Supplementary Information

Supplementary Methods

Image Quantification for Fixed Samples

Fixed sample image analysis was performed in Fiji software[1]. In-house macros were used to perform batch analysis of images. For PSM immunostaining data, noise of raw images was first reduced by the “Despeckle” function. We next used “ROF Denoise” Gaussian denoising function with theta parameter set to 25 for cell nuclei. After background subtraction and local contrast enhancement, a nuclear mask was created from the channel with “IJ Isodata” thresholding method. The cytoplasmic mask was defined as complementary to the nuclear mask. Nuclear and cytoplasmic masked versions of proteins of interest were calculated by multiplying the created masks with data channels. Segmented lines of interest (LOIs, ~30 μm wide) following the curvature of PSM tissue started from the mid-point between tail bud and posterior end of notochord and ended at the anterior end of imaged area. For the analysis requiring data alignment from the anterior boundary of PSM, PSM tissue was assured to be fully covered during image acquisition. Segmented LOIs were drawn starting from the somite-PSM boundary and ending at the tail bud. “Plot Profile” function was used for the intensity measurements through LOI. In heatshock data, spatial average values of other proteins were reported from the ~61 μm long PSM tissue, anterior of notochord tip and the slice medio-laterally centering the PSM tissue was used. For ppERK, Her7-Venus and β-Catenin proteins, maximum intensity projection of 3 z-stacks was used for analysis from the slices exhibiting gradient peaks.

Her7-Venus, ppERK, and nuclear β-Catenin data were background corrected using the trough values of one-cell window smoothened expression profiles along the PSM as reference. Tissues lacking a clock reporter (Df(Chr05:*her1,her7*)$^{b567/b567}$, Df(Chr05:*her1,her7*)$^{b567/+}$, wild-type) were phase sorted according to their PSM sizes; as the PSM tissue keeps growing in between somite stages with tail elongation (Fig. 1b). Smoothing of finalized PSM plots (over x=6.2 μm neighbor window) were done in GraphPad Prism software.

For immunostaining of late-stage embryos (pFAK, F59, Phalloidin), “background subtraction”, “remove outliers”, and “local contrast enhancement” tools were used before standard deviation projection of F59 or pFAK channels. Projection images were then binarized with thresholding. “Simple neurite tracer” plugin[2] was used to trace the fiber lengths in F59 staining, and myotome boundaries in pFAK staining.

For *in situ* hybridization of *xirp2a* data, “light background subtraction”, “remove outliers”, and “local contrast enhancement” tools were followed by binarization of staining with local thresholding. “Simple neurite tracer” plugin was used to trace the somite boundaries; boundary deficiencies were calculated from fractional occupancy of these traced boundaries in binary data (Extended Data Fig. 7a-d).

Image Quantification of Time-Lapse Data

For observation of clock dynamics alone, maximum intensity z-projection of nuclear masked mVenus signal was used for kymograph analysis using the “Time Lapse LOI Interpolator” plugin in Fiji software[1]. 50 pixels width lengths of interest (LOIs) were drawn along the PSM aligning from the tailbud.

For simultaneous imaging of segmentation clock and ERK activity, time-lapse movies were preprocessed in NIS Elements 9.0 as follows: Cell nuclei and membrane channels were denoised using “Denoise a.i” and were bleach-corrected using “Equalize intensity in time”. The nuclear and membrane channels were local contrasted with 3 μm size and 50 power. Tissue movements were aligned in z and time axis using the “Alignment” feature.

After the preprocessing in NIS Elements, time lapse movies were fed to Imaris 9.8.0 software. Using ‘ImarisCell module’, PSM cells were segmented and tracked. Segmented cells between 100 to 2000 μm^3 of cell and 30 to 500 μm^3 of nucleus volumes were retained. Most reliable tracking results were obtained with ‘Brownian Motion’ algorithm. We allowed cell tracks to have gaps in time up to 3 frames.

