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Fgf8 dynamics and critical slowing down may account for the temperature independence of somitogenesis

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Somitogenesis, the segmentation of the antero-posterior axis in vertebrates, is thought to result from the interactions between a genetic oscillator and a posterior-moving determination wavefront. The segment (somite) size is set by the product of the oscillator period and the velocity of the determination wavefront. Surprisingly, while the segmentation period can vary by a factor three between 20 °C and 32 °C, the somite size is constant. How this temperature independence is achieved is a mystery that we address in this study. Using RT-qPCR we show that the endogenous \emph{fgf8} mRNA concentration decreases during somitogenesis and correlates with the exponent of the shrinking pre-somitic mesoderm (PSM) size. As the temperature decreases, the dynamics of \emph{fgf8} and many other gene transcripts, as well as the segmentation frequency and the PSM shortening and tail growth rates slows down as $T-T_c$ (with $T_c = 14.4$ °C). This behavior characteristic of a system near a critical point may account for the temperature independence of somitogenesis in zebrafish.
somitogenesis is the process of segmentation of the antero-posterior axis in vertebrates. In zebrafish this process starts at about 10 hpf and ends at 24 hpf. During that developmental interval, as the embryo elongates, pairs of somites synchronously and periodically pinch off from the anterior part of the PSM in an anterior to posterior series until 31 pairs of somites are formed. The period between somites, but surprisingly not their size, is strongly temperature dependent.

The formation of somites in zebrafish is preceded by the establishment of a segmental pre-pattern in the anterior PSM accomplished by a stripe of gene expression that is thought to result from the interactions between a genetic oscillator and a posterior-moving determination wavefront. This pre-pattern determines the position of the next somite. The Clock and Wavefront framework first proposed by Cooke and Zeeman in 1976, is currently used to describe the output of the complex genetic network underlying the formation of this pre-pattern. In this framework, periodic oscillations (the segmentation clock) that move anteriorly (in the PSM reference frame), pass through a determination wavefront moving posteriorly (in both the lab and the PSM reference frames) and stop oscillating. As a result, a stripe of genes such as mesp2 are activated to establish the future boundary of the following somite. In this model, the size of a somite is determined by the distance traveled by the determination wavefront during one cycle of the segmentation clock (see Fig. 1).

The segmentation clock driving the differentiation of the PSM into somites at the determination wavefront, has been amply studied and described and is not the subject of this work. Rather we here focus on the determination wavefront. In contrast to the segmentation clock whose details are species dependent, the main putative actors of the wavefront (e.g., fibroblast growth factors (Fgf), retinoic acid (RA)) are conserved in vertebrates (from fish to mammals, including snakes and amphibians). Previous studies have shown that as cells exit from the progenitor domain at the posterior end of the PSM, they stop transcribing Fgf genes. Thus, Fgf mRNA progressively decays as cells move towards the anterior of the PSM and an Fgf gradient is formed. This mRNA gradient is translated into a protein gradient and into a MAPK activity gradient along the PSM (as Erk, a MAPK protein is activated downstream of the FGF receptor).

Spurred by these experiments we have decided to investigate the dynamics of the PSM in fish embryos subjected to various perturbations of the putative actors of the wavefront (Fgf, RA, Erk, etc.). For these investigations we used time-lapse and fluorescent microscopy on live wild-type or transgenic embryos expressing either a fluorescent reporter of Erk activity or an exogenous source of Fgf8. In agreement with experiments on tail explants, our results imply that somite formation results from a coupling between the somitic oscillation clock and the local spatial (but not temporal) gradient of Fgf8 (or Erk activity). Expanding on these previous observations, we observe that the PSM size varies as the logarithm of the Fgf8 mRNA concentration, a result that has a simple explanation if the Fgf8 mRNA concentration decays exponentially in both space and time.

In addition, we took advantage of the external development of zebrafish embryos to manipulate the temperature of development. As the temperature T is lowered we find that the dynamics of Fgf8 and many other genes implicated in somitogenesis and cellular differentiation slow down by the same factor, namely as $(T−T_c)$ (with $T_c=14.4 \, ^\circ \text{C}$). Slowing down by this factor is also observed for the segmentation frequency $f_s$, the spatio-temporal dynamics of Erk and the PSM shrinkage and tail growth rates. These observations suggest that the temperature invariance of the developmental program during somitogenesis could be a simple reflection of the critical slowing down of some cellular metabolic networks near a critical temperature $T_c$.

