Species-specific segmentation clock periods are due to differential biochemical reaction speeds

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Short Title: Species-specific oscillation periods

1 One Sentence Summary: *In vitro* segmentation clocks reveal why the human oscillation
2 period is longer than that of mouse

3
4 This week in Science: Human development is in general slower than that of mouse. The
5 segmentation clock is the oscillatory gene expression that regulates body segment
6 formation, and the oscillation period differs among species. Matsuda et al. studied the
7 mechanism by which the human segmentation clock displays an oscillation period of 5-6
8 hours whereas the mouse period is 2-3 hours. The cells at the segmentation clock stage
9 were induced from human and murine pluripotent stem cells and compared. Several
10 biochemical reactions, including protein degradation and delays in gene expression
11 processes, were found to be slower in human cells as compared with the mouse
12 counterparts. These slower reaction speeds in human cells lead to the slower tempo of the
13 human segmentation clock.

Abstract

While mechanisms of embryonic development are similar between mice and humans, the tempo is slower in humans. To investigate interspecies differences in developmental tempo, we recapitulate murine and human segmentation clocks that display 2-3 hours and 5-6 hours oscillation periods, respectively. Our interspecies genome swapping analyses indicate that the period difference is not due to sequence differences in the HES7 locus, the core gene of the segmentation clock. Instead, we demonstrate that multiple biochemical reactions of HES7, including the degradation and expression delays, are slower in human cells than those in mouse. With the measured biochemical parameters, our mathematical model successfully accounts for the 2-3 fold period difference between the species. We propose that cell autonomous differences in biochemical reaction speeds underlie species-specific developmental tempi.

Main Text

Different animal species develop with different tempi(1). The mouse gestation period is ~20 days while that of human takes ~9 months. At each developmental stage, the order of developmental processes and the underlying mechanism are largely conserved between the species, but the progression speed differs. For instance, murine and human nervous systems generate multiple neuronal subtypes in identical order, but the sequential neurogenesis takes longer in humans(1-3). We propose to call such interspecies differences in developmental tempo despite conservation of the mechanism, as developmental ‘allochryny’.

The cause of allochryny remains a mystery due to lack of an appropriate model system. As embryos of different species differ in their sizes, geometries, and nutrients, direct interspecies comparison is challenging. Investigating developmental dynamics in human embryos is also ethically difficult. To overcome the difficulties, we decided to use *in vitro* differentiation of pluripotent stem cells and compare the same type of cells under similar culture conditions between different species. As an example of well-defined developmental time, we focused on the segmentation clock, oscillatory gene expression that regulates the timing of sequential formation of body segments called somites out of presomitic mesoderm (PSM) cells(4, 5). The oscillation period (i.e., duration for one cycle) of the segmentation clock is species-specific: ~30 minutes in zebrafish, ~90 minutes in chickens, 2-3 hours in mice, and 4-6 hours in humans(5, 6). In this study, we recapitulated murine and human segmentation clocks *in vitro*, showing that the allochryny of oscillation periods is derived from cell autonomous differences in the speeds of biochemical reactions between the species.

Cell autonomous period difference between species

To compare murine and human segmentation clocks *in vitro*, we induced PSM-like cells from mouse embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) (fig. S1A and Fig. 1A). The detailed induction protocol and characterization of PSM-like cells are described in our recent reports(7, 8), and several other groups have also reported similar PSM induction protocols(9-13). The induced PSM-like cells are hereafter referred to as PSM. To visualize the segmentation clock, we introduced a HES7 promoter-luciferase reporter(8, 14), detecting clear synchronized oscillations of HES7 expression in both murine and human PSM (Fig. 1A and movie S1). Consistent with previous *in vivo* and *in vitro* measurements(6, 8, 12-15), the mouse PSM oscillated with a period of 122 ± 2 min (mean $\pm$ sd) whereas human PSM exhibited a 322 ± 6 min period (Fig. 1, B to D, and fig. S1B). Thus, our induced PSM serves as an ideal *in vitro* platform to investigate the cause of the 2-3 fold period difference between murine and human segmentation clocks.

