TOOLS AND TECHNIQUES

A toolbox to study epidermal cell types in zebrafish

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ABSTRACT

Epithelia provide a crucial protective barrier for our organs and are also the sites where the majority of carcinomas form. Most studies on epithelia and carcinomas use cell culture or organisms where high-resolution live imaging is inaccessible without invasive techniques. Here, we introduce the developing zebrafish epidermis as an excellent in vivo model system for studying a living epithelium. We developed tools to fluorescently tag specific epithelial cell types and express genes in a mosaic fashion using five Gal4 lines identified from an enhancer trap screen. When crossed to a variety of UAS effector lines, we can now track, ablate or monitor single cells at sub-cellular resolution. Using photo-cleavable morpholino oligonucleotides that target gal4, we can also express genes in a mosaic fashion at specific times during development. Together, this system provides an excellent in vivo alternative to tissue culture cells, without the intrinsic concerns of culture conditions or transformation, and enables the investigation of distinct cell types within living epithelial tissues.

KEY WORDS: Epithelia, Zebrafish, In vivo

INTRODUCTION

Epithelia provide an essential protective barrier for the organs they enucleate. Barrier function is vital to organ function and identity, and therefore, defects in this barrier can lead to a variety of diseases such as asthma and colitis (Hereng et al., 2012; Swindle et al., 2009; Xiao et al., 2011; Zeissig et al., 2007). Furthermore, because cells comprising most epithelia continually turn over by cell death and cell division at some of the highest rates in the body (Blanpain et al., 2007; Hooper, 1956; Pelletier and Sanchez Alvarado, 2007), defects in cell turnover can lead to the most common solid epithelial tumors, or carcinomas. Additionally, epithelial shape changes are central to morphogenetic movements during embryonic development (Gumbiner, 1992; Montell, 2008; Pilot and Lecuit, 2005). For these reasons, mechanisms driving epithelial morphogenesis and carcinoma development are under intensive study. However, cell lines do not replicate the function and behavior of epithelia in vivo and introduce concerns with respect to the genetic alterations required to immortalize cells in culture. Moreover, in vivo live imaging of simple epithelia in vertebrates is difficult because of their limited accessibility. To surmount this, researchers have cultured tissues ex vivo (Chua et al., 2014; Mahe et al., 2013; Schwan et al., 2013), which might alter the native environment, or have introduced windows for imaging or fiber optic cameras (Flusberg et al., 2008; Ritsma et al., 2013), which cause a wound that can also alter any process under investigation.

The epidermis of developing zebrafish, Danio rerio, overcomes these limitations and provides an experimentally accessible and genetically tractable model for studying epithelia. The optical clarity of developing zebrafish makes it ideal for imaging single cell movements in vivo in real time. The developing epidermis is a bilayered epithelium that sits atop a basement membrane during embryonic and early larval stages (Dane and Tucker, 1985; Le Guelllec et al., 2004; Webb and Kimelman, 2005) (Fig. 1) and comprises cells that express defined markers also found in mammalian epithelia and share similar basic structure to those coating human organs such as the breast, lung and prostate (Macias et al., 2011; Pignon et al., 2013; Rock et al., 2010). Additionally, the epidermis of the developing zebrafish contains several specialized cell types that allow for the exchange of oxygen, ions and macromolecules, similar to mammalian bilayered epithelia (Hwang, 2009; Jeyto et al., 2014; Schwerte, 2010). Mammalian genes involved in epithelial morphogenesis, maintenance and disease conditions are conserved in zebrafish (de la Garza et al., 2013; Fukazawa et al., 2010; Lee and Kimelman, 2002; Li et al., 2011; Sabel et al., 2009; Sonawane et al., 2009). Further, gene function within the epidermis can be readily probed using mutations, morpholinos and chemical inhibitors. These attributes make the developing zebrafish an ideal system to study epithelial biology.

