regulate (either increase or decrease) the expression of wild-type connexins or to inhibit the abnormal mutant expression of connexins. Future studies where connexins are overexpressed could be employed in an attempt to overcome the detrimental effects of gap junction coupling caused by the expression of connexin mutants, thus ameliorating the underlying skin disease caused by the mutant connexin. The key premise for this approach to work will require that an exogenous connexin remains unaffected by the endogenous mutant and can serve the functional role(s) of the mutated connexin in the appropriate epidermal strata. In addition, elucidating the mechanisms by which various connexin mutants cause skin disorders will be critical for effective therapeutic design. For example, if a specific connexin-related skin disease is caused by disruption of cell-to-cell adhesion between keratinocytes, overexpression of connexins may strengthen the weakened contacts between keratinocytes. If, however, a connexin-related skin disease is caused by deficient transport of metabolites through gap junction channels, increasing the expression of another connexin family member that can rescue the defective transport may be the approach required. In the case of wound healing, effective connexin-based therapies to enhance the healing response time are currently being performed (53) and will likely need to temporally target-specific connexins expressed in keratinocytes and/or dermal fibroblasts.

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