**Results**

**Kinetics of somitogenesis impairment by Fgf-pathway inhibition.** We monitored the dynamics of Erk during somitogenesis in transgenic zebrafish (DREKA) embryos expressing a fluorescent reporter which cytoplasmic localization increases upon increasing Erk phosphorylation. Changes in Erk activity (i.e., phosphorylation) are thus well reflected and reported by changes in the ratio between cytoplasmic and nuclear fluorescence (Fig. 2a–c). As previously reported we observed that the domain of Erk activity shrank over time (Fig. S1a, b), with transitions at the segmentation clock period from a high activity to a low activity domain at a distance of about 3–4 somites down from the last somite, i.e., 3–4 segmentation periods prior to the appearance of a new somite (Fig. S1c). This transition in Erk phosphorylation is the earliest indication of commitment to differentiation into somites.

Having confirmed the role of Erk as an early somitic marker, we then studied the response of an embryo to perturbations of the Fgf pathway (of which Erk is a downstream effector) by pharmacological inhibition of the pathway with SU5402. Embryos exposed to a low concentration of SU5402 (50 μM), display an increase in the next jump of the Erk domain of activity (i.e., a larger somite three periods later) (Fig. 2a), followed by

**Fig. 1 Schematics of somitogenesis.** a The Clock and Wavefront model: antagonistic gradient of Fgf8 (originating from the posterior PSM, green) and RA (originating from the somites, violet) define a wavefront which interacts with a particular phase of the segmentation clock (in the PSM, red) to generate somites at periodic times and positions. b Kymograph of somitogenesis from 7 to 20 somites. The tail elongates at a constant rate $V_{tail}$ while the PSM shrinks at a roughly constant rate $V_{PSM}$ resulting in a somite wavefront propagating (in the lab frame) at a rate $V_{front} = V_{tail} - V_{PSM}$. 

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regular jumps (and somites) similar to WT embryos. However, if the embryos are exposed (at the ten somites stage) to 200 μM SU5402 the activity of Erk is completely repressed in all the PSM (compare Fig. 2f, g with the control shown in Fig. 2b, c). In such case, somite formation is impaired three segmentation periods later (i.e., from the 13 somites stage onward) (compare Fig. 2h, i with Fig. 2d, e); regular somites are not observed and expression of Mesp2a (a marker of the last somite) is absent.

Uniform activation of the Fgf pathway impairs somitogenesis. Next, we examined the role of RA in somitogenesis. Embryos were incubated from one cell stage in 10 μM DEAB (an inhibitor of RA synthesis) and exposed or not at 70% epiboly to 10 nM transRA (a physiological level which has been shown to rescue rhombomere formation following DEAB treatment). These perturbations had minimal effects on the domain of activity of Erk (Fig. S2a) and on the dynamics of the somitogenetic wavefront (Fig. S2b). On the other hand, embryos treated with DEAB from the one-cell stage and incubated in 1 μM transRA at the onset of somitogenesis display a strong uniform activation of Fgf8 in the PSM (Fig. S3). Subjecting DREKA embryos to such a high RA concentration from the ten somite stage onward has dramatic effects: the Erk activity is uniformly enhanced throughout the PSM (Fig. 3e, f), and somitogenesis is impaired from 13 somites stage with unclear somite boundaries and no expression of Mesp2a (Fig. 3g, h).

The data shown in Figs. 2 and 3 suggest that a spatial gradient of Erk activity (or Fgf8) is required for proper somite formation. To check whether a temporal variation of Erk activity could also alter somite formation, we next studied somitogenesis in presence of an increasing uniform concentration of Fgf8 from an exogenous source.

Uniform activation of an exogenous Fgf8 source does not affect somitogenesis. Our data in agreement with previous results suggest that somitogenesis is sensitive to the gradient of Fgf8 (or Erk activity) but insensitive to physiological perturbations of the RA concentration. To investigate the role of the Fgf8 variation during somitogenesis, we used a transgenic embryo Tg(uas:fgf8-T2A-cfp) to superpose an increasing but uniform source of Fgf8 on the endogenous one. The exogenous fgf8 gene is turned on (Fig. 4a) when a transcription factor Gal4-ERT is released from its cytoplasmic chaperone complex by binding of cyclofen.