Mean cytoplasmic, nuclear, and cell intensities for observed channels alongside with 3-D positional values of tracked cells for which both cell and nuclei were tracked for 24 or more frames (144 min) were extracted from Imaris. ERK activity and the clock intensity are then calculated in MATLAB as:

$$ERK - KTR_{C/N} = \frac{Mean\ Cytoplasmic\ BFP\ intensity}{Mean\ Nuclear\ BFP\ intensity}$$

$$Clock = Mean\ Cell\ \ YFP\ intensity$$

Harvested cell tracks from Imaris were further verified as follows: In clock-intact embryos, we eliminated the tracks displaying abrupt changes in clock expression phase, indicative of cell track jumps. We finally verified correctly tracked cells manually using MATLAB and FIJI software and eliminated all unsuccessful tracks. These successful tracks were coming from cells getting displaced from pPSM towards anterior over time, falling off the ERK activity gradient at various time points. Once the cells join the pPSM, they usually experience 2-4 cycles of clock oscillations before they reach to mid-PSM. However, we were able to reliably track up to 4 cycles of oscillations in some cells that spent a longer time in the pPSM. To extract ERK activity dynamics from individual cell tracks, we sought to shift tracks in time so that all cells would fall off the ERK activity gradient together. For that purpose, we extracted how the EC50 value of each embryo’s ERK activity gradient moves over time as follows: We first constructed an 8-bit ERK activity image in MATLAB, by scaling the 5th percentile and 95th percentile values of $ERK - KTR_{C/N}$ to 0 to 255. We then created ERK activity kymographs using the ‘Time Lapse LOI Interpolator’ plugin in FIJI. Kymographs were interpolated 10 times along the time axis for precise analysis. We measured the displacement of EC50 of the ERK activity gradient along the kymograph. We then shifted every single cell track in time accordingly so that they would pass the gradient’s EC50 at the same moment (270 min in Fig. 1j-n). In clock-intact data (Fig. 1j, m), we further aligned individual cell tracks within an embryo to match their clock oscillation phases by performing temporal shifts less than 5 frames (~half the clock period). Clock signal was detrended to correct for initial photobleaching observed in YFP channel. To do so, we calculated a lower baseline of Venus signal using peak envelope function in MATLAB and subtracted the calculated baseline from the signal. Phase calculations of clock and ERK tracks (Fig.1l) are performed using Gaussian wavelet analysis with pyBOAT[3] for each individual cell without amplitude normalizations, using tracks up to 222 min in gradient aligned data (before descent of gradient, defined as pPSM). We detected ERK oscillations in all clock-intact cells analyzed. Slope calculations (Fig. 1n) were performed by identifying

the troughs of mid-PSM clock waves and fitting a line to ERK activity of individual cell tracks within this time window. In clock mutants, we used an equal mean duration time window encompassing the decline of the gradient. Tracks not exhibiting a declining ERK trend or shorter than 3 frames are excluded from slope analysis; resulting n=197 cells from clock-intact embryos and n=169 cells from clock mutants.

For SFC of ERK activity, we used deep-imaged PSM movies. After feeding cell segmentation data from Imaris into MATLAB, we populated a cell neighbor list for every cell using Delaunay triangulation. We then calculated SFC of ERK activity of a pair of cells i and k as:

$$SFC_{i,k} = max\left(\sum_{k=1}^{n_i} \frac{ERK\ Activity_k}{ERK\ Activity_i} \Big/ n_i \,, \sum_{j=1}^{n_k} \frac{ERK\ Activity_j}{ERK\ Activity_k} \Big/ n_k\right)$$

where cell i has $k = 1,..,n_i$ neighbors and cell k has $j = 1,..,n_k$ neighbors. We next constructed 8-bit SFC of ERK activity maps as described for ERK activity above. We set an upper limit for SFC value (=1) to be 255 in 8-bit construction (Fig. 4). SFC of ERK movies from each embryo were then analyzed with kymographs as described and kymograph averages of multiple embryos are shown in Fig. 4c.

