Optogenetic rescue of a developmental patterning mutant

Heath E. Johnson[1], Stanislav Y. Shvartsman[2,3], Jared E. Toettcher[1]

[1] Department of Molecular Biology
Princeton University, Princeton NJ 08544

[2] Lewis Sigler Institute for Integrative Genomics
Princeton University, Princeton NJ 08544

[3] Department of Chemical and Biological Engineering
Princeton University, Princeton NJ 08544

Corresponding Author and Lead Contact:

Jared Toettcher
Lewis Thomas Laboratory Room 140
Washington Road
Princeton, NJ 08544
609-258-9243 (phone)
609-258-1894 (fax)
toettcher@princeton.edu

Animal embryos are partitioned into spatial domains by complex patterns of signaling and gene expression. Yet it is still largely unknown what features of a developmental signal (e.g., the minimum duration, spatial extent, or number of distinct concentrations) are essential to be compatible with life. Part of the challenge arises because it has been impossible to "paint" an arbitrary signaling pattern on an embryo to test its sufficiency. Here we demonstrate exactly this capability by combining optogenetic control of Ras/Erk signaling with the genetic loss of terminal signaling in early *Drosophila* embryos. Simple all-or-none light inputs at the embryonic termini were able to completely rescue normal development, generating viable larvae and fertile adults from this otherwise-lethal genetic mutant. Systematically varying illumination parameters further revealed that at least three distinct developmental programs are triggered by different cumulative doses of Erk, and that a wide range of spatial patterns permit normal tissue movements during gastrulation. These results open the door to the targeted design of complex morphogenetic outcomes as well as the ability to correct patterning errors that underlie developmental defects.

Introduction

During animal development the embryo is patterned by gradients of protein activity that define cells' positions along the body axes and within developing tissues (*1*). In recent years, many developmental patterns have been characterized in precise quantitative detail in individual embryos (e.g. Bicoid; Sonic Hedgehog; Erk) (*2-4*). These studies have revealed two hallmarks of developmental patterning. First, patterns are highly reproducible from embryo to embryo, suggesting that even slight changes in signal levels could convey positional information. Second, developmental patterns are dynamic, with spatial distributions that vary as the pattern is first established and then erased. Yet it remains unclear which features of these dynamic, spatially-variable signaling patterns are essential (*5*), or how many distinct activity levels (e.g. protein concentrations or cumulative doses) of a single signaling pattern are "read out" by the genetic networks that serve as signal interpretation systems.

We can envision an idealized experiment to define the essential information contained in a developmental pattern (**Figure 1A**). First, one might prepare a "blank canvas" embryo in which a specific developmental signaling pattern is completely eliminated. On this background one might then "paint" a wide range of precisely-defined candidate input patterns and then monitor their capability to rescue each developmental process. Although constructing such a blank canvas is usually possible using genetic loss-of-function alleles, the field has so far lacked the ability to paint arbitrary spatiotemporal signaling patterns on top of this null state.

We reasoned that the recent development of optogenetic control over cell signaling opens the door to exactly this capability. In principle, an appropriately-tailored light input could be used to

produce any spatiotemporal signaling pattern, enabling a developmental biologist to test for the essential features required to support proper tissue patterning, or allowing a bioengineer to apply non-natural stimuli to implement novel tissue architectures or morphogenetic programs (*6*, *7*). However, despite the promise of this approach, it is still unknown whether the engineered light-sensitive proteins that make up optogenetic signaling systems are able to recapitulate all of the functions of natural developmental signals, and therefore whether a spatial light stimulus might even be capable of fully rescuing the loss of an endogenous signaling pattern.