Embryos uniformly expressing this exogenous source of Fgf8 display two characteristic phenotypes at 24 hpf, Fig. 4b: a mild phenotype (phenotype 1) characterized by enlarged heart and yolk and abnormal development of the head, and a severe phenotype (phenotype 2) characterized by enlarged heart, abnormal development of the head (with often no eyes), lost yolk extension and disordered late somites (>20 s). In contrast with embryos where the transgene has not been activated (similar to WT), upon expression of this exogenous source of Fgf8 on the endogenous one, the exogenous fgf8 gene is released from its cytoplasmic chaperone complex by binding of cyclofen.

Embryos uniformly expressing this exogenous source of Fgf8 are subjected to a high Fgf8 concentration from 13 somites stage onward (Fig. 4c). The continuous expression of the exogenous fgf8 results in a total concentration of transcripts a doubling of the initial (endogenous) concentration after about 8 h (20 somite stage). In spite of the presence of this uniformly expressed and continuously increasing exogenous source of Fgf8 on top of the endogenous one, the somitogenetic wavefront is barely affected...
and the PSM shrinks at the same rate as in WT embryos (Fig. 4e). In conjunction with the data shown in Figs. 2 and 3 this observation implies that the somitogenetic wavefront is sensitive to the spatial gradient of Fg8, not its local concentration, a conclusion reached earlier in different studies13,18.

The endogenous Fgf8 concentration decreases during somitogenesis. If during somitogenesis the morphogen fields translate with the growing tail, namely if they are stationary in the tail moving frame, then one expects the PSM size to remain constant. Since the PSM shrinks one is forced to conclude that the morphogen fields are not stationary in the tail moving frame during somitogenesis: their amplitude and/or their extant18 may vary.

To investigate that question we decided to monitor by RTqPCR the mean endogenous \( \text{fgf8} \) mRNA concentration (\( \text{fgf8} / \text{C2/C3/C10/C11} \) normalized by the mean concentration of \( \text{rpl13a} \), a reference gene) during somitogenesis in the whole embryo and in the PSM. That concentration is related to the measured qPCR threshold
The dynamics of many gene transcripts active during somitogenesis slow down with temperature as $T - T_c$. The variation with temperature of the segmentation period, tail growth rate, PSM shrinkage rate and $fgf8$ decay rate are characteristic of the critical slowing down of a system near $T_c$. If somitogenesis is indeed a critical system, all time-varying gene transcripts should behave similarly, i.e., their characteristic time should scale as $1/(T - T_c)$ or stated differently their variation as a function of the somite stage, $s = t/\tau_s$ should be temperature independent. To address this question, we studied, using RTqPCR, the dynamics of the relative average concentration of many gene transcripts at different temperatures, see Table S1. If they scale with temperature in the same way as the segmentation clock period, the data for a given transcript taken at different temperatures should fall on the same curve.

This is the case for genes known to play a role during somitogenesis[23], see Fig. 8, such as the genes implicated in the segmentation clock[24] (her1, her7, and hes6) and its synchronization[25] (deltaC, deltaD, and notch1a), in segmental patterning (mespa and mespb) and myogenesis (myog) but also for genes involved more generally in cellular differentiation (vox, ventx, nanog, and oct4) (Fig. S6). For genes which $\delta Ct$ value vary linearly with time during somitogenesis we can compute the slope of the linear best fit at various temperatures (Figs. 6a and S7). This analysis confirms the critical slowing down of genes as the temperature is decreased. Notice however that not all genes vary during somitogenesis. For some of them ($fgf4$, $igf2a$, $wnt3$, and $xpc$), the relative mean concentration seems constant between 5 and 21 somite stage (Fig. S8).

**Discussion**

In this work, we have studied some factors affecting the somitogenic wavefront velocity, in particular the PSM shortening rate. The observed impairment of somitogenesis in absence of an Erk activity gradient (either uniformly high or low Erk phosphorylation) suggests, in agreement with recent reports[13,18], that it is the spatial variation of Fgf8 (via its downstream effector, Erk) and not its local concentration which sets the position of the somitogenic wavefront. This conclusion is further confirmed by the observed invariance of somitogenesis in presence of a uniformly increasing concentration of Fgf8. The observed periodic jumps in the anterior boundary of the Erk activity domain moreover imply that commitment to somite formation is triggered when the gradient of Fgf8 falls below a threshold at a certain phase of the segmentation clock.