Phase Groupings of IHC data in Wild-type and Mutant Embryos

Somites segment periodically, whereas the tail elongates continuously (Fig. 1b). For embryos with the same number of somites, those that just formed a somite have a shorter PSM than those about to segment next somite. Therefore, we grouped embryos missing a clock reporter (clock mutants and wild-type) into multiple phases based on their PSM lengths (Extended Data Figs. 1c,e,i) such that for each somite stage, Phase I (shortest PSM) embryos are presumably just segmented, and Phase II are further advanced to segment next somite (have longer PSM) and so on. Because early embryo development is unaffected and the PSM normally elongates in homozygous clock mutants, we were able to match their stages with their siblings (heterozygous or wild-type). We used the last broken boundaries to assess the length of PSM in mutants (Extended Data Fig. 1b). Thus, we grouped IHC data for clock mutants and siblings into three phases (Fig. 1c), for wild-type embryos into two phases for three different stages (Extended Data Fig. 1j).

Simulation Details

Reaction-diffusion equations governing molecule concentrations in simulations are as follows:

$$\frac{d(mFgf)}{dt} = K_{mFgf}^{syn} - K_{mFgf}^{deg} \times mFgf \quad (1)$$

$$Clock = A_C \times \frac{\sin(2\pi t/T(x))+1}{2} \quad (2)$$

$$\frac{\partial Fgf}{\partial t} = K_{Fgf}^{syn} \times mFgf(t-\tau_L) - K_{Fgf}^{deg} \times Fgf - K_{Comp}^{bind} \times Fgf + D \times \left(\frac{\partial^2 Fgf}{\partial x^2} + \frac{\partial^2 Fgf}{\partial y^2}\right) \quad (3)$$

$$\frac{d(Comp)}{dt} = K_{Comp}^{bind} \times Fgf - K_{Comp}^{deg} \times Comp \quad (4)$$

$$\frac{d(Erk)}{dt} = K_{Erk}^{syn} - K_{Erk}^{deg} \times Erk - K_{Erk}^{act} \times Erk \times Comp(t-\tau_C) + K_{Erk}^{inactI} \times pErk \times Inh + K_{Erk}^{inactC} \times pErk \times Clock \quad (5)$$

$$\frac{d(pErk)}{dt} = K_{Erk}^{act} \times Erk \times Comp(t-\tau_C) - K_{pErk}^{deg} \times pErk - K_{Erk}^{inactI} \times pErk \times Inh - K_{Erk}^{inactC} \times pErk \times Clock \quad (6)$$

$$\frac{d(Inh)}{dt} = K_{Inh}^{syn} \times pErk(t - \tau_I) - K_{Inh}^{deg} \times Inh \quad (7)$$

where $mFgf$ is Fgf RNA, Fgf is Fgf protein, $Comp$ is Fgf receptor-ligand complex, $Clock$ is segmentation clock protein, Erk is inactive ERK, $pErk$ is active ERK, Inh is the negative feedback inhibitor protein.

Period of clock, $T(x)$, in Eq. (2) nonlinearly increases from posterior ($x = 1$) to the anterior ($x = 40$), as given by the equation $T(x) = 30 \times \left(1 + \exp\left(\frac{5(x-35)}{L}\right)\right)$, matching at boundaries with experimental data in Extended Data Fig. 2f. For the ligand, in Eq. (3), boundary conditions are defined as follows: $\frac{\partial Fgf}{\partial x}|_{x=0} = 0$ at the posterior end, and $Fgf|_{x=L} = 0$, where L is tissue length, at the anterior end of the tissue.

We experimentally extracted the $drug$ parameter for PD184352 treatments, by fitting the data in Extended Data Fig. 8c, 10d. To do so, we defined a Hill formula for drug efficacy of PD184352 to convert experimental drug concentrations into inhibitory $drug$ parameter in simulations as:

$$drug = 1 + \frac{A}{1 + \left(\frac{C_{PD18}}{C_0}\right)^{-\alpha}}$$

where A is 2.1, α is 0.9, C_0 is 1 μM, and C_{PD18} is the experimental PD184352 concentration (Extended Data Fig. 10e).