Although the zebrafish epidermis is intrinsically easy to image, tools to follow specific cell populations within the epidermis and the ability to perturb gene function in a mosaic manner were previously lacking. Being able to express or disrupt gene function in a subset of epithelial cells has been crucial for discovering numerous fundamental processes in Drosophila (Brand and Dornard, 1995; Fischer et al., 1988; Southall et al., 2008). Using tools established in S. cerevisiae and D. melanogaster (Brand and Dornard, 1995; Giniger et al., 1985), we have created zebrafish epithelial lines using the Gal4 UAS system. Gal4 enhancer trap lines exist in the zebrafish (Davison et al., 2007; Kawakami et al., 2010), however, most screens have focused on the developing nervous system (Asakawa et al., 2008; Satou et al., 2013; Takeuchi et al., 2014). To create an epithelial genetic toolkit, we identified new enhancer trap epithelial lines that can be used to track and ablate different epithelial cell types with spatial and temporal control using gal4 photo-cleavable morpholino oligonucleotides. Together, these tools provide an excellent system to study epithelial development, wound repair, regeneration, pathologies, or any other cell biological process with exquisite molecular and cellular control. Moreover, these tools provide an excellent alternative to cell culture studies that circumvent concerns of transformation and culture conditions.
RESULTS

Identification of GAL4 enhancer lines expressed in distinct cell types of the developing epidermis

To identify promoters driving expression in diverse cell types of the developing zebrafish epidermis, we screened a collection of Gal4 enhancer trap lines from a large-scale non-biased screen focused on identifying novel neural-specific lines in *Danio rerio* (Otsuna et al., 2015). The developing zebrafish epidermis is a bilayer during embryonic and larval development that comprises cells similar in function and markers to those comprising mammalian bronchial epithelia (Fig. 1) and acts similarly in oxygen exchange (McLeish et al., 2010; Schwerte, 2010). The basal layer consists of cells that express the epithelial stem cell marker p63 (Bakkers et al., 2002; Lee and Kimelman, 2002), whereas the periderm is an outer superficial layer of differentiated cytokeratin-positive cells (Gong et al., 2002; Wang et al., 2006) that are derived from the enveloping layer (Fukazawa et al., 2010) (Fig. 1A,B). The superficial layer also contains ion transport cells (ionocytes) (Janicke et al., 2007) and secretory mucous-producing cells (Oehlers et al., 2012) that are distributed throughout the tissue (Fig. 1C). We visually screened for expression in these cell types by crossing each Gal4 line to a *Tg(UAS-E1b:nfsB-mCherry)* line that labels all cells driven by...(continued)
the inserted Gal4 enhancer with red fluorescence (here shown in magenta; Fig. 2A-E). For each line, we screened mCherry expression at 48 hours post-fertilization (hpf), when proliferation is high in both layers of the epidermis and at 5 days post-fertilization (dpf), when proliferation is slow and predominantly in the basal cell layer (Carney et al., 2007). Our analyses identified seven unique lines with reproducible expression in specific epithelial cells within the epidermis. These lines include expression patterns that visually mark the periderm, basal cells, fibroblasts and ionocytes (Table S1).

Additionally, two lines express mCherry in multiple epithelial cell and/or tissue types, which we did not characterize further. Some expression patterns also changed during development. For instance, zc1036A showed expression of mCherry within cells at the outer edge of the developing median fin at day 2, but was greatly reduced by day 5 (Fig. 2D).

To characterize where the different Gal4 lines were expressed, we compared their expression with respect to the superficial keratinocytes of the periderm labeled with GFP by crossing to an existing Tg(krt4:GFP) (Gong et al., 2002) line. The zc1016B line labels a population of superficial cells that reside between the borders of the periderm cells and resemble ionocytes, or cells specific for ion transport across the developing epidermis (Fig. 3A).

Analyses of these cells using MitoTracker (Fig. S1) suggest that they represent the ionocytes (Shono et al., 2011), and therefore, we refer to line zc1016B as ‘GET-ionocyte’ (GET, Gal4 enhancer trap).

Interestingly, we found that the zc1044A line expresses mCherry in 85% of the krt4:GFP+ periderm cells (98/115 of cells), with variable levels of fluorescence among individual cells (63.2% of the cells expressed mCherry at high levels, 26.3% at an intermediate level, and 10.5% at a low level). Given the pattern of expression, we termed this the ‘GET-periderm’ line (Fig. 3B). The lack of mCherry (Fig. 3B) or Kaede (Fig. 4A) expression in all the periderm cells might reflect that this enhancer expresses in only a subset of periderm cells.