The gene regulatory network of the segmentation clock consists of two parts: the intracellular network that gives rise to a cell autonomous oscillation and the intercellular network that synchronizes the oscillations among cells (fig. S2A)(16, 17). We attempted to clarify whether the interspecies period difference stems from the single-cell oscillator or the multicellular synchronized oscillations. While intercellular communication through NOTCH-DELTA signaling synchronizes oscillations (16, 18-20), treatment of PSM cells with a NOTCH signaling inhibitor did not affect the oscillation period significantly (fig. S2, B and C). As WNT and FGF signaling pathways also modulate the segmentation clock(21, 22), we measured the oscillation period in a sparse cell culture, where cells are isolated from the neighboring cells (Fig. 1E, fig. S3, and movie S2). The isolated single PSM cells still displayed the 2-3 fold period difference between the species (mouse: 160 ± 9 min; human: 376 ± 51 min) (Fig. 1F), even though the oscillations at the single-cell level were noisier and slower than the population level oscillations (see fig. S3, B and C). These results indicate that the period difference of HES7 oscillation between mice and humans is cell autonomous, and that the cause of the interspecies difference should lie in the oscillation mechanism at the single-cell level.

Interspecies period difference is not due to HES7 sequence differences

HES7 oscillations arise from a delayed autoregulatory negative feedback loop: HES7 is a transcription repressor that directly inhibits its own promoter with time delays (fig. S2A)(16, 23). We first hypothesized that differences in the sequences of HES7 loci

1 between murine and human genomes might lead to the observed oscillation period
2 difference. To test this hypothesis, we swapped murine and human HES7 loci (Fig. 2A).
3 The sequence of the human HES7 locus, including the promoter, exons, introns, and
4 UTRs, was knocked into the mouse HES7 locus in mouse ESCs (fig. S4), and the resulting
5 cells were induced into the PSM fate. The homozygous knock-in (homo swap) PSM and
6 the heterozygous knock-in (hetero swap) PSM showed slightly longer oscillation periods
7 of 146 ± 7 min and 133 ± 4 min, respectively, as compared with the 124 ± 3 min period
8 of wild-type (wt) mouse PSM (Fig. 2, B and C). Considering that the period of wt human
9 PSM is 322 min, however, the period extension in homo swap mouse PSM is minor. We
10 also created knock-in mice, and the homo swap mice appeared largely normal, even
11 though their vertebrae, which are derivatives of somites, displayed minor defects (fig.
12 S5A). The *ex vivo* measurements of the segmentation clock in homo swap embryos
13 showed a ~ 30 min longer oscillation period as compared with wt embryos (Fig. 2, D and
14 E, and fig. S5B), consistent with the ~ 20 min period extension in the homo swap samples
15 of induced PSM (see Fig. 2C). However, the 20-30 min period extension in homo swap
16 PSM/embryos is far from the ~ 200 min period difference between mice and humans, so
17 the human HES7 locus in mouse PSM gives rise to an essentially mouse-like oscillation
18 period.

19 One potential defect in our experimental design of interspecies genome
20 swapping is, however, that the swapped HES7 region might not be long enough, and that
21 a crucial sequence for the oscillation period might exist upstream of the HES7 promoter
22 we defined. To rule out this possibility, we performed 'knock-out and rescue' assays (fig.
23 S6A). The endogenous mouse HES7 gene was knocked out in mouse ESCs, which led to
24 disruption of HES7 oscillation in the induced PSM (Fig. 2F, KO). Then the disrupted
25 oscillation was rescued by an exogenous construct containing a promoter, exons, introns,
26 and UTRs of the murine or human HES7 locus (Fig. 2F, Rescue mHES7 and Rescue
27 hHES7). Note that these exogenous constructs were integrated into random locations of
28 the genome by transposon vectors. Both murine and human HES7 constructs at random
29 genomic locations restored mouse-like oscillation periods in the mouse PSM (Fig. 2G and
30 fig. S6B). We further attempted a complementary experiment: the endogenous human
31 HES7 gene was knocked out, and the disrupted oscillation in human PSM was rescued
32 with the murine or human HES7 construct (Fig. 2F and fig. S6B). Again, murine and
33 human HES7 constructs were indistinguishable in terms of the restored oscillation periods
34 (Fig. 2H). These results collectively indicate that the 2-3 fold period difference is not
35 caused by the sequence differences between murine and human HES7 loci.