Supplementary Discussion 1: The spatiotemporal dynamics of the determination front

What is the determination front?

The clock and wavefront (CW) model proposed that a molecular oscillator (the clock) controls the pace of segmentation while a smoothly regressing wavefront controls the positions of somite boundaries[4]. In the prevailing CW model, this segmental commitment position (the determination front) corresponds to a concentration threshold of the Fgf signaling gradient which acts permissively for the segmentation clock: “Fgf8 morphogen is the molecular determination front [,…] threshold [of which] drops low enough to permit […] cells at the axial location to be competent to form a boundary. More specifically, the cells at the Fgf8 determination front become competent to respond to the “molecular clock” whose alarm goes off when a boundary should be created.” [5]

Supporting the CW model, Dubrulle et al., 2001[6] and Sawada et al., 2001[7] discovered that Fgf signaling forms a gradient in the PSM and perturbing its activity changes somite sizes. Later studies found that Wnt signaling also establishes a gradient in the tissue and its perturbation also changes somite sizes[8,9]. However, it was unknown whether both gradients jointly form the wavefront (e.g., additive effect of both signaling activity) or one signaling pathway is the wavefront (i.e., directly instructing somite boundaries) while the second influences somite sizes indirectly. It was also unclear which feature of the gradient instructs positional information (i.e., determination front) among alternative information encoding mechanisms attributed to morphogen gradients

in different tissues and organisms (see references in [10]). Thus, the identity of the wavefront remained unclear.

We previously showed that Fgf signaling directly instructs somite boundaries whereas Wnt signaling indirectly acts through Fgf signaling in zebrafish[10]. Moreover, experimental data from both PSM explants and intact embryos refuted a concentration threshold model and showed the spatial fold change (SFC) of ppERK activity marks the determination front in zebrafish[10]. The value of SFC is fixed at the determination front (last presumptive somite boundary) throughout somitogenesis and under all perturbation conditions. In this study, we therefore inspect the spatiotemporal dynamics of this experimentally validated determination front (i.e., the SFC of ppERK).

Where is the determination front located?

Segmental commitment occurs four to five somite cycles ahead of physical separation of somites at early-stage chick and zebrafish embryos. Once a somite boundary is determined, perturbing the activity of ppERK or the segmentation clock cannot block segmentation or change somite sizes[6,7,11]. This segmental commitment (i.e., determination front) occurs much earlier than the establishment of RC polarity. Polarized expression of *Mesp/mesp* and other genes occur in the last one to two presumptive somites depending on stage and organisms[12-14]. Thus, the determination front precedes the establishment of RC polarity. Unlike chicken and zebrafish, segmental commitment positions have not been detailly mapped in mice. However, available data suggest that the determination front in mice is also posterior to the *Mesp* expression[15].

Previous work investigating early somitogenesis stages had claimed that the determination front in zebrafish corresponds to the anterior limit of ppERK gradient (i.e., EC0)[16]. However, later published experiments in chick[17] and zebrafish[10] argued against concentration threshold model. Moreover, the anterior limit of ppERK gradient is close to the true determination front (~EC15) only during early somitogenesis (see Figure S4B in [10]). Thereafter, the determination front climbs up the ppERK gradient as the gradient gets steeper at later stages (~EC70 at 26 somite stage[10]). Thus, neither expression domains of RC polarity genes (i.e., *mesp*) nor constant values of the ppERK gradient mark the determination front.

What are the spatiotemporal dynamics of the determination front?