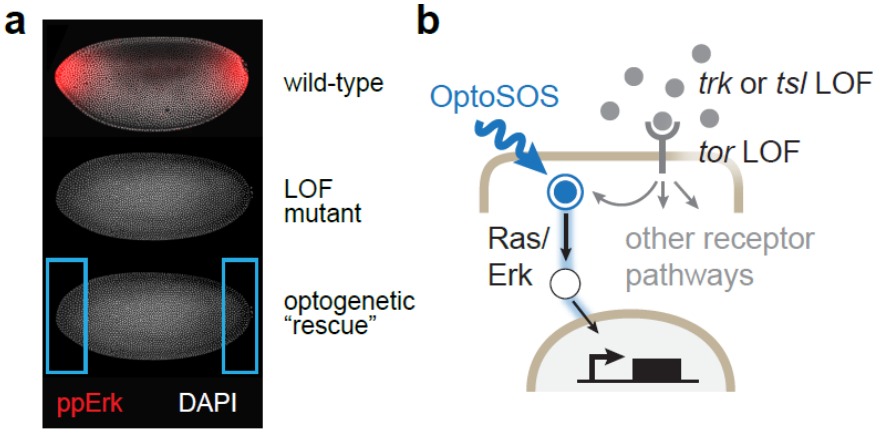

Figure 1. Painting developmental patterns on a blank canvas. (**A**) Upper: phosphorylated Erk (red) in the early *Drosophila* embryo. Middle: Erk signaling is eliminated in loss-of-function Torso pathway mutations such as *trk*[1] shown here. Lower: Direct optogenetic stimulation could in principle be used to 'paint' any signaling pattern on a LOF embryo. (**B**) Receptor-level activation can be bypassed by loss of the upstream components Tor, Tsl and Trk. The light-activated OptoSOS system can be used to directly activate the Ras/Erk pathway in a loss-of-function embryo.

As a first test of this approach, we set out to perform an optogenetic rescue of terminal signaling, the first essential developmental pattern of receptor tyrosine kinase (RTK) activity during *Drosophila* embryogenesis (*8*). The terminal pattern is orchestrated by localized activation of the RTK Torso (Tor) by its ligand Trunk (Trk) at the embryonic anterior and posterior poles (**Figure 1B**), leading to a reproducible terminal-to-interior gradient of Erk kinase activity that is dynamically established over a 2-hour window in early embryogenesis (*4*). This localized pattern of activation is essential: embryos from mothers lacking Tor, Trk, or the

required co-factor Torso-like (Tsl) are defective in a wide variety of anterior- and posterior-localized processes, including the formation of mouth parts and tail structures, the differentiation of many endoderm-derived tissues, and the ability to coordinate tissue movements during gastrulation (*9, 10*). Yet despite decades of study, major questions remain about cells' interpretation of terminal signaling. Some studies have proposed that the naturally-observed gradient of Tor activity is essential for activating distinct target genes and cell fate programs at different embryonic positions (*11-13*), while others posit that terminal signaling represent an all-or-none switch that fires at a single threshold (*14, 15*).

Here, we report the construction of OptoSOS-*trk* embryos that completely lack receptor-level terminal signaling but whose Erk activity can be controlled with light. Moreover, we demonstrate that local illumination is sufficient to fully rescue the entire life cycle from these normally-lethal mutant embryos. OptoSOS-*trk* embryos illuminated at both poles develop normal head and tail structures, gastrulate normally, hatch, metamorphose and mate. We further define the lower essential limits of terminal Erk signaling, demonstrating that at least three distinct developmental switches are triggered at successively increasing Erk activity doses. Finally, we investigate the spatial requirements of terminal patterning in the context of one model embryo-wide response: tissue movements during gastrulation. We find that a wide range of posterior patterns result in normal tissue movements, despite substantial redistribution of cells between lateral and posterior fates. Together, our data provides a first step towards defining the essential information contained in developmental signaling patterns and opens the door to optically programming cell fates and tissue movements with high precision in developing tissues.

Results

Establishing optogenetic Ras as the sole source of terminal pathway activity

We first set out to establish a genetic background where light could be used as the sole source of Erk activity at the embryonic termini, so that its ability to rescue subsequent development could be assessed. Two attributes make terminal signaling an ideal system for such an optogenetic rescue. First, all three components of the receptor-ligand system that initiates terminal signaling (Trk, Tor, and Tsl) are maternal-effect genes, with no essential zygotic requirements (*9*). Thus, in principle, one may be able to rescue the organism's entire life cycle by reconstituting a single developmental pattern. Second, well-characterized optogenetic tools are already available to control the Ras/Erk pathway (*16, 17*), one of the major downstream effector pathways of terminal signaling. Applied to *Drosophila* development, we found that blue light illumination can be used to drive high, uniform levels of Erk phosphorylation in OptoSOS-expressing embryos and expand the expression domains of terminal signaling-associated genes (*18, 19*). A crucial attribute of this optogenetic system is that it directly activates Ras, rendering it independent of upstream receptor-level stimulation (**Figure 1B**). In principle, OptoSOS-based activation could thus be combined with the genetic loss of Torso receptor activity to place Ras/Erk signaling solely under optogenetic control (**Figure 1B**).