By contrast, line zc1036A expressed mCherry in a subset of p63-positive basal cells that do not overlap with the krt4:Gfp cells (0%, 0/105 cells) but co-label with 78% of cells immunostained with the epithelial stem/progenitor cell marker p63 (Senoo et al., 2007; Yang et al., 1999) (218/277 p63+ cells; Fig. 3C-D). Additionally, we engineered a line that expressed GFP exclusively in all basal epithelial cells using a conserved intron enhancer of the endogenous p63 gene in zebrafish (Antonini et al., 2006, 2015; Pashos et al., 2008) (Fig. S2). Immunohistochemical analyses revealed that 100% (159/159 cells) of the p63:EGFP cells were positive for p63 protein, which localized to the nucleus (Fig. 3E). Crossing the zc1036A Gal4 line to the Tg(p63:EGFP) line showed that UAS:nfsB-mCherry expression colocalized with 65% of p63:EGFP basal cells (77/120 cells; Fig. 3F). Therefore, we named the zc1036A line the ‘GET-basal’ line. Together, these tools allow researchers to specifically label the full population of periderm or basal cell layers or defined subsets of them. To fully characterize the expression pattern for the GET-ionocyte, GET-periderm and GET-basal lines, we also generated a high-resolution atlas across different regions of the animal (Fig. S3A-C). We further characterized our UAS-driven tools using the periderm and basal zebrafish lines, as these cell types are likely to be the most widely used for epithelial cell biology.

**UAS lines to study epithelial development**

As many developmental studies require the ability to track specific cells and ablate them, we have compiled a number of different UAS effectors to cross to our Gal4 enhancer trap lines to enable cell tracking and ablation. To track cells over time, we crossed the ‘GET-periderm’ line to a Tg(UAS-E1b:Kaede)1999 line (Davison et al., 2007), to label periderm cells with green fluorescence that can photo-convert upon exposure to blue light and label a subset of cells with red fluorescence (shown as magenta in Fig. 4). By converting the entire larvae, the addition of new cells to the tissue can be followed over time. Cells that are created after photo-conversion will only exhibit green fluorescence within the field of pre-existing cells that express both converted and newly made Kaede protein. Additionally, by limiting the area of exposure to blue light, we can track the fate of a specific population of cells over time (Fig. 4A,B). Using this approach, researchers can track epithelial population dynamics or a targeted subset to study their fates within the developing epidermis.

The Tg(UAS-E1b:nfsB-mCherry)264 reporter line initially used to screen the Gal4 enhancer trap lines also allows these cells to be targeted for apoptosis upon treatment with metronidazole (MTZ) (Curado et al., 2008; Davison et al., 2007). The Escherichia coli nitroreductase enzyme encoded by the nfsB gene is not normally present in zebrafish and is not toxic on its own, but the addition of MTZ creates a cytotoxic byproduct that causes DNA damage and apoptotic cell death (Curado et al., 2008). Thus, specific cells can be ablated in the developing epidermis. We selectively targeted the basal cells for apoptosis during development, as shown by activated caspase-3 immunostaining that are restricted to mCherry-
positive cells, which lead to significant morphological defects in the developing median fin-fold epidermis (Fig. 4D-F). Apoptotic cell death increased in a dose-dependent manner or with a MTZ single low dose over time (data not shown). By contrast, treating wild-type AB zebrafish with MTZ caused no cell death or other phenotypes (Fig. 4C,E). These two Gal4 lines allow specific epithelial cells to be tracked or ablated to study their roles in development over time.