While Niwa et al., 2011[18] showed that the amplitude of ppERK oscillates in mice, ppERK was thought to be steady in zebrafish[16,19]. We here discovered the amplitude of ppERK gradient display clock-dependent oscillations in zebrafish. Our newly generated *her7-Venus* reporter allowing reliable observation of clock oscillations in posterior PSM was inaccessible to earlier zebrafish studies[16,19] to reach this conclusion. Moreover, binarization of the ppERK gradient into ON/OFF states had resulted in over-simplification of the data[16]. In that work, higher levels of the ppERK gradient were called ON separately in each embryo, resulting oversight of oscillations within the high levels. Amplitude oscillations were also missed in live FRET reporter experiments[19]. We can think of several plausible reasons leading to this:

(a) Binning and averaging – Similar to Akiyama et al. [16], representation of the FRET efficiencies in binned groups (3 or 8 bins for different figures) and averaging methods used in the analysis such as median filtering might have resulted such oversight.

Specifically, revealing fast ERK activity oscillations with a noisy live reporter can only work after alignment of the data according to the clock phases. Simultaneous observation of the ERK activity with a reliable clock reporter, as we presented here, was demanded.

(b) Slow response time – A FRET reporter with a slow response time would not recapitulate fast dynamics of the original signal. FRET data showing changes in the ERK activity reflects this sluggishness (see Fig. 1c-e in Sari et al. [19], inhibition of Fgf signaling by using 675 μM SU5402). In pulse experiments, by IHC, we observe ppERK level changes for durations as short as 10 min with a ~22 times lower drug concentration (Fig. 2f, $p<0.0001$). However, the FRET reporter was able to show a significant difference ($p<0.05$) only after 40 min.

Furthermore, designed FRET reporter used CFP and YFP, as donor and acceptor, respectively. On the other hand, all the clock reporters designed up until now required the use of fast-folding and high quantum yield fluorescent reporters (YFP, mVenus, Achilles). This is also a potential problem for future usage of the FRET reporter with existing clock reporters. Due to the limitations of this FRET-based reporter, we generated a new transgenic line by adopting an improved nuclear-cytoplasmic kinase translocation reporter for ERK activity (ERK-KTR(NLS3), in BFP channel, Extended Data Fig. 2a) and combined it with transgenic clock reporter (Her7-Venus, Extended Data Fig. 2d) line.

Quantification of ERK-KTR relies on precise separation of cytoplasm from nuclei and an easy imaging access to the tissue of interest. Tight packing, high posterior motility, and narrow cytoplasmic size of PSM cells together with the spherical shape of zebrafish embryos complicate such quantifications. To overcome those complications, we imaged punctured whole embryos laterally with 0.27 μm x-y and 2 μm z-resolution every 6 minutes in a temperature stabilized on-stage incubation system, for 5-7 hours. Our ERK-KTR reporter precisely recapitulated the ppERK gradient profile (Extended Data Fig. 2b) and responded to SU5402 inhibition of Fgf signaling within 10 minutes (Extended Data Fig. 2c). This precise reporter enabled us to simultaneously observe segmentation clock and determination front dynamics in live embryos. Having confirmed all the dynamics observed in clock-intact embryos, our live-reporter is amenable for measuring ppERK activity and the determination front (SFC of ppERK) longitudinally in the PSM tissue.

Previously, Niwa et al., 2011[18] used *Dusp4* and *Mesp2* expression to indirectly infer information about the ppERK-dependent determination front. However, these genes also receive inputs from other signaling pathways or the segmentation clock (Wnt/Hes7 to *Dusp4* and Notch/Tbx6/Hes7/Her7 to *Mesp2*). Their expression domains do not correspond to the determination front (see discussion above). A direct reporter for the determination front dynamics was long needed. The ERK-KTR transgenic line we present here is a state-of-the-art reporter to track the determination front in the PSM tissue and ERK activity in other tissues. By quantifying the regression dynamics of SFC of ppERK, we showed here that the clock discretizes positional information, and this effect is sufficient and essential for sequential instruction of somite boundaries.

