The Lifeact-EGFP Quail: A New Avian Model For Studying Actin Dynamics *In Vivo*

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Abstract

Here we report the generation of a transgenic Lifeact–EGFP quail line for the investigation of actin organization and dynamics during morphogenesis *in vivo*. This transgenic avian line allows for the high-resolution visualization of actin structures within the living embryo, from the subcellular filaments that guide cell shape to the supracellular assemblies that coordinate movements across tissues. The unique suitability of avian embryos to live imaging facilitates the investigation of previously intractable processes during embryogenesis. Using high-resolution live imaging approaches, we present the dynamic behaviours and morphologies of cellular protrusions in different tissue contexts. Furthermore, through the integration of live imaging with computational segmentation, we reveal the dynamics of cells undergoing apical constriction and the emergence of large-scale actin structures such as supracellular cables and rosettes within the neuroepithelium. These findings not only enhance our understanding of tissue morphogenesis but also demonstrate the utility of the Lifeact–EGFP transgenic quail as a new model system for live *in vivo* investigations of the actin cytoskeleton.

Introduction

During morphogenesis, tissue is formed by a complex interplay of changes in cellular geometry and cellular movements. The forces required to shape developing tissues are generated by actomyosin networks that are coupled to cell-cell and cell-matrix junctions (Clarke and Martin, 2021, Perez-Vale and Peifer, 2020). Contractions of the actomyosin network allow cells to pull on their neighbours or the matrix. These active forces at the cellular level are translated into dramatic changes in shape at the tissue level which underly morphogenesis.

Remodelling of the actin cytoskeleton is the basis of a toolbox of common morphogenetic processes that drive tissue formation. These range from the subcellular scale, such as cellular protrusions, to changes in shape at the cellular scale, up to multicellular structures such as actin cables and cellular rosettes. Live imaging of actin dynamics has emerged as a powerful tool to unravel these processes.

To visualise actin dynamics in real-time during morphogenesis in a highly accessible vertebrate model, we generated the TgT2[UbC:Lifeact-EGFP] transgenic quail. This transgenic line, expressing an EGFP fusion of the actin-binding Lifeact peptide (Riedl et al., 2008), allows for the high-resolution visualization of actin structures within the living embryo, from the subcellular filaments that guide cell shape to the supracellular assemblies that coordinate movements across tissues. The unique suitability of avian embryos to live imaging facilitates the investigation of previously intractable processes during embryogenesis. We present novel insights into the dynamic behaviours of cellular protrusions in different tissues, apically constricting cells, and the assembly of multicellular structures. These findings not only enhance our understanding of tissue morphogenesis but also demonstrate the utility of the TgT2[UbC:Lifeact-EGFP] transgenic quail as a new model system for live *in vivo* investigations of the actin cytoskeleton.

Results and Discussion

Generation of a transgenic Lifeact-EGFP quail line

To avoid potential overexpression artifacts, we used the low-expression ubiquitin promoter to drive Lifeact-EGFP expression in our transgenic quail line (TgT2[UbC:Lifeact-EGFP]). The UbC-Lifeact-EGFP cassette was inserted in between 5′ and 3′ Tol2 (T2) transposable elements to facilitate stable integration into the quail genome (Fig. 1A). Using a direct injection technique (Barzilai-Tutsch et al., 2022, Serralbo, 2020, Tyack et al., 2013) we transfected the blood-circulating primordial germ cells *in vivo*. Fifty wild-type embryos at stage HH16 (E2.5) were injected in the dorsal aorta with a mix of lipofectamine 2000, the UbC-Lifeact-EGFP plasmid and a pCAG-Transposase construct. A male founder was selected and mated with wild-type females to establish the line. The TgT2[UbC:Lifeact-EGFP] quails were viable, phenotypically normal and fertile. The transgene inheritance followed a Mendelian distribution, indicating that the gene was likely integrated at a single location in the genome. We could detect robust Lifeact-EGFP expression from Hamilton and Hamburger stage 3 (HH3) onwards (Hamburger and Hamilton, 1951) (Figs. 1B, S1). The Lifeact-EGFP fluorescence overlapped with Phalloidin-568 staining in fixed embryos and was enriched in regions of increased Phalloidin-568 intensity (Figs. 1B, C, D).