**UAS lines to study cell biology**

These new epidermal lines, aside from being useful for studying epithelial developmental cell biology, could also be used to replace many cell culture studies by tracking specific fluorescently labeled cytoskeletal proteins. By simply crossing our epidermal Gal4 lines to a growing list of UAS-driven cytoskeletal markers, one can readily follow cell or sub-cellular movements in the periderm or other epithelial cells. For instance, we used spinning-disc confocal microscopy to film basal cells dividing with the GET-basal line crossed to a UAS-GFP-alpha tubulin gene (UAS-Gtuba2) to fluorescently label microtubules (Asakawa and Kawakami, 2010) (Fig. 5A). Basal epidermal cell division could be clearly visualized and proceeded with similar kinetics (~76 min) to those seen in cultured HeLa cells (Mackay et al., 2010). During development this technique could be used to follow mitotic spindle orientation, which can play a crucial role in cell fate decisions (Hernandez and Tirnauer, 2010) and epithelial stratification (Lechler and Fuchs, 2005).

Additionally, we have developed UAS effector lines to fluorescently label F-actin; Lifeact-EGFP (Riedl et al., 2008) and UtrCH-mCherry (Burkel et al., 2007) to track cell division, extrusion, migration and other epithelial movements during development (Table S2, Fig. S4). We created stable transgenic lines that show fine actin-based microridges in periderm cells and enrichment at cell–cell contacts. For example, we can readily follow the extrusion of a cell fated to die, which acts to expel cells without disrupting the barrier function of the epidermis (Fig. 5B) (Eisenhoffer et al., 2012; Rosenblatt et al., 2001). These tools allow real-time imaging of subcellular processes that underlie many epithelial cell shape changes required for morphogenetic movements during development or for any cell biological analysis. Additionally, should researchers want to achieve further mosaic expression of these cytoskeletal reporters, they could simply inject the DNA plasmids expressing UAS-fluorescent tag–cytoskeletal protein into the Gal4 line of interest.

Our new Gal4 lines can also be used to investigate distinct subsets of epithelial cells after genetic manipulation. The CRISPR/Cas9 system (Cong et al., 2013; Haurwitz et al., 2010; Mali et al., 2013) has been shown to reliably insert or delete DNA at precise sites within the zebrafish genome (Hwang et al., 2013; Irion et al., 2014). One strategy for tissue-specific genetic manipulation is to control the expression of the Cas9 protein using a known enhancer element (Ablain et al., 2015). Here we created a UAS-driven nuclear-localized CaXX that has eGFP-CAAX expression from a 2A peptide (Ablain et al., 2015). Here we created a UAS-driven nuclear-localized Cas9 that has eGFP-CAAX expression from a 2A peptide to facilitate epithelial cell-type specific loss-of-function studies in the zebrafish (Fig. 5C,D). Additionally, this UAS line can be used with any Gal4 line to facilitate genome editing in a wide variety of cells and tissues with the zebrafish.

**Spatial and temporal control of gene expression using Gal4 photo-cleavable morpholinos**

Whereas the Gal4–UAS system can spatially control gene expression of our effector lines, expression of some genes might disrupt embryogenesis. To circumvent this problem and enable expression of genes later in development, we introduced a photo-cleavable morpholino to gal4 (gal4-pMO) (Eisenhoffer et al., 2012; Tallafuss et al., 2012) (Fig. 6A). Using the gal4-pMOs, gene expression can be triggered at later times to entire embryos or to specific cell populations by illuminating only a few cells, as seen in Fig. 4A with Kaede conversion. To drive gene expression at later time points or in specific targeted cells, we injected gal4-pMO into Ei(Ga4-VP16)×Tg(UAS–E1b:nfsB-mCherry) to one-cell-
stage embryos and monitored them for fluorescence during development. After 350 nm light exposure to cleave the morpholino and allow Gal4-driven expression, we found that 56% of larvae expressed mCherry within 48 h of light exposure (72 hpf) (Fig. 6B-H). Expression is first seen at the distal edges of the median fin epidermis and in the oral epithelium (Fig. 6G,H). By contrast, >90% of gal4-injected embryos that were not photo-converted had no detectable fluorescence with a standard fluorescence dissecting microscope by 24 hpf (Fig. 6D,E). However, ~30% of embryos had low levels of fluorescence when examined at higher magnification on a compound microscope, suggesting incomplete knockdown of Gal4-mediated expression. The efficiency of Gal4 activation is dependent on morpholino concentration combined with intensity and duration of the light pulse during conversion. Recovery of Gal4-driven expression in subsets of cells is attributed to the inherent mosaic expression observed in our enhancer trap lines. This approach could also be used in Gal4 lines that show ubiquitous expression throughout the tissue to increase the number of cells that reactivate Gal4 after exposure to UV light. These results show that photo-cleavable morpholinos directed against gal4 can spatially and temporally control gene expression in the epidermis of developing zebrafish using the newly identified lines. These tools will facilitate mosaic and clonal analysis of distinct cell populations within a living epithelial tissue.