In summary, the determination front is marked by an SFC threshold but not a concentration threshold of ppERK[10]. We now present that, despite oscillation and gradual regression of ppERK levels, the SFC value maintains its position in between

consecutive segmental commitments. Thus, the SFC mechanism together with clock is functioning as an analog to digital converter for a reliable positional information from the dynamics of ppERK gradient. Importantly, we show here the hierarchy between the clock and gradient and mechanistically explain how a continuously regressing and oscillating gradient encodes positional information at discrete locations.

Supplementary Discussion 2: Clock-dependent Oscillatory Gradient (COG) model

Fgf gradient steadily regresses along the PSM (Fig.3k, left, orange) due to localized *fgf* transcription in tail bud and tail elongation. In clock mutants, the ppERK gradient simply follows the regression of Fgf gradient (Fig.3k, middle, green). The SFC of ppERK ($p_{neighbor}/p_{cell}$ =1+Δp/p) depends both on the absolute ppERK level (p) and the local slope of the gradient (Δp). With a steadily regressing ppERK gradient in mutants, the critical SFC position (black stars) also steadily regresses (Fig.3k, middle, black dashed arrows). In wild-type (Fig.3k, right), when the clock expression (magenta) increases in the mid-PSM (Fig.3k, top), it decreases local ppERK levels (p↓). Simultaneously, Fgf signaling recovers ppERK in cells trailing behind the clock stripe resulting in a steeper gradient between cells and their neighbors in mid-PSM (Δp↑, curved green arrow). These changes jointly trigger a sudden increase in SFC (Δp/p) hence the determination front displaces to its next position (Fig.3k, top to middle). When the clock is highly expressed in aPSM (Fig.3k, middle row) or emerges again in the pPSM (Fig.3k, bottom, magenta arrows), it does not affect the SFC of ppERK in mid-PSM. SFC of ppERK stalls during those two phases of the clock (black dashed arrow) because while regressing Fgf gradient decreases the ppERK levels, the elevated slope also relaxes back in the mid-PSM. The decreases of both p and Δp stalls the SFC in its position (Fig.3k, bottom). With cyclic clock expression throughout somitogenesis, this process converts the gradual regression of ppERK levels in mid-PSM into discrete dynamics of the determination front. The SFC readout mechanism extracts robust positional information from the oscillatory dynamics of the ppERK gradient.

Supplementary Discussion 3: Integration of the clock and gradient
How can an oscillatory gradient encode positional information?

Non-PSM cells without a clock were induced to form somite-like epithelial aggregates[20]. However, these ectopic structures formed simultaneously and were not organized into an array of segments. Thus, the segmentation clock is necessary for sequential somite segmentation *in vivo*. However, how the clock instructs segmental commitment and how it is integrated with the ppERK gradient remained unknown.

According to the original CW model, there is no network hierarchy between the clock and wavefront; cells integrate spatial and temporal information as an AND logic gate. However, Niwa et al., 2011[18] discovered that ppERK activity oscillates in mice and its oscillation depends on the clock (i.e., Hes7). Later on, studies in zebrafish[16,19] found that the regression dynamics of the ppERK gradient changes in the presence of the clock (i.e., Her1 and Her7). Conversely, Ishimatsu et al., 2010[21] and Diaz-Cuadros et al., 2020[22] showed that ppERK activity influences the temporal dynamics of the clock in zebrafish and mice. Overall, these studies showed that the clock and ppERK gradient

crosstalk. However, the functional significance of this crosstalk had not been elucidated. Crosstalk does not imply a hierarchy between the clock and gradient. In the absence of a hierarchy, it is still possible that both the clock and gradient control different target genes to independently instruct temporal and spatial information. Thus, how the spatiotemporal information is integrated to drive sequential somite segmentation remained elusive. We here showed the clock acts hierarchically upstream of the gradient to instruct somite boundaries.