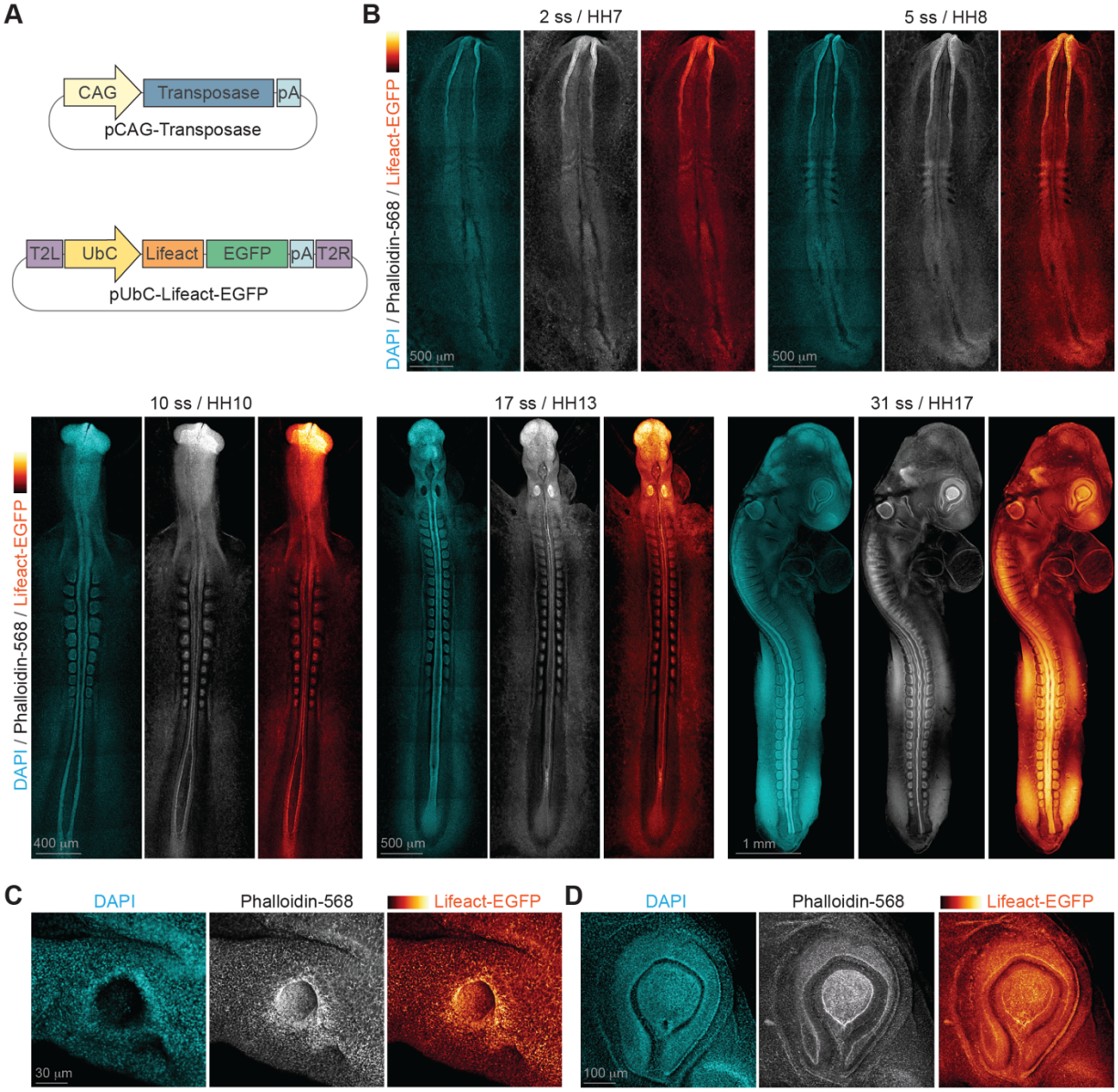

Figure 1. The endogenous actin cytoskeleton is fluorescently labelled in the developing TgT2[UbC:Lifeact-EGFP] transgenic quail embryo. (A) Plasmid vectors used to generate the transgenic line. (B) The Lifeact-EGFP signal matches labelling of the actin cytoskeleton by Phalloidin-568 throughout early embryonic development. (C, D) Higher magnification images demonstrate enriched Lifeact-EGFP signal in regions of increased Phalloidin-568 labelling.

Live imaging of actin dynamics during morphogenesis

We performed live imaging of the TgT2[UbC:Lifeact-EGFP] quail line at a range of scales from the subcellular to the tissue level to examine the dynamics of the actin cytoskeleton during common morphogenetic tissue processes.

Imaging cellular protrusions

Many actively migrating cells reorganize their actin cytoskeleton and generate polarized protrusions (Schaks et al., 2019). Although this has been visualised in multiple tissues (Omelchenko, 2022, Lamb et al., 2020, Olson and Nechiporuk, 2021, Mishra et al., 2019), the actin cytoskeleton has never been imaged live at high resolution during fusion of the early heart tube *in vivo.* Vertebrate heart formation begins with the migration of bilateral sheets of cardiogenic mesoderm towards the midline where they fuse to form the primitive heart tube (Rosenquist, 1966, Stalsberg and DeHaan, 1969, Kirby, 2007). Here we performed high spatiotemporal resolution live imaging of mesodermal cardiac progenitors crawling over the underlying endoderm of the Anterior Intestinal Portal (AIP) in the TgT2[UbC:Lifeact-EGFP] quail embryo at HH7 – HH8 stage (E1). The bilateral collectives of cardiac progenitor cells form many filopodia which are enriched in Lifeact-EGFP and protrude outwards, making contact with the surrounding tissues (Fig. 2A, Supplementary movie 1). The filopodia are on average 9.6μm +/- 1.1μm in length and highly dynamic with an average persistence time of 270 s +/- 23 s. Fusion of the bilateral heart fields is initiated when progenitor cells from each side first make contact via their filopodia (Fig. 2A insets). We did not find any lamellipodia suggesting the cardiac progenitors use a filopodial mechanism to migrate, potentially similar to the contact-dependent asymmetry of cell–matrix adhesion mechanism that drives filopodial-mediated migration of *Drosophila* myotubes (Bischoff et al., 2021). Active endodermal shortening around the AIP has been proposed to account for most of the movement of the mesodermal progenitors towards the midline (Varner and Taber, 2012). We find that the cardiac progenitors migrate over the endoderm at a speed of 4,99 +/-0,09 um min^{-1}, and the AIP shortens by 4.24 +/-0.05 um min^{-1}, suggesting active mesodermal migration is only a minor contributor to the motion of the bilateral heart fields.