Interspecies period difference is due to different biochemical reaction speeds

We then hypothesized that differences not in the sequences but in the biochemical reaction speeds of HES7 between murine and human cells might lead to the observed oscillation period difference. The most important biochemical parameters that affect the HES7 oscillation period are the degradation rates and delays in the regulatory feedback loop of HES7 (Fig. 3A)(16, 24-27). In fact, genetic manipulations that modulate the degradation or delay of HES7 affect the oscillation period in mice(24, 26). Delays due to splicing and export of mRNAs are also reported to be different among mice, chickens, and zebrafish(27). Thus, we measured the degradation rates of HES7 mRNA and protein (i.e., δ_m and δ_p in Fig. 3A) as well as the several delays in the HES7 feedback loop (τ_{Tx} , τ_{In} , τ_{TI} , and τ_{Rp} in Fig. 3A) in PSM cells, exploring which parameter(s) are different between mice and humans. To measure the degradation rate of HES7 protein (δ_p), we overexpressed either murine or human HES7 sequence and then halted its expression (Fig. 3B). Interestingly, both murine and human HES7 proteins were degraded more slowly in human PSM as compared with mouse PSM, meaning that the changes in the degradation rate depend on the differences not in the HES7 sequences but in the cellular environments (i.e., whether HES7 is hosted in a murine or human cell). The half-life of HES7 protein in mice is reported to be 22 min(24), consistent with our measurements where half-lives in murine and human PSM were 21 ± 0.8 min and 40 ± 4 min, respectively (Fig. 3C and fig. S7A).

To measure the delay caused by the transcription and translation of HES7, we induced the expression of HES7 and estimated the onset time by fitting the results to a standard gene expression model where transcription and translation occur in a linear manner with the corresponding delays (Fig. 3D and fig. S7, B and C). The transcription and translation delay (τ_{TxTI}) of HES7 was longer in human PSM (30 ± 1 min) as compared with mouse PSM (17 ± 2 min) (Fig. 3E). The fitting also allowed us to estimate the degradation rate of HES7 mRNA (δ_m), showing slower mRNA degradation in human PSM (half-life: 16 ± 0.3 min) as compared with mouse PSM (half-life: 10 ± 0.3 min) (fig. S7D). Note that the HES7 gene used in these measurements did not include the introns (see Fig. 3, B and D). Since introns affect mRNA splicing and therefore serve as another source of delays(25-27), we measured the delay caused by HES7 introns by creating HES7 promoter-luciferase reporters with (w/) and without (w/o) introns (fig. S8A) and estimating the phase difference between the oscillations of the two reporters (Fig. 3F and fig. S8B). Again, the HES7 intron delay (τ_{In}) was longer in human PSM (37 ± 3 min) as compared with mouse PSM (13 ± 3 min) (Fig. 3G). Roughly consistent with our measurements, the intron delay or splicing delay in mouse embryos is reported to be 12-

19 min(25, 27). Finally, to measure the delay for HES7 to start repressing its own promoter, we induced the expression of HES7 and estimated the onset of decline in the HES7 promoter activity (fig. S9). Fitting the results to an open loop repression model where the induced HES7 protein represses the expression of HES7 promoter-luciferase reporter suggested that the HES7 repression delay (τ_{Rp}) is negligible in both murine and human PSM.

To confirm that the degradation rates and delays measured in murine and human PSM can indeed explain the interspecies period difference, we built a simple mathematical model of HES7 oscillation(16) based directly on the following parameters: δ_p , δ_m , τ_{TxTl} , τ_{In} , and τ_{Rp} (Fig. 4A). Mathematical analyses of the model showed that the oscillation period depends on these measured parameters (i.e., degradation rates and total delays), and that other parameters, such as the transcription and translation rates and the repression threshold, essentially do not affect the period (text S1)(16). Even though one unmeasured parameter, the repression Hill coefficient, potentially affects the oscillation period (text S1), varying this parameter within a realistic range did not change the period dramatically (Fig. 4B). Remarkably, our simulation of oscillations with the murine parameters showed periods of ~150 min whereas that with human parameters showed ~300 min periods (Fig. 4B), reproducing the 2-3 fold period difference between actual murine and human PSM (see Fig. 1D). These results mean that the slower biochemical reactions of HES7 (i.e., slower degradations and longer delays) in human PSM as compared with mouse PSM are sufficient to quantitatively account for the longer oscillation period of the human segmentation clock.