We established embryos of three genotypes that each harbor our OptoSOS system and exhibit diminished or absent receptor-level activity: embryos produced by mothers homozygous for the trk^{1} amorphic allele or tsl^{691} hypomorphic allele, or embryos which carried a Tor RNAi construct (**Supplementary Methods**). We then monitored tissue movements during gastrulation in the presence and absence of blue light to assess whether optogenetic stimulation could

compensate for the loss of terminal signaling in each case (**Figure S1**). Terminal signaling is required for invagination of the posterior midgut during gastrulation, and we previously found that illuminating OptoSOS embryos expands the domain of contractile midgut tissue across more than 80% of the embryo (*18*). All three alleles failed to undergo posterior midgut invagination in the dark, indicative of strong loss-of-function of terminal signaling (**Figure S1**). Blue light illumination restored tissue invagination in OptoSOS-*trk* and OptoSOS-Tor^{RNAi} embryos, but not OptoSOS-*tsl* embryos (**Figure S1**), consistent with a recent report suggesting that Tsl plays an additional, terminal signaling-independent role in gastrulation (*20*). These preliminary experiments suggested that OptoSOS-*trk* and OptoSOS-Tor^{RNAi} embryos are able to transition complete loss of terminal signaling in the dark to a strong gain-of-function phenotype upon illumination. However, to avoid potential concerns of incomplete knockdown or off-target effects due to the Torso RNAi construct, we chose to focus on OptoSOS-*trk* embryos for all subsequent experiments.

Local illumination completely rescues normal development in OptoSOS-*trk* embryos

We next set out to determine whether light was sufficient for restoring various terminal signaling-associated embryonic structures in OptoSOS-*trk* embryos, and if so, which features of the light pattern might prove to be essential. We began with a simple light pattern: binary, all-or-none illumination of the anterior or posterior pole. We matched the pattern's duration (90 min), spatial range (15% embryo length) and light intensity to roughly match the parameters observed for doubly-phosphorylated Erk during endogenous terminal signaling (**Figure S2**) (*4*). Although such a stimulus eliminates both the complex temporal dynamics and spatial gradient of the endogenous terminal pattern, even this simple all-or-none pattern was sufficient to restore head

structures that were indistinguishable from those in wild-type embryos when light was applied to the anterior pole (**Figure 2A**). Similar results were obtained upon posterior illumination, which was sufficient to restore the formation of tail structures such as posterior spiracles as well as the 8th abdominal segment (**Figure 2B**).

To assess rescue of many other terminal signaling-dependent fates and processes that are difficult to monitor individually, we next applied similar all-or-none light patterns at both poles of individual embryos and visualized the remainder of their development by differential interference contrast (DIC) microscopy. Remarkably, a majority of embryos illuminated in this manner were able to gastrulate normally, complete the remainder of embryogenesis, and hatch from the imaging device (**Figure 2C**; **Movie S1**). The larvae that hatched on the microscope were collected and maintained in standard tubes, where they proceeded normally through each instar, pupated and produced normal adult flies (**Figure 2D**). We reasoned that optogenetically-rescued female adult flies produced in this manner should still be *trk*-null, so embryos produced by these females should still harbor phenotypes consistent with the loss of terminal signaling (e.g., head defects, absence of the 8th abdominal segment and tail structures). Indeed, 100% of embryos laid from light-rescued mothers failed to hatch, and cuticle preparations revealed the *trk* phenotype in all progeny (**Figure 2D**). Taken together, these data confirm the optogenetic rescue of terminal signaling in *Drosophila* embryogenesis. Simple illumination patterns were sufficient to overcome lethal defects in body segmentation, tissue morphogenesis, and cell differentiation to restore the entirety of the fly's life cycle.