To examine cellular protrusions in a different tissue context we turned to the developing neural tube. Unlike the stereotypical filopodia of the cardiac progenitor cells, cellular protrusions of varying morphologies are proposed to be required for correct neural tube closure (Rolo et al., 2016). Despite many descriptions of these protrusions in fixed specimens as cytoplasmic threads, ruffles, filopodia, blebs or lamellipodia over the last 50 years (Waterman, 1976, Bancroft and Bellairs, 1975, Geelen and Langman, 1979, Rolo et al., 2016), there has been very little live imaging of their dynamics. We performed high spatiotemporal resolution live imaging of TgT2[UbC:Lifeact-EGFP] quail embryos during spinal neural tube closure at stage HH9 – HH10 (E1) (Ainsworth et al., 2009). Clusters of protrusions highly enriched for Lifeact-EGFP were clearly visible protruding into the open lumen of the neural tube (Fig. 2B). The morphology of these protrusions was variable, appearing to consist of both lamellipodia and filopodia (Figs. 2B, C). The protrusions were highly dynamic, constantly changing in shape and area for periods of minutes, and up to more than an hour until the approaching zippering point of the neural tube reached them (Figs. 2D, E, Supplementary movie 2).

Building on the observed dynamic protrusions in the developing neural tube, we shifted our focus to the migrating surface ectoderm which is also crucial for neural tube formation (Christodoulou and Skourides, 2022, Maniou et al., 2021, Galea et al., 2017, Marshall et al., 2023, Moury and Schoenwolf, 1995). As the neural tube is closing, the surface ectoderm migrates over it towards the embryonic dorsal midline. During this migration, transient apertures in the ectodermal sheet were observed (Fig. 2F). High-speed spinning disk confocal imaging of the TgT2[UbC:Lifeact-EGFP] quail embryo revealed distinctive oscillations of the Lifeact-EGFP at cell junctions and the formation of lamellipodia as surrounding cells tried to close the gap (Fig. 2G, Supplementary movie 3). The fluctuating cell junctions are indicative of pulsed actomyosin contractions and occur at intervals of approximately 2 minutes (Fig. 2H). Simultaneously, a retrograde actin flow at an approximate rate of 2.6 μm min^{-1} was identified, propelling the formation of lamellipodia (Figs. 2H, I). These lamellipodia, in turn, facilitated the forward movement of cells, contributing to the resolution of the temporary openings and sealing of the ectodermal sheet.