Generality of slower biochemical reactions in human cells

Next, we explored how universal our finding of slower biochemical reactions in human cells is. To test whether it is specific to the HES7 gene, we measured the degradation rates of six other genes, transcription factors expressed at the PSM stage(9) (Fig. 5A, fig. S10, and fig. S11). GBX2, MSGN1, and TBX6 proteins showed slower degradation rates in human PSM as compared with mouse PSM, whereas CDX2, EVX1, and Brachyury T did not show a significant interspecies difference (Fig. 5A). We also measured the transcription and intron delays (τ_{Tx} , τ_{In}) (Fig. 5B, fig. S12, and fig. S13). TBX6, GBX2, and MSGN1 showed longer delays in human PSM whereas EVX1 did not (Fig. 5B). These results suggest that the slower biochemical reactions in human PSM as compared with mouse PSM can extend at least to several other genes, but not to all genes. Note that the same genetic construct (depicted in Fig. 5, A and B) was used for the measurements in murine and human cells, meaning that the observed difference in reaction parameters

1 is not due to genomic sequence differences of the target gene but due to different cellular
2 environments between the species.

3 Finally, to test whether the slower biochemical reactions in human cells are
4 specific to PSM cell type, we induced neural progenitor cells (NPCs) from mouse ESCs
5 and human iPSCs (Fig. 5C)(28, 29). Most of the induced NPCs were PAX6 positive (Fig.
6 5C), even though they may constitute slightly different cell populations between the
7 species. We measured the degradation rates of neural marker genes(29) in both murine
8 and human NPCs (fig. S14 and fig. S15). All the four proteins tested, OTX2, FOXG1,
9 PAX6, and SOX1, showed slower degradation rates in human NPCs as compared with
10 mouse NPCs (Fig. 5D). We also measured the transcription and intron delays of OTX2,
11 FOXG1, and SOX1 (fig. S16 and fig. S17), demonstrating slightly longer delays in human
12 NPCs for all three genes (Fig. 5E). Compared to PSM cells, NPCs tended to show larger
13 interspecies differences in degradation rates and smaller differences in delays, even
14 though the number of genes tested is too small to draw any conclusions. These results
15 suggest that slower biochemical reactions in human cells can be generalizable not only to
16 the PSM fate but also to other cell types. We propose that murine and human cells possess
17 species-specific cellular environments that affect speeds of several biochemical reactions
18 including degradations and delays (Fig. 5F), potentially causing other interspecies
19 differences in developmental tempo. The cellular environments can mean any gene set or
20 any cellular characteristic, such as the metabolic rate and cell size.

21 **Discussion**

22 In summary, we have shown that the human segmentation clock exhibits 2-3 times slower
23 oscillations than that of mouse, because of slower biochemical reactions of HES7 in a
24 human PSM cell. It is noteworthy that multiple reaction parameters of HES7, including
25 the degradation rates, transcription/translation delay, and intron delay, displayed
26 interspecies differences and collectively accounted for the 2-3 fold period difference.

27 We have also revealed the existence of several other genes than HES7 that show
28 interspecies differences in reaction speeds in PSM cells or NPCs. Interestingly, a recent
29 report has shown that human motor neuron generation is 2-3 times slower than that of
30 mouse, and the key regulatory genes exhibit slower protein degradation rates in human
31 motor neural progenitor cells(3). Oscillation dynamics and biochemical reaction speeds
32 of p53-Mdm2 network are also reported to be slower in a human cell line as compared
33 with a mouse line(30). These reports together with our results imply a general principle
34 of allochrony.
35

An obvious question remaining is the mechanism by which human cells display slower biochemical reactions. We attempted to find commonalities in sequences or functionalities among the genes that either showed or did not show differential reaction speeds between murine and human cells, but the number of genes tested was not large enough to yield significant results. Thus, while this study has revealed cell autonomous mechanisms of developmental allochry, their deep origin remains a mystery.