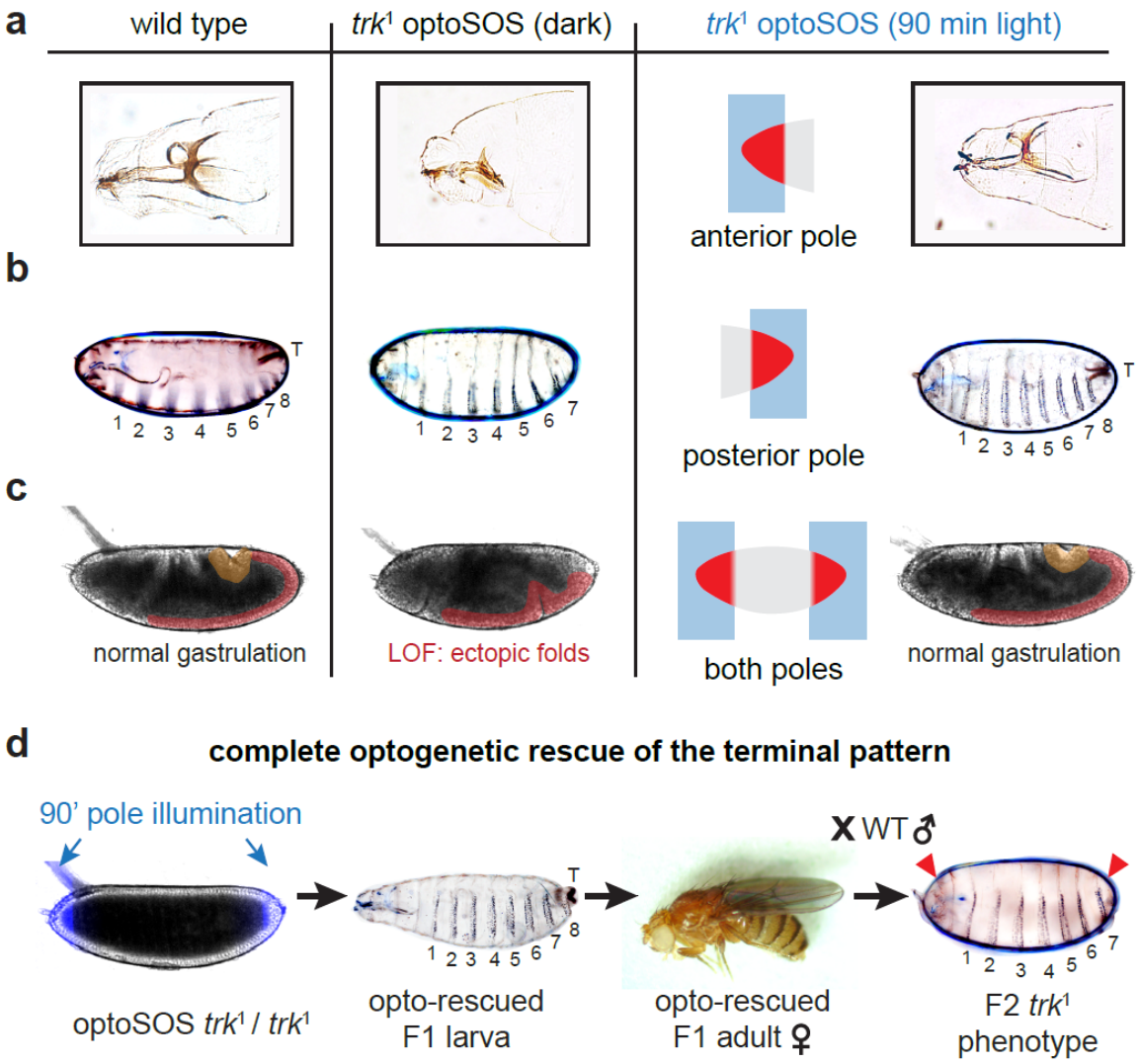

Figure 2. Light-controlled terminal signaling rescues normal development. (**A**) Head structures are formed normally in wild-type embryos but are absent or truncated in embryos lacking terminal signaling. Anterior illumination rescues wild-type head structures. (**B**) Tail structures (marked as "T") and the 8th abdominal segment, missing in the absence of terminal signaling, are rescued by posterior illumination. (**C**) Rescue of normal morphogenetic movements during gastrulation, posterior midgut invagination (yellow highlight) and germ band elongation (red highlight) by illumination at both poles. (**D**) Illuminating embryos at the termini with 90 min of blue light rescues the entire fly life cycle. Embryos hatch, eclose, and mate. The embryos produced by female light-rescued flies show the *trk* mutant phenotype, as expected from this maternal-effect gene.