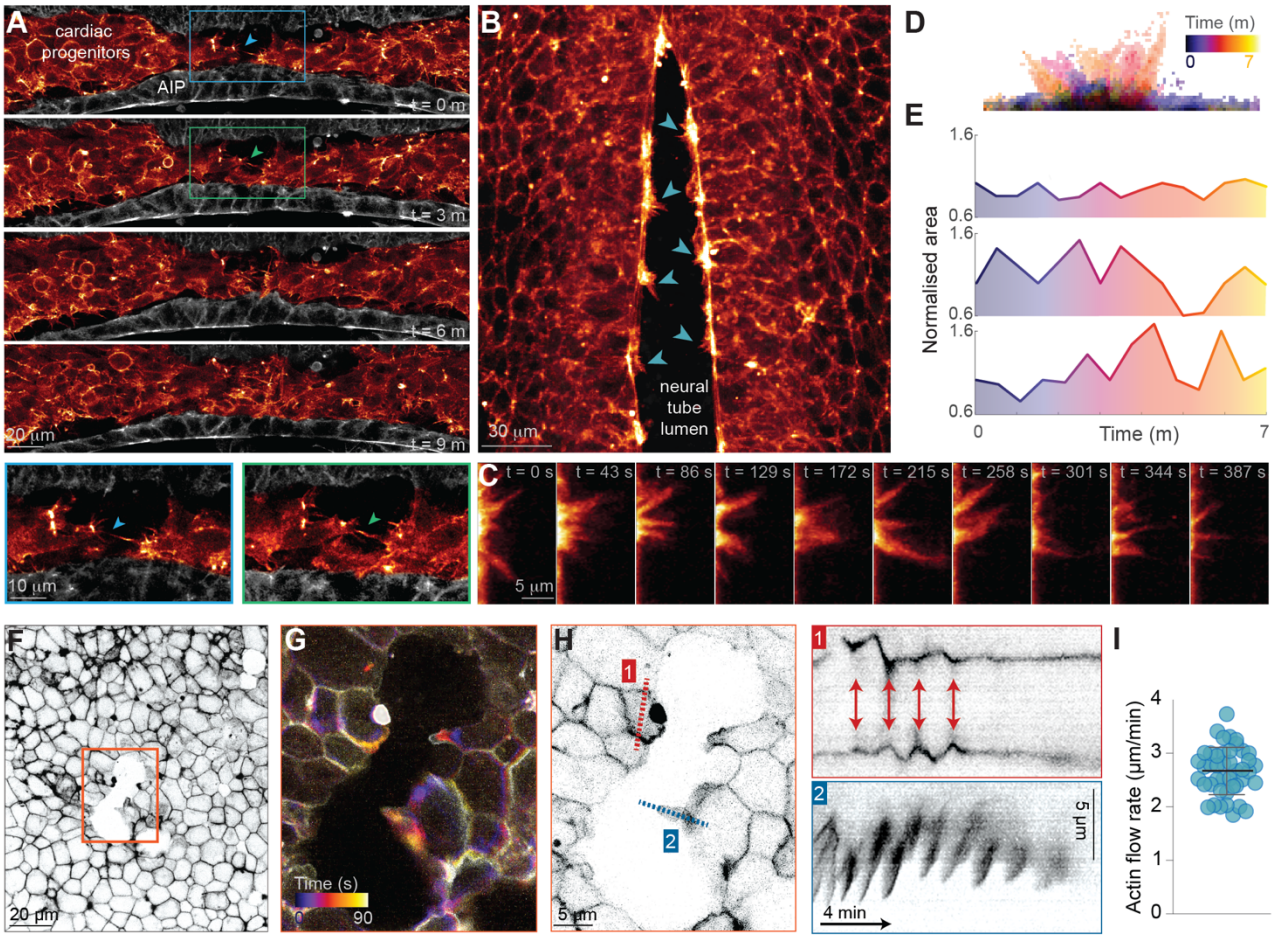

Figure 2. Live imaging reveals dynamics of cellular protrusions in different tissue contexts. (A) Mesodermal cardiac progenitor cells extend filopodia towards the surrounding tissues as the bilateral heart fields migrate towards the midline over the Anterior Intestinal Portal (AIP). Blue insert shows timepoint prior to contact between leading cells from each side. The cells extend filopodia towards each other (blue arrow). Green insert shows contact via a filopodium (green arrow) between the leading cardiac progenitor cells from each side. The tissues surrounding the cardiac progenitor cells are pseudocoloured for clarity. (B) Cellular protrusions of varying morphologies extend from the edges of the neural folds into the open lumen of the neural tube. (C) High spatiotemporal resolution imaging reveals the highly dynamic nature of the neural tube protrusions. (D, E) Computationally masking the protrusions allowed measurement of the area change over time, three examples shown in graph. (F) The ectodermal sheet contains transient apertures indicated by orange box. (G) Higher magnification of inset from F pseudocoloured with a temporal code to reveal dynamic actin-based structures. (H) Kymographs made from regions indicated demonstrate contractile pulsations in cell junctions (1) and formation of lamellipodia by actin flow (2). (I) Speed of actin flow measured at lamellipodia.

Together, our live imaging confirmed that the TgT2[UbC:Lifeact-EGFP] quail line is an excellent model system for studying the rapid remodelling of the actin cytoskeleton during protrusive cell behaviours *in vivo*.

Actin dynamics during apical constriction

Another common morphogenetic process during tissue development is apical constriction, in which cells change geometry by shrinking their apical surface (Martin and Goldstein, 2014). Epithelial remodelling requires apical constriction in a wide variety of contexts to attain the correct form and architecture of the tissue. Different dynamic patterns of actomyosin network contraction can drive apical constriction (Martin and Goldstein, 2014). Studies in invertebrates have shown that pulsed contractions of a medioapical meshwork drive shrinkage of the apical cell surface (Martin et al., 2009, Solon et al., 2009). However, a purse-string-like contraction of a circumferential cellular actomyosin cable has long been proposed to drive apical constriction in vertebrates (Baker and Schroeder, 1967, Schroeder, 1970). Recent work demonstrated that pulsatile contraction of a

transient apical actin network drives stepwise shrinkage of the apical surface in constricting cells in the *Xenopus* neuroepithelium but this has not yet been investigated in the avian neural plate (Christodoulou and Skourides, 2015).