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Figure 1 Period difference between murine and human segmentation clocks

(A) HES7 reporter activities in murine and human presomitic mesoderm (PSM)-like cells in time-lapse imaging with a luminescent microscope. Kymographs indicate spatio-temporal signals along the line between points A and B. Scale: 400 μ m. See movie S1. (B) Oscillatory HES7 reporter activity measured with a luminometer. A collective signal from a 35 mm dish. Mean $\pm$ sd (shadow). $n = 3$. (C) Overlay of murine and human detrended signals. (D) Oscillation periods estimated from fig. S1B. (E) Time-lapse imaging of sparsely seeded single PSM cells. See movie S2. Scale: 20 μ m. (F) Periods of single cells estimated from fig. S3. (D, F) Mean $\pm$ sd. P-values from two-sided student's t-test.

Figure 2 Swapping murine and human HES7 loci

(A) Homologous recombination between murine and human HES7 loci. (B) HES7 reporter activities in mouse PSM containing human HES7 locus. Wt: mouse HES7/mouse HES7; Hetero swap: human HES7/mouse HES7; Homo swap: human HES7/human HES7. (C) Periods estimated from (B). Wt human PSM period shown in Fig. 1D is displayed as a control. P-values from two-sided Dunnett's test. (D) Time-lapse imaging of *ex vivo* tail bud cultures of E10.5 mouse embryos containing human HES7 locus. (E) Periods estimated from fig. S5B. P-value from two-sided paired t-test. (F) Knock-out and rescue assay. Endogenous HES7 genes were knocked out (KO), and the disrupted oscillations were rescued with an exogenous construct of either mouse HES7 (Rescue mHES7) or human HES7 (Rescue hHES7). See fig. S6A. (G, H) Periods estimated from fig. S6B. P-values from two-sided student's t-test. (B, F) Mean $\pm$ sd (shadow). $n = 3$. (C, E, G, H) Mean $\pm$ sd.

Figure 3 Measuring biochemical parameters of HES7

(A) Negative feedback loop of HES7. Delays and degradation rates of the loop were measured in the indicated panels. τ_{TX} : Transcription delay; τ_{In} : Intron delay; τ_{TI} : Translation delay; τ_{Rp} : Repression delay; δ_m : Degradation rate of mRNA; δ_p : Degradation rate of protein. (B) Degradation assay of HES7. The transcription of HES7 fused with NanoLuc (NLuc) was halted upon Doxycycline (Dox) addition (top), and the decay of NLuc signal was monitored with a luminometer (bottom). (C) Inverse degradation rates of HES7 protein estimated from fig. S7A. (D) Expression delay assay of HES7. The transcription of HES7-NLuc was induced upon Dox addition (top), and the onset of NLuc signal was monitored (bottom). (E) Transcription/translation delays of HES7 estimated from fig. S7, B and C. (F) Intron delay assay of HES7. Reporter constructs without (w/o)

and with (w/) HES7 intron sequence were introduced simultaneously (top and middle), and the cross correlation of two reporters were calculated (bottom). See fig. S8A. (G) Intron delays of HES7 estimated from (F) and fig. S8B. (B, D, F) Mean $\pm$ sd (shadow). n = 3. (C, E, G) Mean $\pm$ sd. P-values from two-sided student's t-test.

Figure 4 Simulating oscillations with measured biochemical parameters

(A) HES7 oscillations simulated with the parameters measured in murine or human PSM. Hill coefficient was set as n = 3. The other parameters are in table S1. (B) Periods estimated by computing the power spectra of simulated oscillations with different values of Hill coefficient.