At least three doses of terminal signaling trigger distinct developmental programs

The rescue of all anterior and posterior tissue responses by a single light dose is consistent with two different models of terminal cell fate choice. First, all terminal fates may be triggered

by a single ‘decoding module’, activating all cellular responses at a single threshold of pathway activation (*15*). Alternatively, individual fates may be rescued one by one as the signaling dose crosses distinct fate-specific thresholds (*12*). To discriminate between these possibilities, we set out to map which terminal fates were triggered as the light dose was progressively increased. We found that even the lowest light dose tested – a 5 min light pulse – was sufficient to restore tail structures in a majority of embryos (**Figure 3A**; **Figure S4**). Even a 5 min pulse delivered globally to the entire embryo was sufficient to rescue tail structures at their proper embryonic position, revealing that these structures are triggered by a developmental switch that does not require any local spatial information from the terminal pattern. Other terminal phenotypes, including abdominal segmentation and posterior midgut invagination, were unaffected by the 5 minute Erk pulse, and still exhibited their terminal loss-of-function phenotype.

As we progressively increasing the light dose, we observed the sequential rescue of additional developmental processes. The 8th abdominal segment was restored as the posterior light stimulus was increased to 20 min (**Figure 3B**), and normal gastrulation movements were restored at 45 min of posterior illumination (**Figure 3C**). A similar dose was also required at the anterior pole for the formation of head structures. We thus conclude that Ras/Erk activity is interpreted into at least three all-or-none developmental programs with thresholds spanning nearly an order of magnitude (5 min – 45 min) (**Figure 3D**). Our data are thus consistent with a ‘multiple-threshold’ model of terminal signal interpretation: different doses of terminal signaling are able to trigger qualitatively-different downstream response programs (*12*). Importantly, the multiple-threshold model is perfectly compatible with our prior observation of optogenetic rescue by a single, high dose of light. That is because phenotypes appear to be rescued