The shortening of the PSM therefore implies that the Fgf8 gradient decays with time. To investigate that issue we have measured the mean $fgf8$ mRNA concentration during somitogenesis (in whole embryo and in the PSM). Our observations point to an exponential decrease of the mean $fgf8$ mRNA concentration with a typical time scale of $\tau \sim 11$ somitogenic
periods: $\langle [fgf8m] \rangle \sim \exp(-t/\tau)$. Since the PSM shrinks at a constant rate $V_{PSM} \sim 24 \mu m/somite$: \( \Delta V_{PSM} = V_{PSM}\tau \), the mean $fgf8$ mRNA decay can also be expressed as a function of the PSM size: $\langle [fgf8m] \rangle \sim \exp(-\frac{\Delta V_{PSM}}{V_{PSM}}\lambda)$, with $\lambda = \tau V_{PSM} \sim 260 \mu m$.

These results are consistent with a source-sink mechanism for the Fgf8 gradient, where the $fgf8$ mRNA is generated in the progenitor domain with a time decreasing concentration (see Supplementary Note 1) and is degraded on a typical length-scale $\lambda$ towards the anterior part of the PSM: $\langle [fgf8m(x,t)] \rangle = F_0 \exp(-x/\lambda - t/\tau) \equiv u(x,t)$, where $x$ is the distance to the tail end. Thus, the measured mean concentration $\langle [fgf8m] \rangle$ varies exponentially with time, as indeed observed:

$$\langle [fgf8m] \rangle = \int_{0}^{\infty} dx [fgf8m(x,t)] \sim \exp(-t/\tau)$$

Our results suggest that a new somite $n$ is formed at distance $x_{n}$ from the tail end when the gradient of $Fgf8(x,t)$ falls below a certain threshold, $\eta_t$ at a given phase of the somitogenesis clock: $t = n\tau_s$.

$$\eta_t = -\frac{dFgf8}{dx}(x_{n},n\tau_s)$$
If the concentration of Fgf8 protein is a monotonous function of its mRNA: \( \text{Fgf8}(x,t) = G(\text{fgf8m}(x,t)) \), then:

\[
\eta_i = \frac{\lambda}{\lambda} G_0(\eta_i) \exp\left( -\frac{x_0}{\lambda} \frac{n\tau}{\eta_i} \right) G_0(\eta_i)
\]

In other words: \( x_0 = x_0 - n\tau\lambda = x_0 - n\tau V_{PSM} \) with \( x_0 = \lambda \ln(G_0(\eta_i) F_0 / \eta_i \lambda) \). Namely the PSM shrinks at a velocity entirely determined by the dynamics of Fgf8 (the ratio between its degradation length-scale \( \lambda \) and its decay rate \( \tau \)) (Fig. S9 and Supplementary Note 1). Notice that if the threshold (i.e., \( x_0 \)) is altered by a drug such as SU5402 from somite stage \( n \) onwards, only the size of the first somite after the perturbation \( t_{h+1} = x_{h+1} - x_0 \) will be altered (as \( x_{h+1} \) and \( x_0 \) are associated to different thresholds (i.e., \( x_0 \)), while later somites will have a regular size (same threshold, i.e., \( x_0 \)), as indeed observed\(^{18} \). It may be interesting to check in other model organisms if \( \left( \text{fgf8m}(n) \right) \) decays exponentially with time and if differentiation into somite is set by its gradient. If so one expects the \( \text{fgf8} \) mRNA concentration to correlate with the PSM size as observed here, i.e., as \( \exp(-\Delta PSM/\lambda) \).

Our results are also compatible with a recent report of a scaling relation between somite and PSM size in tail explants\(^{13} \), if the time dependence of the \( \text{fgf8} \) mRNA concentration in these tail explants does not decay exponentially (Fig. S9 and Supplementary Note 1).

We have further observed that the relation between the \( \text{fgf8} \) decay time, \( \tau \), and the PSM shrinkage rate \( V_{PSM} \): \( V_{PSM} = \frac{\lambda}{\tau} \) was conserved over a temperature range, where \( V_{PSM} \) varies by a factor two, implying that this relation established initially at 27 °C is not fortuitous. We observed that the \( \text{fgf8} \) decay rate, the spatio-temporal behavior of its downstream effector (pErk), the PSM shrinkage and tail growth rates and the clock frequency, all display the characteristic behavior of a system near its critical temperature at \( T_c \). All rates slow down as \( T \rightarrow T_c \). We have generalized that observation to many time-varying genes implicated in somitogenesis and cellular differentiation. These results imply that somitogenesis shares the characteristic dynamics of a system near criticality\(^{26} \).
Fig. 8 Variation with temperature of various gene transcripts. The \( \delta \)Ct of genes (her, her7, hes6, deltaC, deltaD, notch1a, myog, mespa, and mespb) known to play a role in somitogenesis relative to reference genes (rpl13a or \( \beta \)-actin) are plotted as a function of somite stage. Notice that as a function of somite stage the data taken at different temperatures collapse on the same curve. For some genes for which \( \delta \)Ct appears to vary linearly with somite stage (exponentially decreasing concentrations) we display the best fit. For others the continuous curve is just a guide to the eye. The increasing concentration of myog (decreasing \( \delta \)Ct values) is consistent with its role as a differentiation factor during myogenesis which follows somite formation.