Figure 5 Generality of slower biochemical reactions in human cells

(A) Degradation assay of other genes expressed at the PSM stage. The transcription of a gene of interest (GOI) fused with NLuc was halted, and the decay of NLuc signal was monitored (top). Inverse protein degradation rates were estimated from fig. S11 (bottom). HES7 degradation rate shown in Fig. 3C is displayed as a control. (B) Expression delay assay of other genes expressed at the PSM stage. The transcription of FLuc-GOI fusion construct flanked by a stop codon was induced, and the onset of FLuc signal was monitored (top). Sums of the transcription delay of fusion construct, intron delay of GOI, and translation delay of FLuc were estimated from fig. S13 (bottom). (C) *In vitro* differentiation of NPCs from mouse ESCs and human iPSCs. Scale: 200 μ m. (D) Degradation assay in NPCs. Inverse protein degradation rates were estimated from fig. S15. (E) Expression delay assay in NPCs. Delays were estimated from fig. S17. (F) Proposed scheme. Murine and human cells have different cellular environments that affect the speeds of multiple biochemical reactions. TxTl: transcription and translation. (A, B, D, E) Mean $\pm$ sd. n = 3. P-values from two-sided student's t-test.

Fig. 1.

Fig. 2

Fig. 3

Fig. 4

Fig. 5

text S1 Parameter dependence of the oscillation period in the model

The mathematical model of the HES7 system that we use to describe the behavior of the segmentation clock is [1]:

$$\frac{dm}{dt} = \frac{\beta}{1 + \left(\frac{p(t-\tau_m)}{K}\right)^n} - \delta_m m \quad (\text{S1})$$

$$\frac{dp}{dt} = \alpha m(t - \tau_p) - \delta_p p, \quad (\text{S2})$$

where m and p are the concentrations of HES7 mRNA and protein, respectively, α and β are the translation and transcription rates, K is the repression threshold, n is the repression Hill coefficient, δ_m and δ_p are the degradation rates of the mRNA and protein, which we have measured experimentally as explained in the main text. The mRNA delay τ_m is composed by the repression delay τ_{Rp} , the transcription delay τ_{Tx} , and the intron delay τ_{In} , all of which we have measured experimentally as well. The protein delay τ_p , in turn, corresponds to the translation delay τ_{Tl} , which was also quantified using our experimental observations.

This system has a fixed point (m^*, p^*) for which the two derivatives above are zero, which obeys:

$$\frac{\beta}{1 + \left(\frac{p^*}{K}\right)^n} = \frac{\delta_m \delta_p p^*}{\alpha} \quad (\text{S3})$$

The stability of this fixed point can be analyzed by assuming the following temporal response to a small perturbation (a, b) :

$$m(t) = m^* + a \exp(\lambda t) \quad (\text{S4})$$

$$p(t) = p^* + b \exp(\lambda t) \quad (\text{S5})$$

Introducing expressions (S4)-(S5) into Eqs. (S1)-(S2), linearizing around $a = b = 0$, and imposing that a solution of the form (S4)-(S5) exists with nonzero a and b , leads to the following transcendental characteristic equation for the eigenvalues λ :

$$(\lambda + \delta_m)(\lambda + \delta_p) + \frac{n(\delta_m \delta_p)^2}{\alpha \beta} \frac{p^{*n+1}}{K^n} \exp(-\lambda(\tau_m + \tau_p)) = 0, \quad (\text{S6})$$

In general the eigenvalues are complex numbers $\lambda = \sigma + i\omega$. The eigenvalue with highest real part determines the stability of the fixed point, with $\sigma < 0$ corresponding to a stable fixed point, and $\sigma > 0$ to an unstable one. The corresponding imaginary part establishes the frequency at which the system oscillates towards the fixed point (if $\sigma < 0$) or away from it (if $\sigma > 0$). In the case of an unstable fixed point with $\omega \neq 0$, the system usually falls on a limit cycle whose period can be expected to be close to $2\pi/\omega$. Taking these considerations into account, Eq. (S6) shows that **the period of the HES7 oscillations does not depend on the transcription and translation delays separately, but only on the total delay $\tau_m + \tau_p$.**

For the parameters that we consider in this paper, $p^* \gg K$, as can be seen in Fig. S1, which represents graphically the solution of Eq. (S3) as the crossing point between its left-hand side

(blue line) and right-hand side (orange line). In the limit $p^* \gg K$, Eq. (S3) has the following approximate solution:

$$p^{*n+1} = \frac{\alpha\beta K^n}{\delta_m \delta_p} \quad (\text{S7})$$

Figure S1: Graphical determination of the fixed point of the HES7 model used in this paper, for the parameters corresponding to the mouse (left) and human (right) cells.