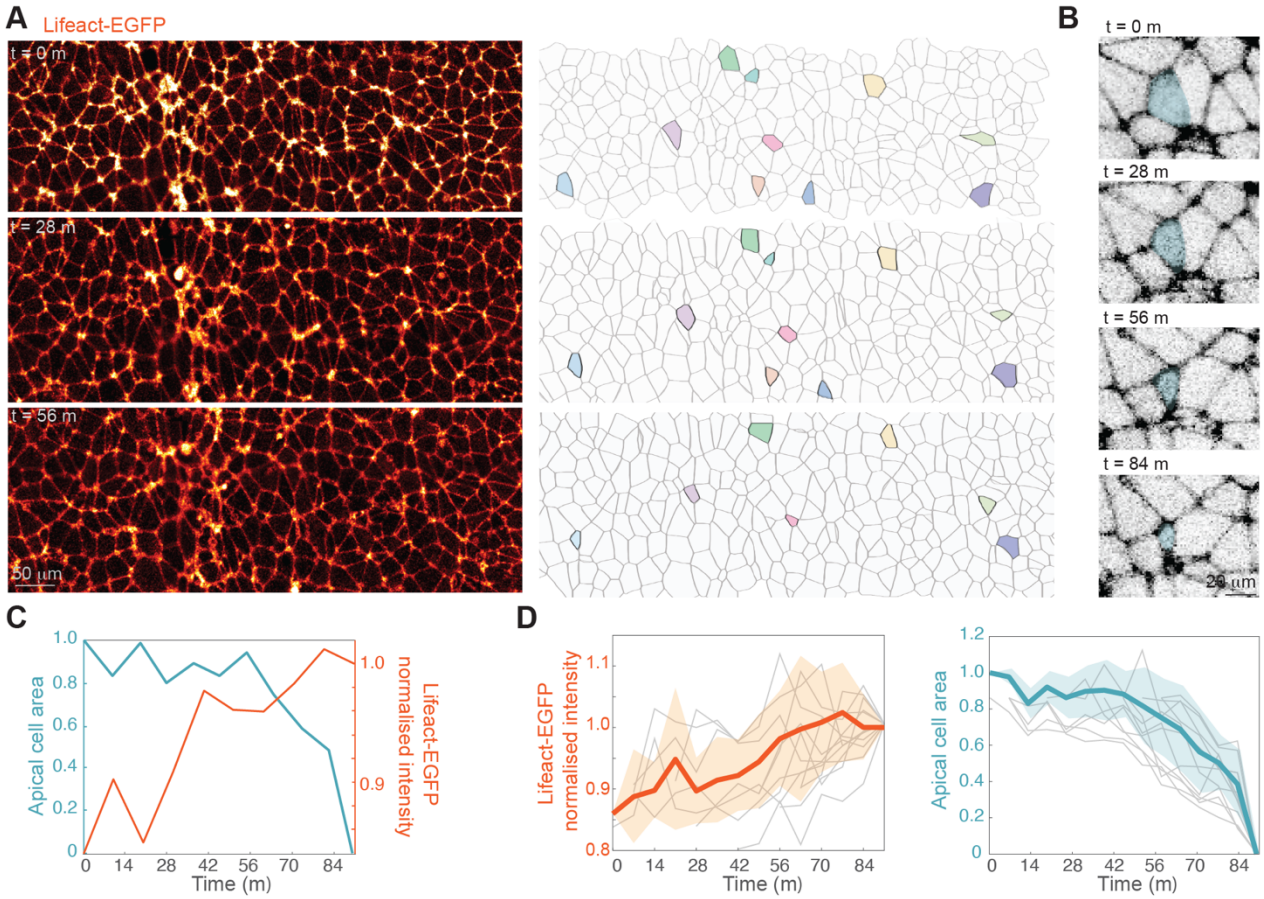

Figure 3. Cells undergo pulsatile apical constriction in the developing neuroepithelium. (A) Computational segmentation of the Lifeact-EGFP signal in live imaging of the neuroepithelium identifies cells undergoing apical constriction. (B) Time series of an apically constricting cell (blue cell in segmented image in A). (C) The ratio of Lifeact-EGFP intensity at the apical cortex relative to the cell junctions increases in a pulsatile manner as the apical cell area undergoes stepwise shrinkage, example of a single cell. (D) Graphs show mean (coloured line) +/- s.e.m (shading) and individual measurements (grey lines) for the normalised Lifeact-EGFP intensity and apical cell area over time.

To visualise the actin cytoskeleton and cell dynamics during apical constriction we performed live imaging of the neuroepithelium in the open region of the neural tube of TgT2[UbC:Lifeact-EGFP] quail embryos at HH8 – HH9 (E1) (Fig. 3A). Computational segmentation was performed to identify cells undergoing apical constriction. To achieve this, we first used a custom local Z projection strategy to project the curved apical surface of the neuroepithelium onto a 2D plane. Next, we used the Cellpose 2.0 segmentation algorithm to identify cell boundaries labelled by Lifeact-EGFP. Using a custom MATLAB script, we identified cells undergoing apical constriction – defined as cells with a decrease in apical cell area of 90 % over 90 min time (rate higher than 0.01 min^{-1}) (Fig. 3B). Measuring the ratio of Lifeact-EGFP signal at the apical cortex relative to the cell junctions revealed an average increase of 19 +/- 9 % during the first 50% of the reduction in apical cell area (Figs. 3C, D). Furthermore, the identified cells did not undergo a constant decrease in apical cell area but instead showed a more pulsatile pattern consistent with a ratchet-like mechanism (Figs. 3C, D). Pulsatile ratchet-like apical constrictions have been well-studied in *Drosophila* where they drive rapid apical constriction *en masse* during gastrulation and dorsal closure (Martin et al., 2009, Mason et al., 2013, Solon et al., 2009). However, the apical constriction events identified here are more similar to those recently described during epiblast cell ingression at the mouse primitive streak where isolated cells constrict their apical surface over a 25 – 90 minute period (Francou et al., 2023).

Formation of supracellular actin cables and multicellular rosettes

The formation of supracellular actin cables and multicellular rosettes is associated with tissue reorganization in a variety of species (Miao and Blankenship, 2020, Harding et al., 2014). Supracellular actin cables mechanically link multiple cells to allow the coordinated application of tension across the tissue. They can also reinforce the structure of entire tissues and increase their resistance to mechanical strain (Duda et al., 2019). Myosin-driven contraction of supracellular actin cables constricts the lateral edges of the cells along the cable, pulling them into a rosette structure with a centrally shared interface. These multicellular rosettes are a common feature during morphogenesis of various organs (Gompel et al., 2001, Lienkamp et al., 2012, Villasenor et al., 2010).