degraded by treatment with DNaseI (Qiagen) for 20 min at room temperature. Total RNAs were eluted in 14 µL nuclease-free water (Qiagen). RNA concentration and purity were first assessed using Nanodrop (ThermoFisher). Sample quantity and purity were estimated by measuring the ratios of spectrophotometric absorbance 280/260 nm and 260/230 nm. RNA quality and integrity were further analyzed by capillary electrophoresis (Fragment Analyzer, Agilent Technologies) to determine the RNA quality number (RQN) for each sample. Defined on a scale ranging from 1 to 10, the mean RQN of the 194 samples was 9.9, indicating very good RNA quality. RNA were stored at \(-80^\circ\)C before reverse transcription.

Reverse transcription. RNA samples were diluted at 10 ng/µL by adding nuclease-free water. cDNA synthesis was performed using Reverse Transcription Master Mix from Fluidigm® according to the manufacturer’s protocol in a final volume of 5 µL containing 40 ng total RNA. Reverse transcription was performed using a Nexus Thermocycler (Eppendorf®) following the temperature protocol: 5 min at 25°C, 30 min at 42°C followed by heat-inactivation of the reverse transcriptase for 5 min at 85°C and immediately cooled to 4°C. cDNA samples were diluted 5× by adding 20 µL of low TE buffer [10 mM Tris; 0.1 mM EDTA; pH = 8.0] (TEKNOVA®) and stored at \(-20^\circ\)C before specific target pre-amplification.

Specific target pre-amplification. Each diluted cDNA was used for multiplex pre-amplification in a total volume of 5 µL containing 1 µL of 5× Fluidigm® PreAmp Master Mix, 1.25 µL of cdNA, 1.25 µL of pooled TaqMan® Gene Expression assays (Life Technologies, ThermoFisher) with a final concentration of each assay of 180 nM (0.2x) and 1.5 µL of nuclease-free water. The cdNA samples were subjected to pre-amplification following the temperature protocol: 95°C for 2 min, followed by 18 cycles at 95°C for 15 s and 60°C for 4 min. The pre-amplified cDNA was diluted 5× by adding 20 µL of low TE buffer and stored at \(-20^\circ\)C before qPCR.

High throughput qPCR. Quantitative PCR was performed using the high-throughput platform BioMark® HD System and the 48.48 GE Dynamic Arrays (Fluidigm®). The expression of 48 target genes was quantified in 108 samples by quantitative PCR on five 48.48 microfluidic chips. Each of the five chips contained a non-template control (NTC), and a serial dilution of a cdNA sample has been used both as a standard curve to determine efficiencies and inter-chip calibrator. The Ct obtained from the standard curve and an internal control were identical among the five chips allowing inter-chip comparisons. Six microliter of Sample Master Mix (SMM) consisted of 2.7 µL of 5× diluted pre-amplified cDNA, 0.3 µL of 20× GE Sample Loading Reagent (Fluidigm) and 3 µL of TaqMan® Gene Expression PCR Master Mix (Life Technologies, ThermoFisher). Each 6 µL Master Mix Assay (MMA) consisted of 3 µL of TaqMan® Gene Expression assay 20× (Life Technologies, ThermoFisher) and 3 µL of 2× Assay Loading Reagent (Fluidigm). Five microliter of each SMM and each MMA premixes were added to the dedicated wells. The samples and assays were mixed inside the chip using MX IFC controller (Fluidigm). The loaded Dynamic Array was transferred to the Biomark® real-time PCR instrument and subjected to PCR experiment (25°C for 30 min and 70°C for 60 min for thermal mix; 50°C for 2 min and 95°C for 10 min for hot start; 40 cycles at 95°C for 15 s and 60°C for 1 min). The parameters of the thermocycler were set with ROX as passive reference and single probe FAM-MGB as fluorescent detector. To determine the threshold cycle Ct, data were processed by automatic threshold for each assay, with linear derivative baseline correction using BioMark Real-Time PCR Analysis Software 4.0.1 (Fluidigm). The quality threshold was set at the default setting of 0.65.