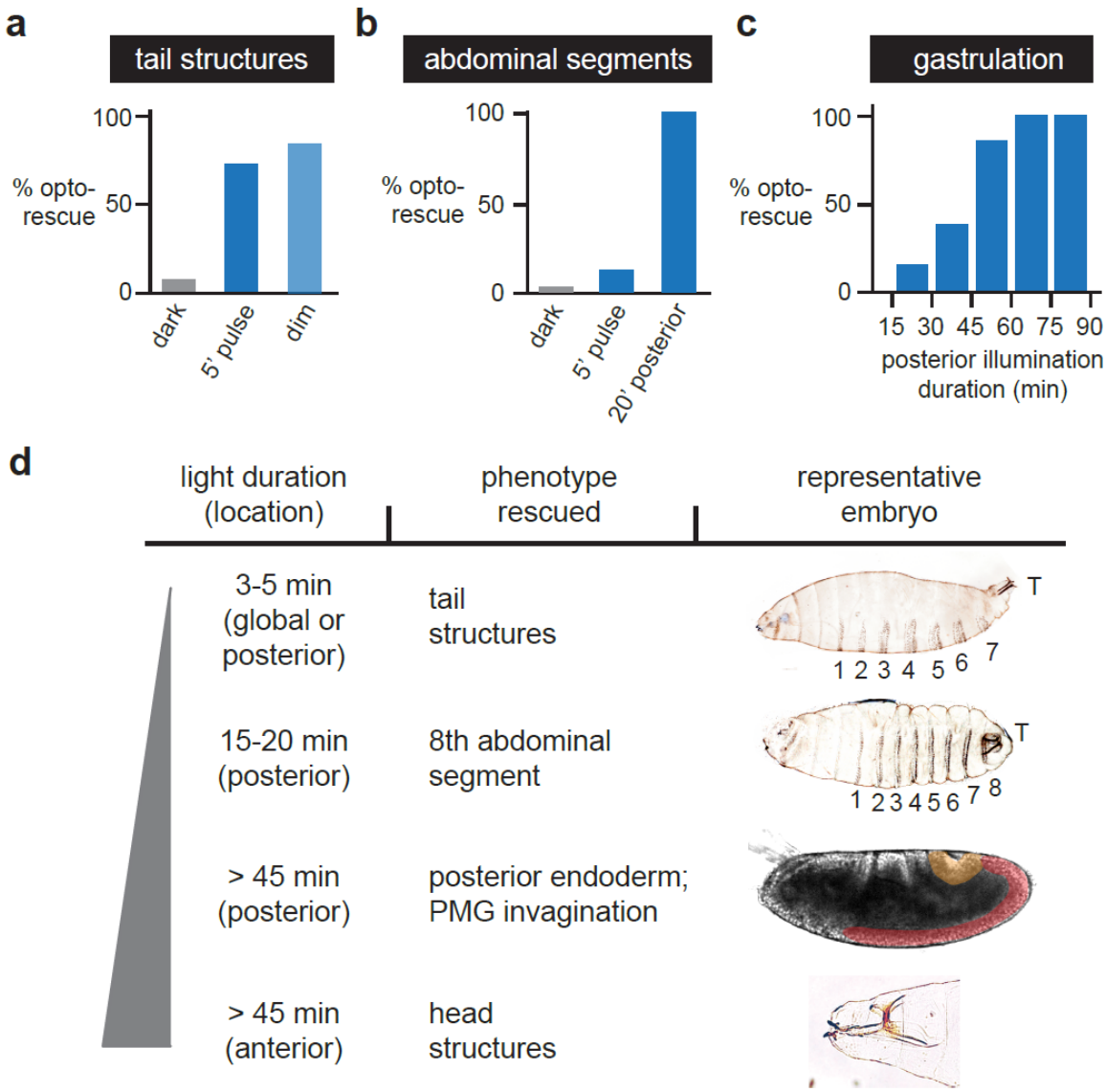

Figure 3: Three doses of terminal signaling trigger distinct developmental programs. (**A**) The fraction of embryos with normal tail structures was quantified from cuticle preparations from embryos incubated in the dark, subjected to a 5-minute light pulse ("5' pulse"), or subjected to continuous, low-intensity Ras/Erk signaling driven by 1 sec blue light pulses delivered every 120 sec ("dim"). (**B**) The fraction of embryos with 8 abdominal segments was quantified from cuticle preparations from embryos incubated in the dark, subjected to a 5-minute light pulse ("5' pulse") or illuminated for 20 min at the posterior pole. (**C**) Posterior tissue movements during gastrulation (yellow and red highlights) were scored by differential interference contrast (DIC) imaging of individual embryos illuminated at the posterior pole for different durations. (**D**) Developmental sequence of terminal phenotypes rescued at progressively higher doses of signaling. In each case, the dose, spatial position, developmental phenotype and representative image of a rescued embryo are shown.

independently from one another, so a given light dose rescues all developmental processes that are triggered at thresholds at or below this level.

Gastrulation movements are robust to variation in the spatial range of terminal patterning

The preceding experiments define the temporal requirements for terminal signaling, but what rules govern its permissible spatial parameters? We can again envision two extreme models. First, it is possible that only a precise spatial range of terminal signaling can support normal development, by balancing the proportion of cells committed to terminal and non-terminal fates. At the other extreme, a diverse set of spatial patterns could funnel into a proper developmental outcome (*21*), as in the case of the Bicoid morphogen gradient as the gene dosage is varied (*22*) or the Shh gradient in the neural tube of Gli3$^{-/-}$ mice (*23*). To test these possibilities, we next set out to map how the spatial domain of terminal signaling affects one model developmental response: tissue morphogenesis during gastrulation.

Prior to gastrulation, terminal signaling triggers differentiation of posterior endoderm tissue. This tissue then moves across the embryo's dorsal surface during gastrulation and germ band elongation, a process that is thought to be driven by a combination of 'pushing' by elongating ventral tissue (**Figure 4A**; red) and 'pulling' by invagination of the posterior endoderm itself (**Figure 4A**; yellow) (*24*, *25*). Terminal signaling is absolutely required, as embryos derived from *trk*-mutant mothers completely fail to undergo posterior invagination and germ band elongation, leading to buckling of the elongating tissue along the embryo's ventral surface (*9*).