Earlier studies on ppERK dynamics had indicated differences between mice and zebrafish: Niwa et al., 2011[18] reported ppERK gradient amplitude to oscillate in pPSM and display traveling waves in mid-PSM in mice, while Akiyama et al., 2014[16] reported a non-oscillatory ppERK gradient displaying stepwise regression in mid-PSM. Species-specific differences between ppERK gradient dynamics put the determination front concept under scrutiny and initiated a search for alternative models[17,23]. As oscillations were unexpected for a signaling gradient instructing the determination front, Niwa et al., 2011[18] fully switched the roles of the Notch/Hes signaling and Fgf/ppERK signaling: They proposed ppERK oscillations and Notch stripes regulate the periodicity of somites in time (i.e., the clock) and space (i.e., the determination front; see also[24]), respectively. Our findings here showed these differences between zebrafish and mice were superficial, revealing a previously unnoticed conservation in ppERK gradient dynamics. We further showed that the clock decreases ppERK levels as a causal link. We then revealed an oscillatory signaling dynamics can still provide positional information through the fold-change detection (SFC) mechanism. Therefore, the ppERK gradient can function as a unifying segmentation mechanism among vertebrates.

Case for SFC mechanism among alternative signal detection models

In this study, we assessed whether the SFC mechanism can explain how an oscillatory gradient encodes positional information for somite boundaries. We also highlighted the obvious failure of concentration threshold model in resolving this problem. Alternative signal detection models (temporal fold-change, gradient slope, and time-integration/averaging) were already scrutinized in our previous work and failed to explain numerous experiments e.g., cell non-autonomous segmental commitment of PSM cells in response to FGF signal changes in their neighbors[10]. Here we will briefly discuss whether those alternative models could explain the results presented in this study.

Is it possible that segment boundary positions can be instructed by a sharp difference in ERK activity during a segmentation cycle (i.e., temporal fold-change (TFC))? If we consider the outcomes of the rescue experiments, PSM cells do not seem to be responding to TFC of ppERK for segmental commitment: Global pulsatile drug inhibition drives pPSM oscillations of the ppERK levels (Fig. 2f,g). However, TFC of ppERK in pPSM is much higher with SU5402 pulses (up to %70.2±3.8 decrease for $x_{PSM} \leq 150$ μm) than their wild-type counterparts (up to %39±12 decrease, Extended Data Fig. 10a). Moreover, pPSM cells of pulsatile SU5402 treated embryos experience higher TFC of ppERK than even the mid-PSM (determination front) cells of wild-type embryos (%46±12 decrease, Extended Data Fig. 10a), yet they do not commit to segmentation precociously in the pPSM. Instead, we observe matching number of boundary inductions (Fig. 2h,j), only by the cells located in mid-PSM during the pulses

(Extended Data Fig. 6b,c). Furthermore, TFC detection mechanism, similar to the concentration threshold, would be sensitive to signal oscillations within a single embryo. On the other hand, SFC detection of ppERK relies on responding to the signal ratio between neighboring cells. Therefore, SFC detection prevents pPSM cells committing to segmentation prematurely: because ppERK levels go down and up together in all cells, the SFC value does not increase (Fig. 4b,c,e). Signal comparison among neighbors could also filter out extrinsic sources of noise.

Alternatively, slope detection as well could buffer out amplitude oscillations of the ppERK gradient. However, slope detection predicts opposite results from two experiments giving the same outcome in this study: 1-) In *her7-Venus* reporter embryos, when clock expression increases at the determination front the ppERK gradient steepens (Extended Data Fig. 10b, Extended Data Fig. 4b,f,g). 2-) In pulsatile inhibition experiments with mutants, the slope of the ppERK gradient decreases (Extended Data Fig. 10c, Fig. 2f). Both reduction of slope (drug pulse in mutants) and increase of it (clock reporter line), followed by a relaxation, results in the same outcome: segmental commitment (Extended Data Fig. 10b,c). This argues against a slope detection model to explain observed data.