Inserting expression (S7) into the characteristic equation (S6) leads to:

$$(\lambda + \delta_m)(\lambda + \delta_p) + n\delta_m\delta_p \exp(-\lambda\tau) = 0, \quad (\text{S8})$$

where $\tau \equiv \tau_m + \tau_p$ is the total delay of the system. Considering again that the imaginary part of the eigenvalue with the highest real part gives us an estimate of the oscillation period, we can observe from Eq. (S8) that **the period of the HES7 oscillations does not depend on the values of the translation and transcription rates α and β , nor on the repression threshold K .**

Writing the eigenvalue λ as a complex number as mentioned above, $\lambda = \sigma + i\omega$, and separating the real and imaginary parts of Eq. (S8), we obtain two coupled transcendental equations for σ and ω :

$$\omega^2 - (\sigma + \delta_m)(\sigma + \delta_p) - n\delta_m\delta_p \exp(-\lambda\tau) \cos(\omega\tau) = 0 \quad (\text{S9})$$

$$\omega(2\sigma + \delta_m + \delta_p) - n\delta_m\delta_p \exp(-\lambda\tau) \sin(\omega\tau) = 0 \quad (\text{S10})$$

These two equations can be solved graphically by looking for the crossing points between the two curves defined by the left-hand-side terms of Eqs. (S9) and (S10). This graphical analysis, shown in the left panel of Fig. S2, reveals a solution for which the real part of the eigenvalue is positive (see zoom in right panel), corresponding to a unstable spiral which will be surrounded by a limit cycle whose frequency we can approximate by the value of ω given by the x coordinate of the crossing point.

Figure S2: Graphical determination of the eigenvalue of the fixed of model (S1)-(S2), for the parameters corresponding to the mouse case. The right panel is a zoomed-in version of the left panel.

We can obtain the value of the oscillation frequency at the bifurcation by dividing Eq. (S9) by Eq. (S10) with σ set to 0, which leads to the following transcendental equation:

$$\tan(\omega\tau) = \frac{\omega(\delta_m + \delta_p)}{\omega^2 - \delta_m\delta_p} \quad (\text{S11})$$

This expression shows that **at the bifurcation point, the period of the oscillations does not depend on n , but only on the degradation rates of the mRNA and the protein, and on the total delay.**

Equation (S11) also allows us to discern the relative contributions to the oscillation period of the delay τ and the degradation terms δ_m and δ_p . To that end, we solve graphically the equation around the unstable fixed point found in Fig. S2 above, as shown in the left panel of Fig. S3. As

Figure S3: Left: graphical determination of the frequency of the oscillations at the bifurcation, for the parameters corresponding to the mouse case. Right: dependence of the period on the fold-change of the delay (red), degradation rates (blue) and expression strengths (grey). The value 1 corresponds to the parameters of the mouse case.

can be seen in that plot, the crossing point between the left- and right-hand sides of the equation

(magenta and green, respectively) is dominated by the location of two vertical asymptotes, shown by dashed lines of the corresponding colors. The magenta asymptote occurs at $2\pi/\tau$, and the green one at $\sqrt{\delta_m\delta_p}$. This shows that both the delay τ and the degradation rates δ_m and δ_p will affect in a similar manner the crossing point between the two curves, and thus the corresponding value of ω (and of the period $2\pi/\omega$). This is shown systematically in the right panel of Fig. S3, which shows how the oscillation period at threshold varies when rescaling either the delay τ (red), both degradation rates δ_m and δ_p together (blue), and both expression strengths α and β together (grey). As expected, the period does not depend on the expression strengths, and varies in a similar manner (although in opposite sense) for the degradation rates and the delay. Both an increase in delay and a global decrease in the degradation rates lead to an increase in period. This is the situation in our fitting of the human cell experiments, with respect to those of mice.

References

- [1] J. Lewis, “Autoinhibition with transcriptional delay: a simple mechanism for the zebrafish somitogenesis oscillator,” *Current Biology*, vol. 13, no. 16, pp. 1398–1408, 2003.