In the avian embryo, supracellular actomyosin cables and epithelial rosettes are thought to be important for the formation of the neural tube (Nishimura et al., 2012, Nishimura and Takeichi, 2008). The planar polarized assembly of supracellular actomyosin cables is proposed to enable polarized tissue contraction along the mediolateral axis. This is thought to allow the tissue to bend only along the anteroposterior axis of the embryo, rather than bending radially if the contraction was isotropic (Nishimura et al., 2012). However, this idea is based on computational modelling and studies on fixed tissue which is prone to shape artifacts arising from the fixation. Using live imaging, we visualised the formation of supracellular actin cables and rosettes in the neuroepithelium of the TgT2[UbC:Lifeact-EGFP] quail embryo at HH8 – HH9 (E1) (Fig. 4A). We focused on the region caudal to the closed neural tube, where the neural plate was still relatively flat. The transverse view of the 3D stack revealed a slight curvature along the midline of the neural plate (Fig. 4A, t = 0 h, x,y view). We identified supracellular actin cables spanning 10 or more cells in a mediolateral direction and followed these cables using live imaging. During the next two hours, the supracellular cables contracted and the curvature of the neural plate increased along the mediolateral axis (Figs. 4A, t = 2 h, x,y view, B). Next, we used computational segmentation and a custom MATLAB script to identify multicellular rosettes consisting of 5 – 8 cells (Fig. 4C). Surprisingly, even at the start of the imaging period when the neural plate was only slightly curved, most cells were already part of a multicellular rosette. 83.1% of these rosettes contained 5 cells, 14.3% contained 6 cells and only 2.6% contained 7 cells (Figs. 4C, D). After two hours, the total number of rosettes had increased by 42.9%. Furthermore, the distribution had shifted towards higher-order rosettes with only 61% containing 5 cells but 29.1% containing 6 cells, 8.1% containing 7 cells and 1.8% containing 8 cells. As the actin cables forming the rosettes contracted, the Lifeact-EGFP intensity (normalised to cable area) increased, suggesting continued actin accumulation during cable contraction (Fig. 4E).