**DISCUSSION**

A major challenge for epithelial biology studies is analyzing cell behaviors in vivo. The developing zebrafish epidermis provides an ideal system for investigating epithelia, yet tools to mark and track specific cell types were previously lacking. Here we describe novel Gal4 enhancer trap lines that can be used to visualize specific epidermal cells in developing zebrafish to enable time lapse imaging, overexpress genes of interest, and target cells for ablation. Importantly, we identified new lines with expression restricted to the outer periderm or basal cells, two commonly studied cell types in epithelial cell biology. Our methods allow researchers to easily study specific cell populations within a living epithelial bilayer.

By combining new and existing UAS effectors with our Gal4 lines, any biological process in epithelia can be studied at subcellular resolution. For example, using our system, one can now follow or ablate specific epithelial cells during development. Additionally, because of the exquisite subcellular resolution of processes visualized by our UAS-driven fluorescent cytoskeletal lines, we believe that these lines could supplement studies using
epithelial cell culture lines and eliminate various concerns about their intrinsic transformed states. Finally, zebrafish epidermis could provide an excellent model for epithelial bilayers, a primary site of malignancy formation in the human body.

An important, yet unexpected, aspect of our Gal4 lines is that they are expressed in subsets of both the periderm and basal layers of the developing zebrafish epidermis. Many studies in epithelia rely on expression or knockdown in a mosaic fashion when trying to resolve whether a protein functions in a cell-autonomous manner, or so that function can be probed in genes that would be lethal if expressed in all cells. Therefore, having these lines express intrinsically in a mosaic fashion could be an additional asset for many epithelial studies. In cases where mosaic expression is not desired, the krt4 and our novel p63 enhancer elements express ubiquitously in the periderm and basal layers, respectively.

The tools for the zebrafish epidermis presented here provide an excellent system to investigate pathologies in epithelial bilayers. For example, our Gal4 enhancer trap lines can be used to manipulate gene expression within specific epithelial cell types, as well as follow their movements and behaviors under physiological conditions and during pathogenesis or carcinogenesis. Recent studies have demonstrated that mutations in conserved tumor suppressor genes or the sphingosine-1-phosphate signaling pathway lead to altered epithelial cell behaviors and hallmarks seen in aggressive tumors (Gu et al., 2015; Marshall et al., 2011; Reischauer et al., 2009), highlighting the utility of the zebrafish epidermis to model the earliest events of carcinoma formation and progression.

In conclusion, we have identified five novel Gal4 enhancer trap lines that can drive expression of any gene in different cells of the developing zebrafish epidermis. Using UAS effector lines, we developed methods to track or ablate cells in the periderm, and observe subcellular microtubule and actin dynamics in either cell layer. Finally, we showed that photo-cleavable morpholinos can be used to spatially and temporally control expression of any gene driven by a UAS element in specific epithelial cells. The Gal4 enhancer trap lines described here have been deposited in the Zebrafish International Resource Center (ZIRC) for public distribution (Table S1). Additionally, the UAS effector lines are available for distribution as plasmids (Table S2). Together, these tools provide a set of reagents that will allow researchers to study specific subsets of epithelial cells and their dynamics during development, repair or carcinogenesis and will provide an in vivo alternative to study processes at resolutions typically found in cell culture.

MATERIALS AND METHODS

Zebrafish husbandry and maintenance

Zebrafish, *Danio rerio*, were maintained at 28.5°C in water with a pH of 7.5, and kept on a 14 h/10 h light/dark cycle according to Westerfield (2007). The zebrafish used in this study were handled in accordance with the guidelines of the University of Utah Institutional Animal Care and Use Committee.