Normalization and quantification. The reference gene rpl13a (or \( \beta \)-actin when mentioned in the text) has been chosen after pair-wise correlation analysis using Bestkeeper algorithm23. The difference in the number of quantification cycles between the genes of interest (GOI) (in various conditions) and a reference gene (GRF, i.e., rpl13a or \( \beta \)-actin) was \( \Delta \)Ct = C\text{t}_{\text{GOI}} - C\text{t}_{\text{GRF}}.\) The normalized transcripts abundance (averaged over three PCR replicates) was calculated as \( \Delta \frac{1}{Ct} = \log_{2} (\text{norm} (\frac{\text{r}^{\text{rpl13a}}}{\text{r}^{\beta\text{-actin}}})) / \text{norm}(\text{r}^{\text{rpl13a}}/\text{r}^{\beta\text{-actin}})) \sim -\ln(\text{norm}(\text{r}^{\text{rpl13a}}/\text{r}^{\beta\text{-actin}}))).\) The list of genes studied is described in Table S1.

IHC on zebrafish embryos. The embryos obtained in the various conditions and the different stages mentioned in the text were fixed in 4% PFA overnight at 4°C, followed by dehydration with 100% methanol at \(-20^\circ\)C for more than 1 day. After gradual rehydration of methanol and wash with PBS/Tween 0.1%, the embryos were incubated in a blocking solution: 1%Trition, 1% DMSO, 1% BSA and 10% sheep serum (Sigma) in PBS on a shaker for 1 h at room temperature, followed by incubation with 1:400 Phospho-ERK Monoclonal Antibody as the primary antibody (Thermo, MA5-15173) on a shaker overnight at 4°C. After extensive washing with PBS/Tween and incubation in blocking solution, a second antibody (anti-Rabbit conjugated to Alexa Fluor 488, Invitrogen) diluted 1:200 was added overnight at 4°C. After washing with PBS/Tween, images were taken on a Nikon Ti microscope equipped with a Hamamatsu Orca camera.
Whole-mount in-situ hybridization (ISH). Whole-mount in-situ hybridization with digoxigenin-labeled riboprobes was performed as described previously. The antisense riboprobes were synthesized from template plasmids (gift of P. Rosa, IBENS) containing fgf8 full length cDNA. The antisense riboprobes were synthesized from template plasmids (gift of P. Rosa, IBENS) containing fgf8 full length cDNA or xirp2a.

Drug treatments. Wild type embryos were incubated in 10 μM DEAB (with or without addition at 70% epiboly of 10 nM all-trans RA). The double transgenic embryos Tg(ubi:Gal4ERT;cry:CFP;uas:fgf8-T2A-cfp;ubi:Eos) were incubated at 70% epiboly in 3 μM cyclofen (gift of J. Lielli) diluted in EM. DREKA embryos were treated with 50 μM, 200 μM of SU5402 (Sigma-Aldrich) at 10 somite stage for 1 h and 10 nM and 1 μM of all-trans RA (Sigma-Aldrich) from ten somite stage until observation. As controls, siblings were kept in EM.

Fluorescent microscopy. DREKA embryos were imaged on a Leica sp5 confocal microscope with a 20x water objective in GFP channel. All other fluorescent images were taken on a Nikon Ti microscope equipped with a Hamamatsu ORCA V2+ camera and a 10x plan fluo objective. Filter setting of CFP: excitation at 438 ± 24 nm, emission at 483 ± 32 nm; mEosFP and Alexa 488: excitation at 497 ± 16 nm, emission at 535 ± 22 nm.

Time lapse video. All embryos were dechorionated before bud stage using acute tweezers. The embryos were mounted in an agarose mold and kept in a temperature incubator at 27 ± 0.3 °C during imaging, following published protocols. Temperature incubator at 27 °C (Fig. S4) and over 2.

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Author contributions
W.Z. and D.B. planned and analyzed the experiments. W.Z. and P.S. performed most of the experiments. W.Z., B.D., V.C., and M.De. performed the RT-qPCR experiments. S.V., V.M., and M.Di. developed some of the zebrafish lines used here. W.Z., P.S., B.D., and D.B. wrote the paper.

Competing interests
The authors declare no competing interests.

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