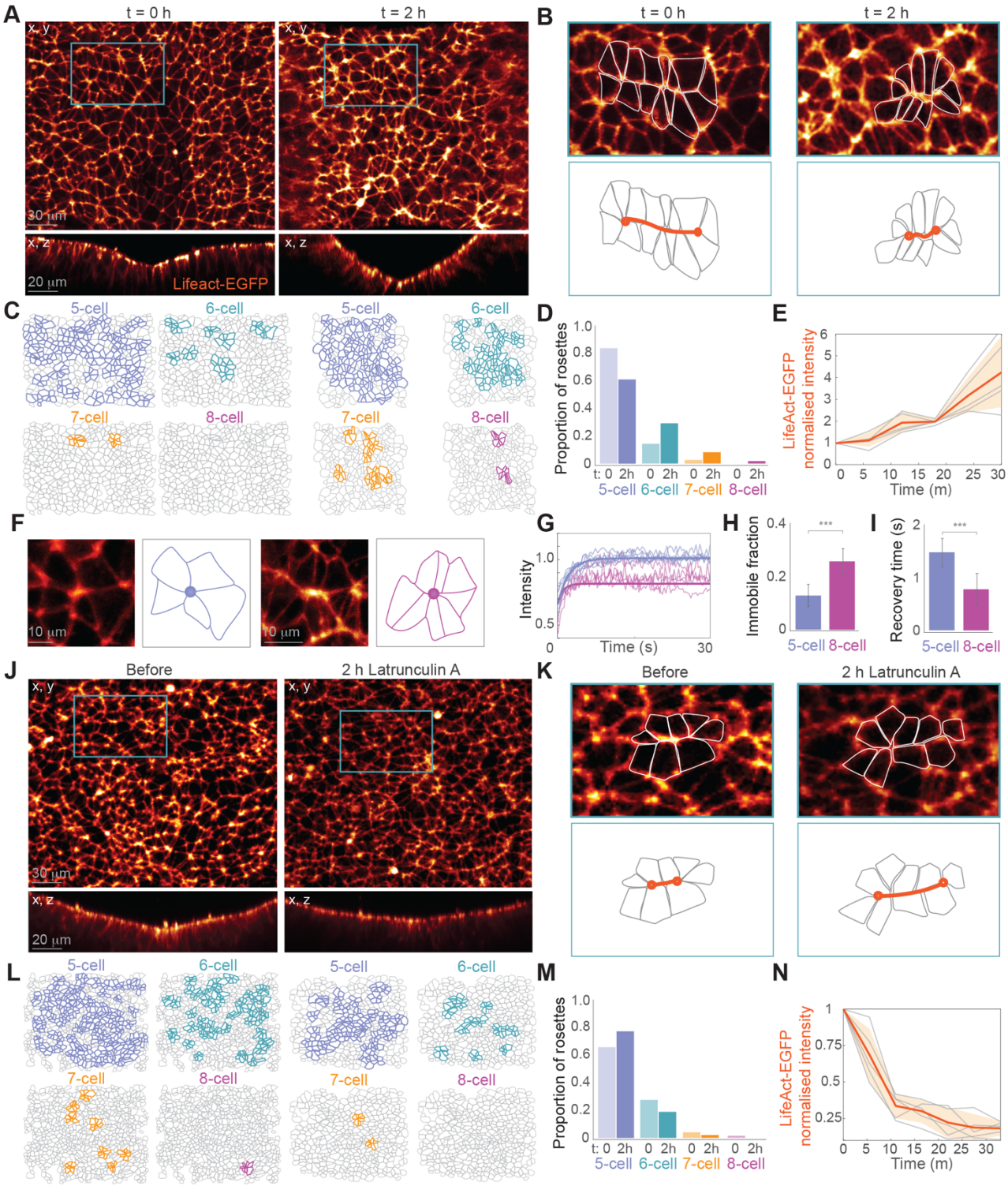

Figure 4. Formation of supracellular actin cables and multicellular rosettes in the developing neuroepithelium. (A) Supracellular actin cables (blue boxes) form in the relatively flat neural plate and contract within 2 hours, concomitant with bending along the midline (x,z views). (B) Insets show supracellular cables indicated in A. (C, D) Computational segmentation reveals an increasing number and complexity of rosettes over time. (E) The intensity of Lifeact-EGFP increases as the supracellular cable contracts (normalised to cable area). (F – I) FRAP of the actin at the central vertex shows a higher immobile fraction and a faster recovery time in 8-cell rosettes compared to 5-cell rosettes. Graphs show mean +/- s.e.m. (J, K) Treatment with Latrunculin A causes extension of supracellular actin cables and flattening of the neural plate. (L, M) Latrunculin A reduces the number and complexity of rosettes. (N) The intensity of Lifeact-EGFP localised to the actin cable is decreased by Latrunculin A treatment (normalised to cable area). Graphs in E and N show mean (orange line) +/- s.e.m. (orange shading) and individual cable measurements (grey lines).

To examine the dynamics of the actin remaining at the centre of the multicellular rosettes following contraction of the supracellular cables we used Fluorescence Recovery After Photobleaching (FRAP). Comparing 5-cell rosettes to 8-cell rosettes revealed a higher immobile fraction and a decreased recovery time in the higher-order rosettes (Figs. 4F - I). Our results show that a greater proportion of the actin molecules are stationary and not exchanging with the surrounding pool of actin within the centre of the 8-cell rosettes. This may indicate a higher degree of actin stabilization or stronger binding interactions that prevent the actin molecules from diffusing away. However, within the mobile pool of actin, there is also a higher rate of actin turnover or a more efficient replenishment in the centre of the higher-order rosettes, possibly due to greater accessibility or a higher rate of actin polymerisation at the site. Together, these findings suggest that the actin structures linking the cells in the centre of the higher-order rosettes are rapidly remodelled, but once actin monomers are integrated into the structure, they become part of a stable network that resists further exchange. These complex dynamics may reflect tuning of the actin remodelling within multi-cellular rosettes to balance structural integrity with the flexibility required for ongoing morphogenesis.

To perturb the supracellular actin cables and rosettes, we treated the embryos with the actin polymerisation inhibitor Latrunculin A (Coue et al., 1987). Following two hours of treatment, we observed a decrease in mediolateral curvature of the neural plate and extension of the supracellular actin cables (Figs. 4J, K). Latrunculin A treatment also decreased the total number of rosettes by 43.5% (Fig. 4L). Moreover, there was a corresponding shift from higher-order towards lower-order multicellular rosettes (Figs. 4L, M). During Latrunculin A treatment there was a decrease in the Lifeact-EGFP intensity in the supracellular cables, consistent with a reduction in actin polymerisation (Fig. 4N). Together, our results support a role for supracellular actin cable contraction and rosette formation in the anisotropic bending of the neural plate during neural tube formation. The increasing number and complexity of the multicellular rosettes during neural plate bending suggests that the persistence of these structures is higher than the transitory rosettes involved in tissue remodelling in *Drosophila* (Blankenship et al., 2006). It is possible that the accumulation of higher-order rosettes in the neural plate may also contribute to the reported decrease in tissue fluidity during neurulation (Bocanegra-Moreno et al., 2023, Yan and Bi, 2019).

By employing cutting-edge live imaging of a new Lifeact-EGFP transgenic quail model, we have investigated the dynamics of the actin cytoskeleton across various tissues *in vivo*. Our approach has allowed us to observe cellular protrusions and dynamic behaviours like apical constriction and the formation of complex actin structures, including supracellular cables and rosettes, in the developing embryo. The insights gained from this research offer a deeper understanding of processes involved in tissue morphogenesis and confirm the TgT2[UbC:Lifeact-EGFP] transgenic quail as an invaluable resource for real-time, *in vivo* study of the actin cytoskeleton.

Materials and methods

Generation of transgenic Lifeact-EGFP quail line

The direct injection technique was performed as described previously (Serralbo, 2020, Tyack et al., 2013). Briefly, plasmids were purified using a Nucleobond Xtra Midi EF kit. 1 µl of injection mix containing 0.6 µg of pUbC-Lifeact-EGFP Tol2 plasmid, 1.2 µg of CAG Transposase plasmid and 3 µl of Lipofectamine 2000 CD (ThermoFisher Scientific) in 90 µl of OptiPro was injected in the dorsal aorta of 2.5-day-old embryos. Eggs were then sealed and incubated until hatching. Chicks were grown for 6 weeks until they reached sexual maturity. Semen from the male was collected as described previously (Serralbo, 2020). Genomic DNA from semen was extracted and PCR was performed to test for the presence of the transgene. Males showing a positive band were kept and crossed with wild-type females. F1 offspring were selected by genotyping 5 days after hatching by plucking a feather.

Maintenance of quails

Transgenic quails were hosted and bred at the University of Queensland according to local animal ethical policies. Fertilized quail eggs from transgenic quail lines were collected daily by the animal facility. Eggs were kept at 14°C in a fridge before use.

