folding of a domain is preceded by interaction with its substrate, the associated changes in entropy can improve binding kinetics. All these properties are relevant in the function of the carboxy-terminal domain of Tom22.

Whatever the precise mechanism by which the TOM complex promotes protein translocation through the outer membrane, the following points seem clear: Tim50 can activate the TOM complex, to promote interactions between the carboxy-terminal domain of Tom22 and a substrate protein. Tim21 is antagonistic, binding the carboxy-terminal domain of Tom22, either after the substrate has been displaced, or displacing the substrate as a consequence of binding. The net result is that substrate proteins in transit through the outer membrane rapidly make productive interactions with the TIM23 complex.

The TIM23 complex is a smart machine. The paper by Chacinska *et al*. [4] is a culmination of work from several labs that now makes clear the TIM23 complex can select and take a substrate protein from the TOM complex and initiate its insertion into the inner membrane. It can respond to the presence of stop-transfer sequences and then shunt the substrate from the translocation channel. In the absence of stop-transfer signals, the TIM23 complex can switch to engage the motor subunits of the PAM complex and thereby complete the translocation of the substrate into the matrix.

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Department of Biochemistry and Molecular Biology, Bio21 Institute of Molecular Sciences and Biotechnology, University of Melbourne, Parkville, Australia, 3010.

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Cell Biology: Master Regulators of Sealing and Healing

The protective layer of the epidermis in *Drosophila* (cuticle) and mice (stratum corneum) are structurally unrelated. Yet new evidence suggests a conserved transcription factor, Grainyhead, controls both their development and the means by which both structures repair themselves.

Brian Stramer and Paul Martin

Amazingly, while the eyes and hearts of *Drosophila* and mammals are constructed in entirely different ways and are morphologically quite distinct, their development appears to be under the control of similar master-regulatory transcription factors: Small eye/Pax6 for eyes [1], and Tinman/Nkx2-5 for heart [2]. From two new papers [3,4], it appears that this theme of signalling conservation controlling analogous structures and processes in these two very different phyla may also be true for epidermal barrier formation and even for wound healing.

The new studies [3,4] have shown that the CP2 transcription factor Grainyhead is required for assembly of the complex chitin coat of the fly as well as the outer stratum corneum of the epidermis in mice. Furthermore, in both flies and mice, Grainyhead is rapidly upregulated in the epithelium if either of these protective outer layers are damaged, and it appears to be necessary for successful healing.

The embryonic epidermis of *Drosophila* is composed of a simple squamous epithelium covered by a protective barrier called the cuticle, which is made of a mesh of crosslinked proteins and chitins [5]. Two enzymes, DOPA decarboxylase and tyrosine

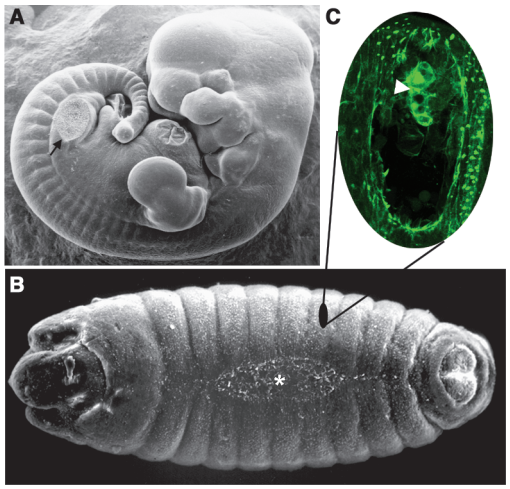

Figure 1. Skin of mice and flies develop and heal alike.
(A) Scanning electron micrograph of a day 12 mouse embryo with a repairing hindlimb amputation wound (arrow). (B) Scanning electron micrograph of a Stage 15 *Drosophila* embryo undergoing dorsal closure (asterisk). (C) High magnification image of an embryonic *Drosophila* wound in which the epithelium and inflammatory cells (arrowhead) have GFP tagged actin.

hydroxylase, are required to catalyze the production of the quinones necessary to cross-link the cuticle proteins creating a final rigid protective barrier [5].

Mace *et al*. [3] showed that these enzymes are rapidly switched on in epidermal cells surrounding wounds in the epithelial surface of *Drosophila* embryos. So not only are these enzymes necessary for cuticle formation during development, but they may similarly be required for epidermal repair.

Previous studies in flies showed that wound healing recapitulates morphogenesis at the level of the cytoskeletal machinery needed to close epidermal holes — actin pursestrings and dynamic filopodial zippers [6,7] (Figure 1) — but the new studies [3,4] now provide evidence for a novel transcriptional regulatory process which is used during development and reused to repair wounds.

The enhancer elements that mediate transcriptional upregulation of the DOPA decarboxylase and tyrosine hydroxylase genes during the wound response have consensus binding sites for Grainyhead, which is localized to the epidermis of *Drosophila* embryos and is required for developmental DOPA decarboxylase expression; not surprisingly, Grainyhead mutant flies have a defective cuticle [8]. DOPA decarboxylase reporter constructs lacking the Grainyhead binding sites fail to be activated around wounds. Furthermore, Grainyhead mutant fly embryos only weakly induce DOPA decarboxylase expression at wound sites, confirming that Grainyhead signalling is key to wound expression of DOPA decarboxylase.