Gal4 enhancer trap screen

To generate the enhancer-trap lines, the *Et(Gal4-VP16;myl7:GFP)* transgenic line and embryos were exposed to UV light at 28-32 hpf. (B-H) Representative bright-field (B,D,F) and fluorescent (C,E,G,H) images 48 h after exposure to UV, at 3 dpf. Box in G denotes area of magnification for H. B-H represent data from three independent experiments. Scale bars: 250 μm in B-G, 50 μm in H.
F0 embryos were raised to maturity and then crossed to a Tg(UAS-E1b:nsfB-mCherry) reporter line. The F1 transgenic embryos were identified based on epithelial fluorescent mCherry expression patterns and imaged at 2 and 5 days post-fertilization. Embryos positive for epithelial mCherry expression were raised and then outcrossed to the F3 generation for further characterization.

**p63 enhancer cloning and transgene construction**
Multiz tracks on the University of California Santa Cruz Genome Browser revealed an intronic region in the zebrafish p63 gene with multiple regions of homology in intron 4 of mouse p63, which contains multiple enhancers that direct tissue-specific expression in transgenic mice (Antonini et al., 2006, 2015; Pashos et al., 2008). The 5564 bp region containing these sequences was amplified with the primers: For, TCGATATGCCCTTGATGACCT; Rev, TCCTGATAGTTGCTGACAC. The resulting PCR product was cloned into pDONR221 and subsequently into pGW_cfosEGFP universal expression plasmid (Fisher et al., 2006). The PCR product was also cloned into the ToI2 p5E vector (Kwan et al., 2007).

**Generation of UAS reporters**
Lifectact-EGFP, Lifectact-mCherry, UtrCH-mCherry and nCas9n were all cloned into the middle entry vector of the ToI2 kit (ToI2 kit plasmid #218) (Kwan et al., 2007). They were then combined with E1b-UAS S' and poly A' entry clones into the ToI2 pDEST_CG2 (ToI2 kit plasmid #395) destination vector. The Cas9 middle entry clone was combined with the E1b-UAS S' and p3E-2A-EGFP-CAAX-poly A (ToI2 kit plasmid #458) into the pDESTToI2p2A (ToI2 kit plasmid #394) destination vector. Injectors were performed with 25 pg of the purified plasmids along with 50 pg of transposable mRNA into wild-type AB strain. Et(Gal4-VP16)p10444, Tg(UAS-E1b:nsfB-mCherry)p264 or Et(Gal4-VP16)p10614, Tg(UAS-E1b:nsfB-mCherry)p264 developing zebrafish embryos at the 1-cell-stage. Potential carriers were identified by fluorescence expression and raised to adulthood.

**Kaede photo-conversion**
Et(Gal4-VP16)p10444, Tg(UAS-E1b:Kaede)p1999 embryos were anesthetized and mounted in 1% low melt agarose in E3 medium. Initial images were taken of both the 488 nm (green) and 568 nm (red) channels using a 20× objective. Embryos were then exposed to 350 nm light, with the area of exposure and then allowed to develop at 28.5°C. At 24 hpf, the embryos were then converted on a Nikon 90i Eclipse compound fluorescent microscope using a 10× objective with exposure to 305 nm light for 60 s. Alternatively, embryos were exposed to 350 nm light using the GeneTools lightbox on the highest intensity setting with exposure of 5 min. Either condition resulted in very little toxicity to wild-type uninjected embryos. Sequence for the gal4 antisense photo-cleavable morpholino oligonucleotide: GTCGATAGTACATGTCGTCAT.