On the other hand, the COG model relying on SFC mechanism is able to explain the experiments. Simulating the COG model, we were able to fit the optimal boundary-inducing PD183452 drug regime (Extended Data Fig. 10d) and convert the MEK inhibitor concentrations into inhibitory efficacy of ERK phosphorylation (Extended Data Fig. 10e). These simulations showed that restoring somite segmentation in clock mutants does not require strong oscillations of ppERK gradient amplitude (Extended Data Fig. 10f). Supportively, we had shown somite segmentation can also be restored with moderate amplitude oscillations (Extended Data Fig. 8e) similar to that in wild-type embryos (Fig. 1c). Simulation fits of the COG model further enabled us to simulate short (6'+24') and long (10'+40') pulse experiments with 600 nM PD183452 treatment (corresponding to $drug = 1.81$ in simulations). We observed COG model can explain lower (35%) and higher (48%) amplitude oscillations of ppERK gradient for short and long pulses respectively (Extended Data Fig. 10g, and j) and predict discrete determination front shifts (Extended Data Fig. 10h, and k) matching with experimental data (Extended Data Fig. 10i, and j).

As a last note, segmentation is rapidly restored in clock mutants upon drug pulses, coinciding with the correct location of determination front in wild-type embryos (Fig.2j, Extended Data Fig. 6c). This rapid restoration also argues against time integration-averaging models requiring longer processing durations.

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Supplementary Table 1: Simulation Parameters. Name, description, and values of parameters describing the simulation space and equations.

Supplementary Table 1 | Simulation Parameters

Parameter Name	Description	Value / Range
Rate Equation Parameters		
K_{mFgf}^{syn}	*fgf* mRNA synthesis rate	24 min^{-1} (0^{th} order)
K_{mFgf}^{deg}	*fgf* mRNA degradation rate	0.06 min^{-1} (1^{st} order)
K_{Fgf}^{syn}	Fgf protein synthesis rate	2.4 min^{-1} (1^{st} order)
K_{Fgf}^{deg}	Fgf protein degradation rate	0.6 min^{-1} (1^{st} order)
K_{Comp}^{deg}	Receptor complex degradation rate	2.4 min^{-1} (1^{st} order)
K_{Comp}^{bind}	Binding rate of Fgf protein-receptor complex	0.024 min^{-1} (1^{st} order)
K_{Erk}^{syn}	ERK synthesis rate	30 min^{-1} (0^{th} order)
K_{Erk}^{deg}	ERK degradation rate	0.24 min^{-1} (1^{st} order)
K_{pErk}^{deg}	ppERK degradation rate	0.24 min^{-1} (1^{st} order)
K_{Erk}^{act}	ERK activation (phosphorylation) rate	2.4 min^{-1} (2^{nd} order)
K_{Erk}^{inactI}	ERK inactivation rate by inhibitor protein	0.96 min^{-1} (2^{nd} order)
K_{Erk}^{inactC}	ERK inactivation rate by clock protein	0.96 min^{-1} (2^{nd} order)
K_{Inh}^{syn}	Inhibitor protein synthesis rate	1.2 min^{-1} (1^{st} order)
K_{Inh}^{deg}	Inhibitor protein degradation rate	0.6 min^{-1} (1^{st} order)
D	Diffusion coefficient of Fgf protein	50 μm^2/sec
τ_L	Translation and secretion time-delay of Fgf	6 min
τ_C	Time-delay from receptor complex to ERK activation	6 min
τ_I	Transcriptional and translational time-delay of inhibitor protein	18 min
$drug$	Fold-decrease of ERK activation rate due to inhibitory drug	1.05 – 3.00
$pulse$	Time fraction of drug treatment per cycle	1:2 – 1:6
A_c	Oscillation amplitude of clock signal	35
Simulation Space Parameters		
t	Iteration time step	0.004 min
$L \times W$	PSM length and width	308×*28* µm
V_g	Tail growth speed with cell ingression	0.911 µm/min
Simulation Fit Parameters		
C_0	Half efficacy concentration for PD183452	1 µM