The epidermis of mice and humans is far more complex than that of *Drosophila* embryos; in mammals this layer of the skin consists of a stratified squamous epithelium protected by a structure somewhat analogous to the cuticle in flies [9]. This stratum corneum, much like the *Drosophila* cuticle, is composed of structural proteins and lipids, covalently cross-linked by the enzyme transglutaminase 1, which fulfils the same role as DOPA decarboxylase and tyrosine hydroxylase for crosslinking the fly cuticle proteins [9].

Ting *et al*. [4] showed that Grainy head-like 3 (Grhl3), the mouse homolog of Grainyhead, is expressed in the developing embryonic epithelium from embryonic day 12.5 onward, and that Grhl3 null mice have defects in skin permeability. Grainyhead mutant embryos dipped in dye exhibit a leaky skin at stages when their wild-type siblings are fully impervious to the same dyes.

The reason for this leakiness turns out to be that, just as Grainyhead is needed for cuticle formation in flies, Grhl3 is needed for formation of the stratum corneum in mammalian skin. Ting *et al*. [4] realized that the Grhl3 mutant phenotype is rather similar to that of transglutaminase 1 knockout mice [10,11]. Further analysis of the Grhl3 target by screening random sequences revealed a site identical to *Drosophila's* Grainyhead, and the authors went on to show that the transglutaminase 1 gene contains consensus binding sites for Grhl3.

It is interesting to note that transglutaminase 1 and DOPA decarboxylase/tyrosine hydroxylase are not actual homologs but rather are examples of a convergent evolutionary process that selected for crosslinking enzymes in creation of the outer protective layer of the epidermis; yet the signals driving assembly of these different protective layers appear to be conserved across phyla. The genome of the nematode *Caenorhabditis elegans* also contains a Grainyhead orthologue, Ce-GRH-1, and depletion by RNA interference (RNAi) results in larvae with a fragile epidermal cuticle, similar to the effect of loss of the respective Grainyhead homologue in mouse and flies [12]. Clearly, hunting down the downstream targets of Grainyhead that may regulate morphogenetic sealing processes and/or aspects of the healing response, will be the new goals of several labs in the next few years.

It is obviously premature to give Grainyhead master-regulatory status, as the new studies show its conservation in the differentiation of only a single aspect of the epidermis. But the papers [3,4] leave several unanswered questions that suggest significant additional roles for Grainyhead transcription factors. Ting *et al*. [4] observed a very dramatic failure of Grhl3 mutant mice to re-epithelialise wounds at both E12.5 (about half way through gestation) and later at E16.5. If Grhl3 is really only involved in transglutaminase 1 expression and stratum corneum development, one would not expect a complete failure in re-epithelialization.

Indeed, the barrier function in mice is not even developed until around E18, so why epidermal wound defects found at E12.5? Grhl3 mutant mouse embryos also show defects in neural tube closure [13], an epithelial fusion that shares parallels with the final sealing stages of wound repair [7].

But neural tubes do not contain a stratum corneum, suggesting that Grhl3 may have other roles in epithelial fusion events. Furthermore, overexpression of Grainyhead in *Drosophila* embryos causes a failure in dorsal closure [14], a widely studied epithelial fusion event that has many similarities to wound repair [6,7].

These are not the first reports to suggest a conservation in wound repair signalling between flies and higher vertebrates. The Jun N-terminal kinase (JNK) cascade leading to activation of the transcription factor AP-1 has been widely characterized in tissue repair in both flies and higher organisms [15–17]. Interestingly, the DOPA decarboxylase wound response enhancer has AP-1 consensus sequences, as well as Grainyhead binding sites, and the AP-1 binding sites are necessary for full function of the enhancer, suggesting that this transcription factor may cooperate with Grainyhead. Mace *et al*. [3], however, found that the extracellular signal-regulated kinase (ERK), and not the JNK pathway, appears to be necessary.

One of the holy grails of wound healing research is to determine what are the initial signal(s) that activate all of these cascades. What is upstream of ERK, JNK, Grainyhead, AP-1, small GTPase activation and the other immediate early wound responses, and are these triggering events conserved across phyla? The activation episodes may be growth factor mediated or triggered by mechanical stretching of the wound edge cells as tensions change within the epithelial sheet; in both scenarios, these cues could be shared by analogous morphogenetic episodes like gastrulation, dorsal closure or neurulation [7]. Alternatively, they could be cell damage related cues, for example release of intracellular ATP stores, or signals brought in by inflammatory cells recruited to the wound [18], in which case they might be unique components of the repair response. Either way, adding another conserved repair response to the list lends credence to *Drosophila* as a clinically relevant model of repair. And no doubt, its genetic tractability will lead to new clues into how wound repair may be controlled in the future.

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Departments of Physiology and Biochemistry, University of Bristol, Bristol BS8 1TD, UK.
E-mail: Brian.Stramer@bristol.ac.uk; Paul.Martin@bristol.ac.uk

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Animal Behaviour: Pigtailed Police

A recent study of pigtailed macaques shows that most effective policing interventions in conflict situations are by socially powerful group-members, who sustain relatively low costs by intervening. Questions arise about the ontogenetic and phylogenetic emergence of policing individuals.

James R. Anderson

Monkeys often get involved in each other’s fights. Why should they do this, given that it requires energy and may be risky, especially if one of the opponents turns on them? In most cases, fight interference reflects kin relations and alliances in the group, with individuals coming to the aid of a family member or taking the side of one unrelated group-member against another. The former kind of intervention can be explained in terms of inclusive fitness, as by helping out a relative the interfering individual is promoting the survival of its own genetic material. The latter kind of interference may be an example of reciprocal altruism, with the ally who ‘donated’ the