**Photo-morpholino injections and photo-conversion**
The photo-cleaveable morpholino antisense oligonucleotides used in this study were acquired from Gene Tools, LLC. Et(Gal4-VP16)p10444_Tg (UAS-E1b:nsfB-mCherry)p264 one-cell stage zebrafish embryos were injected with 0.2 nM photo-cleavable morpholino oligonucleotides directed against gal4 and then allowed to develop at 28.5°C. At 24 hpf, the embryos were then converted on a Nikon 90i Eclipse compound fluorescent microscope using a 10× objective with exposure to 305 nm light for 60 s. Alternatively, embryos were exposed to 350 nm light using the GeneTools lightbox on the highest intensity setting with exposure of 5 min. Either condition resulted in very little toxicity to wild-type uninjected embryos. Sequence for the gal4 antisense photo-cleavable morpholino oligonucleotide: GTCGATAGTACATGTCGTCAT.

**Immunohistochemistry**
Zebrafish embryos and/or larvae were fixed and stained according to Eisenhoffer et al. (2012) with primary antibodies against p63 (Ab11149; Abcam, Cambridge, MA; 1:500) or activated caspase-3 (559565; BD Pharmigen, San Jose, CA; 1:700). For staining of mitochondria, embryos were incubated with 500 nM of Mitotracker 488 (M7514; Thermo Fisher Scientific, San Jose, CA; 1:700). For staining of mitochondria, embryos were incubated with 500 nM of Mitotracker 488 (M7514; Thermo Fisher Scientific, Waltham, MA) for 30 min in the dark, rinsed and mounted for imaging.

**Transmission electron microscopy**
Samples were fixed in 3% glutaraldehyde+2% paraformaldehyde+0.1 M sodium cacodylate buffer and treated with 0.1% Millipore-filtered cacodylate buffer tannic acid. They were post-fixed with 1% buffered osmium tetroxide for 30 min, stained in block with 1% Millipore-filtered uranyl acetate. Samples were dehydrated in increasing concentrations of ethanol, infiltrated, embedded in LX-112 medium and polymerized in an oven at 60°C for about 3 days. Ultrathin sections were cut in a Leica Ultracut microtome (Leica, Deerfield, IL), stained with uranyl acetate and lead citrate in a Leica EM Stainer, and examined in a JEM 1010 transmission electron microscope (JEOL, Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using AMT Imaging system (Advanced Microscopy Techniques, Danvers, MA).

**Live imaging**
Embryos or larvae were anesthetized with 0.04% tricaine, mounted in a 10 mm MatTek culture dish with 1% low-melt agarose in E3 embryo medium, and images were then acquired on an inverted Nikon microscope equipped with a Yokogawa spinning disc head, three Coherent solid-state lasers, and an Andor electron-multiplied, cooled CCD camera as in Eisenhoffer and Rosenblatt (2011). Image acquisition and post-processing were performed using Andor iQ software. For high-resolution images of the whole animal at different developmental stages, individual images were acquired that spanned the entirety of the animals in the x, y, and z planes using a 10× objective and the tiling/montage feature within the Andor iQ software. Maximum intensity projections were generated from the resulting data and a single composite image was created from the individual panels using the montage feature in Andor iQ.

**Photo-morpholino injections and photo-conversion**
The photo-cleaveable morpholino antisense oligonucleotides used in this study were acquired from Gene Tools, LLC. Et(Gal4-VP16)p10444_Tg (UAS-E1b:nsfB-mCherry)p264 one-cell stage zebrafish embryos were injected with 0.2 nM photo-cleavable morpholino oligonucleotides directed against gal4 and then allowed to develop at 28.5°C. At 24 hpf, the embryos were then converted on a Nikon 90i Eclipse compound fluorescent microscope using a 10× objective with exposure to 305 nm light for 60 s. Alternatively, embryos were exposed to 350 nm light using the GeneTools lightbox on the highest intensity setting with exposure of 5 min. Either condition resulted in very little toxicity to wild-type uninjected embryos. Sequence for the gal4 antisense photo-cleavable morpholino oligonucleotide: GTCGATAGTACATGTCGTCAT.

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**Competing interests**
The authors declare no competing or financial interests.

**Author contributions**
G.T.E. and J.R. designed the research. G.T.E., O.E.R., and G.S. performed the associated sample preparation, partially supported by the MD Anderson Cancer Center CCSG P30CA016672.