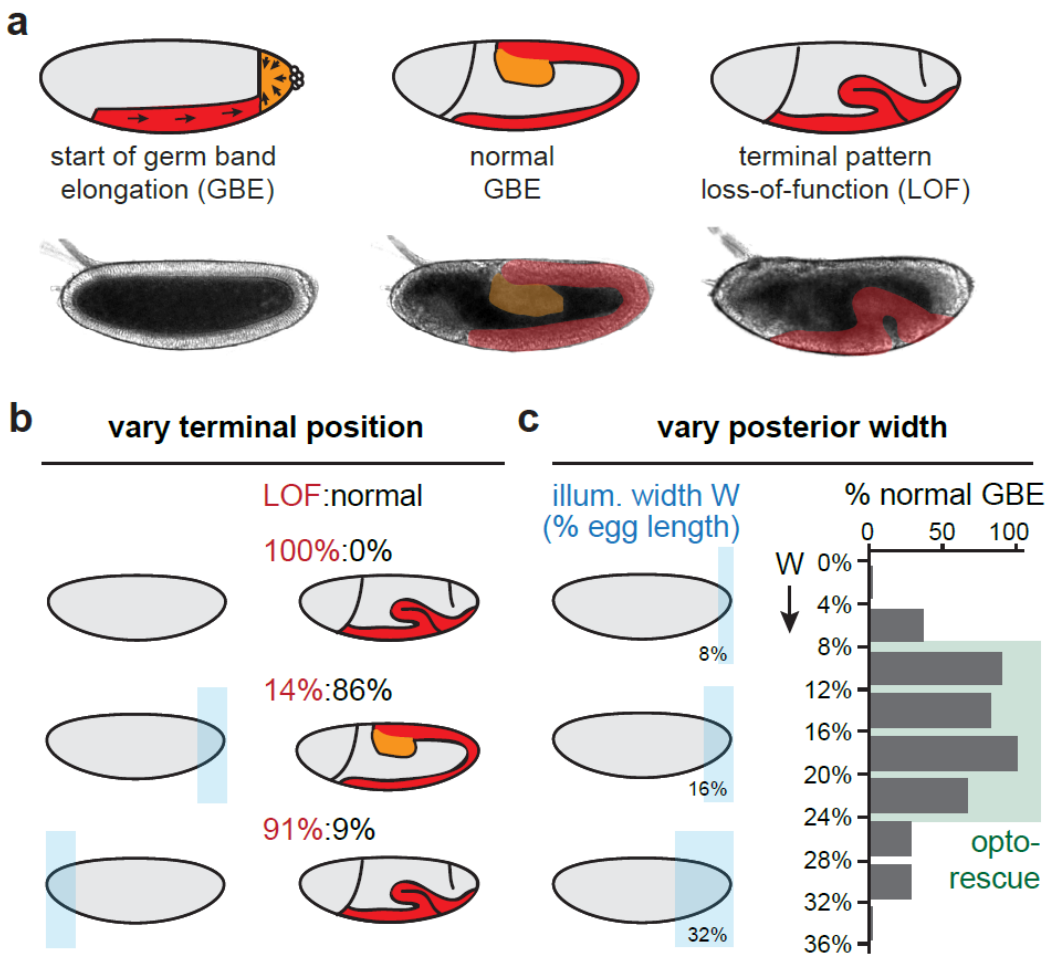

Figure 4. The lower limit of spatial signaling required for tissue morphogenesis. (**A**) Schematic of posterior tissue movements during germ band elongation (GBE). Left: During germ band elongation, cells migrate to the ventral surface (red highlight) and intercalate, while the posterior endoderm (orange highlight) constricts and internalizes. Middle: This results in the large-scale movement of posterior tissue along the dorsal surface towards the head. Right: In the absence of terminal signaling, germ band elongation is blocked, leading to buckling of ventral tissue. (**B-C**) Optogenetic dissection of the spatial requirements for terminal signaling in gastrulation. "Normal" gastrulation was defined as successful posterior invagination and a germ band whose length was within the 95% confidence interval obtained from 50 wild-type embryos. A loss-of-function ("LOF") embryo was defined by a lack of posterior invagination and germ band elongation. (**B**) Gastrulation was scored for embryos that were kept in the dark or illuminated at either the anterior or posterior pole and monitored by differential interference contrast (DIC) imaging. (**C**) Gastrulation was scored for embryos that were illuminated with different posterior pattern widths, from 0-36% of the embryo's length. Illumination widths that produce normal gastrulation in a majority of embryos are marked with a green box. For **B-C**, normal gastrulation is defined by presence of posterior invagination and a germ band that extends to a length within the 95% confidence interval measured across 27 wild-type embryos.