Phalloidin staining

Embryos were fixed at room temperature for 30 minutes in 4% paraformaldehyde (Sigma Aldrich, P6148-500G), and extra-embryonic tissues were trimmed before adding Phalloidin-Rhodamine (1:1000, Abcam, AB235138) and DAPI (1:1000, Sigma Aldrich, MBD0015-15ML) for 3 hours at room temperature. After incubation, embryos were washed and mounted with fluoromount aqueous mounting medium (Sigma Aldrich, F4680-25ML) for imaging on glass slides (Epredia).

Quail embryo culture

Eggs were incubated horizontally at 37.5°C in a humidified atmosphere until the desired stages. After incubation, quail eggs were cooled down for approximately 1 hour at room temperature (RT) before culture. While keeping the egg horizontal by placement onto a customized egg holder, 1 – 2 mL of thin albumin was aspirated out with a 5 mL syringe (Nipro) and a 18G sterile needle (BD PrecisionGlide) from the blunt end of the egg to create a space between the embryo and the shell. A small window (approx.1.0 × 0.5 cm) was cut on the top of the eggshell to facilitate visualisation and staging. Embryos were staged based on morphological criteria as described previously (Ainsworth et al., 2009). Embryos were cultured according to a protocol described elsewhere (Williams and Sauka-Spengler, 2021). Briefly, the egg yolk and albumin were carefully slid out of the shell into a 35 mm petri dish with the embryo resting at the top of the yolk. The albumin covering the surface of the embryo was carefully wiped away with Kimwipe tissue. A piece of 1.5 × 1.5 cm square filter paper with a 0.2 × 1.0 cm hole in the centre was placed onto the surface of the embryo, with the embryo centered in the hole. The filter paper was then cut along the edge with a pair of sharp dissection scissors to release the embryo from surrounding tissues while maintaining biological tension. The filter paper, with the embryo attached, was lifted away from the yolk using fine forceps and placed onto a 35 mm 1 well dish pre-coated with 1 mL agar-albumin. The yolk was washed away from the embryo using preheated Hanks' Balanced Salt solution (HBSS). After washing, the embryo was transferred into another 35 mm 1 well dish pre-coated with 1 mL agar-albumin mixture consisting of 0.3% w/v bacto-agar and 50% v/v albumin. Before further use, the 1 well dish containing the embryo was kept in a humidified environmental chamber at RT.

Live imaging

Following culture, embryos were allowed to rest for at least 1 h at RT before imaging and then transferred dorsal side facing down to a 6-well imaging plate pre-coated with 250 µL of agar-albumin. All live imaging was done at

the IMB microscopy facility (University of Queensland) which is supported by the Australian Cancer Research Foundation.
Live imaging was performed on the following systems: a Zeiss Axiovert 200 Inverted Microscope Stand with LSM 710 Meta Confocal Scanner fitted with dedicated GaAsP 488 nm and 561 nm detectors for increased sensitivity, a Zeiss Axiovert 200 Inverted Microscope Stand with LSM 710 Meta Confocal Scanner, including spectral detection and Airyscan super-resolution detector and Mai Tai eHP 760-1040nm laser with dedicated GaAsP NDD detectors or an Andor Dragonfly spinning disc confocal equipped with dual Andor Zyla 4.2 sCMOS cameras, controlled by Fusion Software (Andor). Microscope environmental chambers were maintained at 37.5°C while imaging.

FRAP

FRAP experiments were performed with a Zeiss 40X (W-Plan Apochromat NA1.10) objective at 5-times magnification. A 4 µm x 4 µm region of interest (ROI) was photobleached with 2-photon laser (Mai Tai Laser System) at 840 nm with 30% laser power and imaged every 0.2 sec for 30sec.
For FRAP analysis, mean fluorescence intensity at the photobleached was corrected by background fluorescence and normalized to a non-photobleached reference. The average of the pre-bleach fluorescence intensities was set to 100%. The normalized mean fluorescence intensities were then fitted with an exponential function, to obtain the decay time and the plateau intensity (Ip). The immobile fraction was calculated as I-Ip. The analysis was performed in MATLAB (MATLAB ver. R2021a).

Computational segmentation

Images were segmented using Cellpose- human in loop workflow (Pachitariu, M. & Stringer, C. (2022). Cellpose 2.0: how to train your own model. *Nature methods*, 1-8.). Cell tracking and rosette detection were performed using custom scripts in MATLAB.

Statistical analysis

Statistical analysis was done using 2-way ANOVA and unpaired t-test as needed. All analysis was done on the Prism Graphpad software (version 10.0.2). For all the statistical analysis: ns, p-value > 0.05; *, p-value ≤ 0.05; **, p-value ≤ 0.01; ***, p- value < 0.0005; **** p-value < 0.0001.

Acknowledgements

Research was conducted in a facility constructed with support from Australian Cancer Research Foundation (ACRF)/Institute for Molecular Bioscience Cancer Biology Imaging Facility, which was established with the support of the ACRF. We thank the IMB Microscopy facility staff.

Competing interests

No competing interests declared.

Funding

M.D.W is supported by a Future Fellowship (FT200100899), a Discovery Project grant (DP220101878) from the Australian Research Council and an Ideas Grant (2013027) from the National Health and Medical Research Council.

Data availability

Data availability: All relevant data can be found within the article and its supplementary information.

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