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**Data availability**
The Gal4 enhancer trap lines described here have been deposited in the Zebrafish International Resource Center (ZIRC) for public distribution (see Table S1)

**Supplementary information**
Supplementary information available online at http://jcs.biologists.org/lookup/doi/10.1242/jcs.184341.supplemental

**References**
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Figure S1. Mitotracker staining reveals enhancer trap line expressed in ionocyte population. Mitotracker staining (magenta) in Tg(krt4:GFP) (green) zebrafish. Co-expression of mitotracker (green) in the zc1016B enhancer trap line (magenta).
Figure S2. Characterization of Tg(p63:EGFP) transgenic zebrafish. Max projection confocal images of Tg(p63:EGFP) transgenic zebrafish at 24hpf and 48hpf.
Figure S3. High-resolution atlas of the identified Gal4 enhancer trap lines. Max projection confocal images demonstrating expression in the whole animal for the GET-ionocyte (A), GET-Periderm (B) and GET-Basal (C) lines. High magnification images of different regions along the animal for each Gal4 enhancer trap line are also shown. A list of cell types each line is expressed in is presented in Table S1. Depending on the experiment and approach, expression in other cell types may complicate perturbation studies. Scale bars, 50μm for the whole animal images, 20μm for the high magnification images.
Figure S4. Epithelial enhancer trap line driving expression of UAS cytoskeletal fluorescent reporter lines. Expression of the UAS cytoskeletal fluorescent reporters by the Periderm-GET line reveals localization of F-actin (A) and membrane bound fluorescent expression (B), respectively, within the developing periderm (48hpf). (A). Expression of UAS:mCherry-UtrCH (magenta) in the Et(Gal4-VP16)zc1044A; Tg(UAS-E1b:Kaede)s1999t (green) line; and (B) UAS:EGFP-CAAX (green) in Et(Gal4-VP16)zc1044A; Tg(UAS-E1b:nsfB-mCherry) (magenta) line.
Table S1. Gal4 Enhancer Trap Lines

<table>
<thead>
<tr>
<th>Line</th>
<th>Epidermal Cell Type Expression</th>
<th>Other</th>
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<tbody>
<tr>
<td>1016B</td>
<td>Ionocytes</td>
<td>Notochord</td>
</tr>
<tr>
<td>1021A</td>
<td>Pectoral Fin, Fibroblasts</td>
<td>Otic Vesicle, Hindbrain, Vasculature</td>
</tr>
<tr>
<td>1036A</td>
<td>Basal Cells</td>
<td>Optic Cup, Olfactory Placode/Bulb, Muscle, Notochord</td>
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<tr>
<td>1038A</td>
<td>Median Fin Epidermis</td>
<td>Glia, Olfactory Placode/Bulb</td>
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<tr>
<td>1044A</td>
<td>Periderm Cells</td>
<td>Glia, Midbrain, Retina, Hindbrain, Hypothalamus, Spinal Neurons</td>
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Table S2. Plasmids Available For Studying Epithelial Cell Biology in Zebrafish

**Enhancers**

<table>
<thead>
<tr>
<th>Name</th>
<th>Enhancer Element</th>
<th>Cell Type Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>p5E-krt4</td>
<td>krt4</td>
<td>Periderm Cells</td>
</tr>
<tr>
<td>p5E-p63</td>
<td>p63</td>
<td>Basal Cells</td>
</tr>
<tr>
<td>p5E-p63cfos</td>
<td>p63+cfos minimal promoter</td>
<td>Basal Cells</td>
</tr>
</tbody>
</table>

**UAS Effectors**

<table>
<thead>
<tr>
<th>Name</th>
<th>Subcellular Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAS:mCherry-UtrCh</td>
<td>F-actin Filaments</td>
</tr>
<tr>
<td>UAS:Lifeact-EGFP</td>
<td>F-actin Filaments</td>
</tr>
<tr>
<td>UAS:EGFP-CAAX</td>
<td>Plasma membrane</td>
</tr>
<tr>
<td>UAS:nCas9n-2A-EGFP-CAAX</td>
<td>Nucleus (Cas9)/Plasma membrane (EGFP)</td>
</tr>
</tbody>
</table>