To assess whether the anterior or posterior terminal pattern is sufficient on its own for normal gastrulation, we monitored morphogenetic movements in individual OptoSOS-*trk* embryos illuminated at either the anterior or posterior pole (**Figure 4B**). We found that gastrulation failed in dark-incubated embryos as well as > 90% of embryos illuminated only at the anterior pole. In contrast, posterior pole illumination triggered normal morphogenetic movements that were quantitatively indistinguishable from wild-type embryos or embryos illuminated at both poles. Having thus determined that posterior terminal signaling is sufficient for gastrulation, we proceeded to systematically vary the width of posterior pattern. We found that the overall size of the posterior invagination scaled in proportion to the illumination width (**Figure S5A-B**), confirming that OptoSOS stimulation is indeed sufficient to program posterior endoderm (*19*). Yet despite the different proportion of terminal vs non-terminal tissue, a majority of embryos underwent germ band elongation that was quantitatively indistinguishable from wild-type controls as the light pattern was varied from 8–24% of the egg's length (**Figure 4C**; **Figure S5C**). These data reveal a surprising degree of robustness in tissue morphogenesis. As the relative population is shifted between two mechanically-active cell populations (i.e., elongating ventral cells and constricting posterior cells), the speed and extent of tissue movement is unaffected. The ability to program tissue movements at any spatial positions of interest will likely make OptoSOS-*trk* embryos a rich resource for informing and challenging models of tissue morphogenesis.

Discussion

Here, we have shown how optogenetic control can be used to systematically interrogate which features of a developmental signaling pattern are compatible with life. We find that a

single pulse of blue light, delivered to both embryonic termini, is sufficient to convert a lethal loss-of-function phenotype to rescue the full *Drosophila* life cycle: embryogenesis, larval development, pupation, adulthood and fecundity.

Our optogenetic rescue result provided two insights into the interpretation of developmental RTK signaling. First, we find that recruiting the catalytic domain of SOS to the plasma membrane recapitulates all the essential developmental functions of Tor receptor tyrosine kinase signaling at the embryonic termini. This complete molecular sufficiency is non-obvious: RTKs activate many intracellular pathways that are bypassed by OptoSOS stimulation (*16*), some of which have been suggested to play roles in early *Drosophila* embryogenesis (*26*). Nevertheless, our result is consistent with reports that RTK-driven gene expression (and thus cell fate determination) is mediated primarily by the Ras pathway (*27*), and that activating Ras pathway mutations are genetic suppressors of Tor partial loss-of-function alleles (*28*).

Second, our use of simple, all-or-none light inputs suggests that the normally-observed gradient of terminal signaling is dispensable. This statement is supported by three additional observations: (i) all-or-none illumination results in a sharp boundary of OptoSOS membrane translocation (*19*); (ii) a live-cell Erk biosensor reveals a sharp boundary of light-induced pathway activity relative to the endogenous terminal gradient (**Figure S3**); and (iii) all embryonic positions are uniformly sensitive to SOS stimulation, so uniform illumination over a field induces uniform pathway activity (*19*). It appears that only two levels of Ras signaling are required – signaling must be “on” above a sufficient threshold at the termini and “off” at interior

positions – contrary to a longstanding view of developmental RTK activation (*12*, *29*) and unlike classical morphogens such as the Bicoid transcription factor (*30*).

Our results suggest that a great deal of the sophisticated information processing in terminal patterning is contained not in the gradient or dynamics of upstream signaling, but rather emerges from downstream transcriptional networks. After all, although simple binary patterns of light are sufficient, we find that distinct developmental events are triggered at vastly different doses (tail structures: 5 min; gastrulation movements: 45 min) and with different spatial requirements (tail structures: no spatial requirement; gastrulation: 40-120 μm pattern width). The order in which phenotypes are rescued is also puzzling: tail structures are formed at the posterior-most position in the embryo, a position where terminal signaling is usually highest, but paradoxically respond to the lowest threshold of terminal signaling. How might a single signal be sensed at such vastly different concentrations and spatial scales? These observations immediately raise an open challenge: we still lack a clear picture of the signal interpretation circuits that respond to different profiles of developmental Erk signaling. Future work combining optogenetic control with high-resolution transcriptional studies (*27*, *31*) to track the localized expression of classic downstream genes (e.g. Tll, Hkb) could be crucial for closing this gap.

There is considerable current interest in defining the rules that govern morphogenesis and patterning, both *in vivo* during embryo development and in engineered organoid-based systems. The optogenetic approaches defined here represent a first step toward the delivery light-based programs to specific cells of interest within multicellular tissues. Such a capability may open the

door to unprecedented control over developmental processes in both natural and synthetic multicellular systems.

Author Contributions

H.E.J., S.Y.S. and J.E.T. conceived and designed the project and wrote the manuscript. H.E.J. performed all experiments.

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